VOL 54 NO 1 (2022)

ACTA MEDICA INDONESIANA

The Indonesian Journal of Internal Medicine

2022

A Publication of The Indonesian Society of Internal Medicine

e-ISSN: 2338-2732
p-ISSN: 0125-9326

Urease, Gastric Bacteria and Gastritis

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Urease is an enzyme produced by diverse bacterial species including normal flora, non pathogens, and pathogens such as *Proteus mirabilis*, *Staphylococcus saprophyticus*, *Klebsiella pneumonia*, *Citrobacter freundii*, *Enterobacter cloacae Helicobacter spp* and *Helicobacter pylori*.[1-4] Urease is central in *Helicobacter pylori* metabolism and virulence, important for colonization in the gastric mucosa.[1] Urease catalyzes the hydrolysis of urea to ammonia and carbamate. This ammonia product can be examined by Urease biopsy test and Urea breath test such as ^{14C}-Urea Breath Test or ^{13C}-Urea Breath Test.[1]

Previously, the Urea breath test was intended to detect an increase in ammonia which is a urease product in the gastric mucosa produced by pathogenic gastric bacteria, such as *Helicobacter pylori*, etc.[1-7]

Acute and chronic gastritis caused by infection with these pathogenic bacteria infection turned out to be positive on Urea breath test.[8-11] Indirectly, the results of the urea breath test are also related to the presence of inflammation in acute and chronic gastritis, regardless of whether the cause is Helicobacter pylori or other urease-producing pathogenic bacteria.[7,8]

The use of the urea breath test indirectly in diagnosing acute and chronic gastritis should be studied further. The use of the urea breath test is indeed very important to assist health services in countries and regions with limited endoscopic facilities, especially developing countries.

We know that the prevalence of *Helicobacter pylori* infection in causing acute and chronic gastritis by examination of Urea breath test in Indonesia is not too high, ranging from 2-11.2%.[5] So that is why more studies on *non-Helicobacter pylori* producing urease pathogens are needed, which can appear as a false positive urea breath test.

Miftahussurur M et al.[5] in their research on 95 dyspeptic patients at Soetomo Hospital Surabaya Indonesia found that the urease levels of positive patients with acute and chronic gastritis were higher than negative patients ($p = 0.001$, $r = 0.353$; $p < 0.0001$, $r = 0.433$). The AUC values of 14C-UBT for detecting acute, chronic, and atrophic gastritis were 0.889, 0.632 and 0.544, respectively. They concluded that 14C-UBT is an adequate diagnostic modality to predict acute or chronic gastritis but not atrophic gastritis.

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Indonesia DIA-RAMADAN Study: A Real-life, Prospective and Observational of Gliclazide MR in Type-2 Diabetes Patients During Ramadan Fasting

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ABSTRACT

Background: *Sulfonylureas (SUs) have been widely used in many countries for T2DM treatment. Gliclazide is one of the SUs with the lowest risk of hypoglycemia; however, the safety and effectiveness of gliclazide MR during Ramadan has not yet been reported in Indonesia. This study aimed to assess safety, efficacy, and tolerability of gliclazide modified release (MR) during Ramadan fasting.* ***Methods:*** *The study was a part of DIA-RAMADAN study, a prospective observational study with subjects of T2DM patients aged >18 years, who had either controlled or sub-optimally controlled blood glucose level, performed Ramadan fasting. Subjects had been treated with gliclazide MR for at least 90 days prior the study, and were examined for their body mass index (BMI), fasting plasma glucose (FPG) and HbA1c levels 6 to 8 weeks before Ramadan (V0) and 4 to 6 weeks after the end of Ramadan (V1).* ***Results:*** *Out of 198 subjects participating in the study, there were only two subjects (1.0%) who reported symptomatic HEs (either confirmed or not confirmed) and no severe HEs had been reported. There were no significant changes in HbA1c and FPG levels ($p>0.05$). Interestingly, there was a reduction of bodyweight (-0.4kg) from pre- to post-Ramadan ($p < 0.001$). Almost no subjects reported discontinuation of gliclazide MR throughout the entire study; however, there was one subject who reported a change of diabetic treatment into diet only.* ***Conclusion:*** *gliclazide MR is safe, well tolerated and can maintain glycemic control effectively for Indonesian patients with T2DM who perform Ramadan fasting.*

Keywords: *type 2 DM, gliclazide MR, Ramadan, Indonesia.*

INTRODUCTION

Indonesia, as a country with the largest Muslim population, has been projected with having 238 million of Muslim population in 2010.[1] With a prevalence of diabetes mellitus (DM) of 10.9% by year 2018, Indonesia has been ranked as a country with the seventh largest diabetes population in the world.[2,3] Fasting during the holy month of Ramadan is an important event for Muslims and considered as one of the five pillars of Islam. Fasting for a long period could potentially affect the metabolic state of diabetes patients, including hypoglycemia, hyperglycemia, ketoacidosis, dehydration and increasing risk of complications.[4] Patients in Indonesia fast for one month during Ramadan with at least 13 to 14 hours of fasting per day.[5] There is no consensus about the most appropriate oral antidiabetic (OAD) agents for patients with T2DM to use during Ramadan.[6]

Sulfonylureas (SUs) remain the most commonly used OAD after metformin in Indonesia.[7] It is well known that SUs were associated with a higher risk of hypoglycemia, which has raised some concerns about their use during Ramadan. Several studies demonstrate that many patients with T2DM may continue to use second-generation SUs and fast safely during Ramadan.[6,8] Several randomised clinical trial (RCT) studies showed that gliclazide has lower risk of hypoglycaemia, even during fasting Ramadan, compared with other SUs. A newer formulation, modified release (MR) of gliclazide showed a lower risk of hypoglycemia, even during a fasting period when compared with other SUs.[6,9,11]

The effectiveness and safety of gliclazide MR has not been studied during Ramadan in a real-life setting. The Indonesia-DIA-RAMADAN study, is a part of Global-DIA-RAMADAN study, which is conducted across countries in the Middle East, Africa, South, and South-east Asia. To our knowledge, our study represents the first real-life study of gliclazide MR in patients with T2DM who perform fasting during Ramadan. The aim of our study was to assess safety (based on HEs), efficacy (based on HbA1c changes), and tolerability of gliclazide MR in Indonesian Muslims with controlled or suboptimal controlled T2DM (HbA1c < 9%) during Ramadan fasting.

METHODS

DIA-RAMADAN was a prospective and observational study conducted at 64 sites in 9 countries across the Middle East, Africa and Asia (Bangladesh, Egypt, India, Indonesia, Kuwait, Malaysia, Pakistan, Saudi Arabia and United Arab Emirates). Seven cities in Indonesia involved in this study were Jakarta, Surabaya, Yogyakarta, Makassar, Aceh, Solo, and Malang. The inclusion criteria were: subjects > 18 years of age with T2DM, body mass index (BMI) of ≥23 and <30 kg/m^2, controlled or sub-optimally controlled T2DM (HbA1C <9%); treated with gliclazide MR for at least 90 days prior to the initiation of the study (inclusion visit), either as monotherapy or in combination with any other diabetes treatment except insulin; experienced with self-monitoring of blood glucose (using blood glucose meter); subjects were willing to perform full (30 days) fast during Ramadan in 2019.

The study was conducted from mid-March 2019 to end of August 2019. The overall treatment duration consisted of pre-Ramadan period (6 to 8 weeks prior to the Ramadan), the Ramadan period (4.5 weeks), and post-Ramadan period (4 to 6 weeks after Ramadan) (**Figure 1**). During the first inclusion visit (V0), patient's demographic data, HbA1c and fasting plasma glucose (FPG) levels and body weight data were collected. Each patient was provided with a diary at V0 for recording the following events: (1) Any changes in their recommended oral antidiabetic therapy; (2) Any hypoglycemia-related symptoms experienced during the study; (3) Any other adverse events (AEs) occurred during the study.

Ethics

This study was approved by Ethical Committee of Health Research Ethics Committee (HREC) Faculty of Medicine, Brawijaya University (Reference no. 013/EC/KEPK/01/2019).

Study Assessment

Gliclazide MR was taken orally once daily at breakfast according to the summary of product

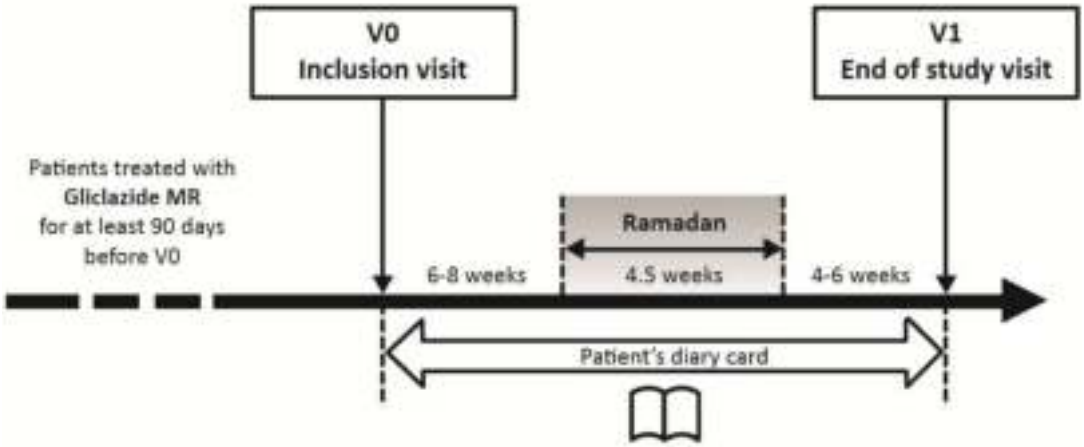

Figure 1. DIA- RAMADAN study protocol

characteristics (SmPC) until the beginning of Ramadan. During Ramadan[11], subjects were advised by their physician to take their gliclazide MR at iftar time (i.e., the post-sunset meal). Dose adjustment was based on direction of the investigator according to routine practice and local guidelines, if applicable. During the study, subjects continued receiving concomitant treatments for comorbid conditions.

Outcome Variables

The primary endpoint of the study was the proportion of subjects with at least one symptomatic hypoglycemia event (HE), either suggestive or confirmed by a measured glucose concentration of ≤ 70 mg/dL. Symptomatic hypoglycemia was defined as the presence of at least one of the following symptoms: sweating, pallor, tremor, intense hunger, pounding heart, visual disturbance, drowsiness, weakness, dizziness, cognitive impairment, unexplained behavior or mood change, confusion, headache; without or with a measurement of blood glucose. Severe hypoglycemia was defined as reported severe cognitive impairment requiring third-party assistance for recovery.[12,13]

Secondary endpoints included changes of the following: HbA1c and FPG levels as well as body weight between V0 and V1; the proportion of subjects with at least one confirmed HE (asymptomatic or symptomatic); the proportion of subjects with at least one HE of any type. Any HE was defined as symptomatic hypoglycemia (confirmed or not) or confirmed asymptomatic hypoglycemia (asymptomatic with a measured glucose concentration of ≤ 70 mg/dL).

Statistical Analysis

Two-sided statistical tests (paired t-test or Wilcoxon Signed Rank test) were applied with type I error (alpha), which was set at 5%. The Wilcoxon Signed Rank test was applied in cases of strong violation of normality. Statistical analyses were performed by Aixial (Boulogne-Billancourt, France). Analyses were conducted using SAS software, version 9.4 or higher (SAS Institute, North Carolina, USA). The values presented as means ± standard deviation unless specified otherwise.

RESULTS

Patient Recruitment

Out of a total of 212 recruited patients, 198 patients were included in the final analysis set. Fourteen patients were excluded from the final analysis for reasons including noncompliance with inclusion/exclusion criteria and withdrawal of consent. Of the 198 patients examined at the inclusion visit (V0), 183 (92.4%) completed the study by attending the end of study visit (V1). The majority of patients who withdrew from the study did so due to non-medical reasons. Among 198 patients, 91 were male (46%) and 107 were female (54%), with average fasting day was 28.7 days. Contributing cities were Jakarta [24%], Surabaya [21%], Yogyakarta [17%], Makassar [16%], Aceh [10%], Solo [7%], and Malang [5%]). All subjects were included in the final analysis set.

Table 1. Baseline characteristics

Variable	Mean (SD)
Gender (%)	
Male	91 (46)
Female	107 (54)
Age (years)	57.4 (8.2)
Diabetes durations	3.9 (3.9)
Diabetes treatment	
Gliclazide MR (monotherapy)	84 (42.4)
Gliclazide MR + 1 Other OAD	84 (42.4)
Gliclazide MR + 2 others OAD	30 (15.2)
Comorbid conditions (%)	
Arterial hypertension	76 (38.4)
Dyslipidemia	84 (42.4)
Established cardiovascular disease	10 (5.1)
Diabetic neuropathy	9 (4.5)
Diabetic nephropathy	2 (1.0)
Diabetic retinopathy	1 (0.5)
Diabetic foot	1 (0.5)
Duration of Fasting (days)	28.7 (3.5)
Laboratory values	
HbA1c, (%)	7.4 (0.9)
FPG, (mg/dL)	134.5 (42.4)
Serum Creatinine, (mg/dL)	0.91 (1.02)

FPG, fasting plasma glucose; OAD, oral anti diabetic; HbA1c, glycated hemoglobin; SD, standard deviation

Baseline Patterns of Antidiabetic Medication Use

Of 198 patients, 84 patients were using gliclazide MR as monotherapy (42.4%) and 114 patients were using gliclazide MR in combination with other antidiabetic agents (**Figure 2**). The mean daily dose of gliclazide MR was 65 mg. No dose modification in gliclazide MR treatment was observed during the study.

Safety and Tolerability

Hypoglycemia event under gliclazide MR treatment during Ramadan fasting in Indonesia was very low; there were only two subjects (1%) experiencing at least one hypoglycemia episode during the period of fasting, i.e., one subject (0.5%) with symptomatic hypoglycemia and one patient (0.5%) with confirmed hypoglycemia. No subjects reported severe hypoglycemia.

Adverse Effects (Other Than HEs)

The most commonly reported AEs in the study were vertigo and gastrointestinal disorders. In clinical experiences, gliclazide MR does not cause vertigo and gastro-intestinal symptoms but we cannot confirm whether these vertigo and gastro-intestinal complaints are related to medication or fasting. No subjects experienced any drug-related AEs during the study.

Tolerability

During the study period, there was only one subject who discontinued the gliclazide MR treatment and changed it with lifestyle modification according doctor's advice. Our results showed that gliclazide was well tolerated by almost all subjects.

Efficacy

HbA1c and FPG levels were examined at visits V0 and V1 (**Figure 3A**). There was no significant change of HbA1c and FPG (p>0.05) levels between both study visits. There were increased number of subjects with HbA1c level of

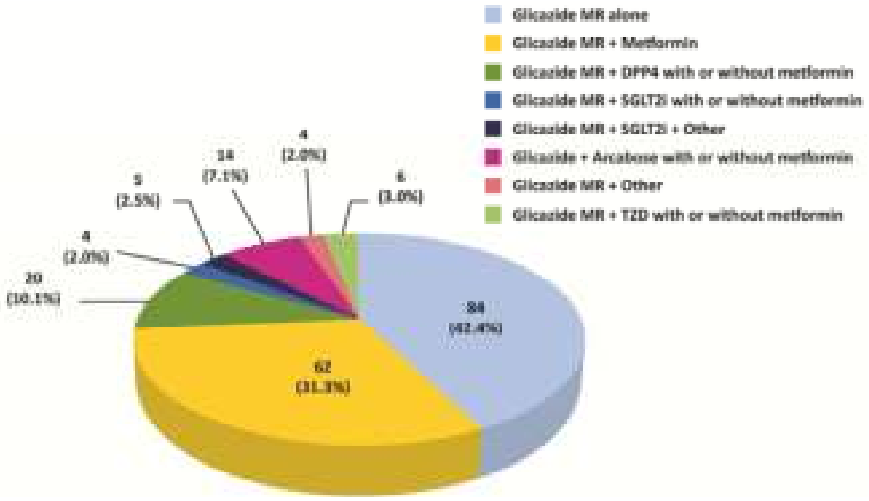

Figure 2. Antidiabetic treatment at baseline (v0). DPP4, dipeptidyl peptidase 4 inhibitor; GLP1 RA, glucagon-like peptide-1 receptor agonist; MR, modified release; SGLT2i, sodium-glucose cotransporter 2 inhibitor; TZD, thiazolidinedione.

<7.5% from 99 to 109 subjects and from 0 to 13 subjects with HbA1c >9%, indicating that there was improved glycemic control in 10 subjects and worsened in 13 subjects. (**Figure 3B**).

(HbA1c [n = 182] and FPG [n = 182] at V1). FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; SD, standard deviation.

Body Weight

Significant reductions in body weight (0.4 kg) were recorded between visits V0 and V1 ($p < 0.001$) (**Figure 4**).

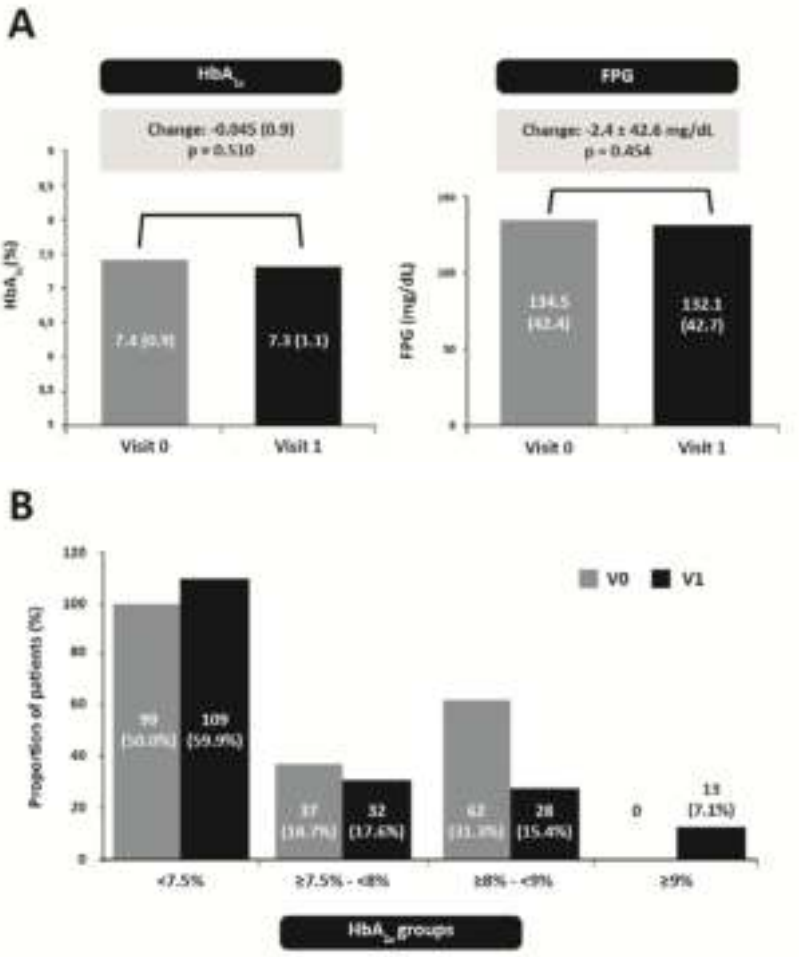

Figure 3. HbA1c and FPG levels at V0 and V1. (A) Mean HbA1c and FPG levels at V0 and V1. (B) Proportion of patients within specified HbA1c range at V0 and V1.

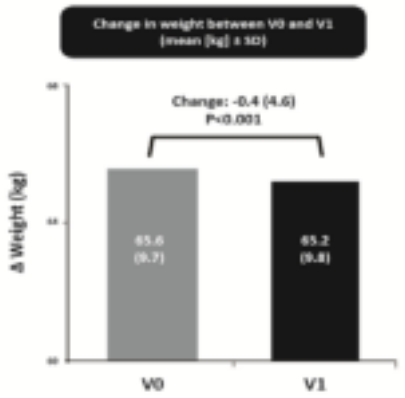

Figure 4. Mean body weight at V0 and V1. (n = 184). SD, standard deviation.

DISCUSSION

Sulfonylureas have been extensively used as treatment of type-2 diabetes for nearly 70 years.[14] The most common choice for second-line therapy in patients who require additional glucose-lowering during metformin monotherapy were SUs and DPP-4is.[15]

To our knowledge, DIA-RAMADAN is the first real-life observational study assessing the efficacy and safety of gliclazide MR 60 mg in patients with controlled or sub-optimally controlled T2DM during Ramadan fasting. In line with the results from previous observational studies, gliclazide (IR or MR) showed a lower incidence of HEs compared with other SUs in this study. A large observational study evaluating the incidence of HEs in patients with T2DM treated with glipizide, glimepiride, gliclazide (IR or MR), or glibenclamide showed that 14% of those treated with gliclazide experienced at least one symptomatic HE during Ramadan, which was notably lower than values reported for other SUs (glipizide [27.6%], glibenclamide [25.6%] and glimepiride [16.8%]).[16]

The reduced rate of HEs with gliclazide compared with other SUs could be explained by differences in its pharmacokinetic and pharmacodynamics properties, mechanism of action and insulin excretion profile.Sulfonilureas stimulate secretion of insulin from pancreatic β-cells via a blockade of ATP-sensitive K-channels (KATP), resulting in membrane depolarisation, calcium influx and insulin release. While glibenclamide provides irreversible inhibition of the Kir6.2/SUR1 KATP channel, inhibition by gliclazide is rapidly reversible.

A previous study examining hypoglycemia in patients with T2DM receiving sitagliptin or an SU during Ramadan showed that incidence of hypoglycaemia was similar between sitagliptin and gliclazide. The proportion who reported symptomatic HEs in sitagliptin group was 6.7% compared with 6.6% of patients treated with gliclazide MR.[6] Additionally, the proportion of patients treated with gliclazide MR with at least one symptomatic HE during Ramadan was reported to be as low as 1.8% in one study (compared with glibenclamide [5.2%] and glimepiride [9.1%]).[10]

The ultimate goal of diabetes treatment during Ramadan is to sustain glycemic control during fasting period with low risk of hypoglycemia in patients with T2DM[17]. Our results presented here show that gliclazide-treated patients experienced a stable level of HbA1c and FPG as well as a significant reduction of body weight between study visits. As no dose changes were reported during the study, patients treated with gliclazide MR can therefore continue with pre-Ramadan dosing levels during fasting. There was a higher proportion of patients having an HbA1c value ≥9% at V1 versus V0 (6.6%) compared to DIA-RAMADAN global study.[18] This different result could be related to change of meal composition during Ramadan since there was no report of any change in diabetic treatment except one subject reporting discontinuation of gliclazide MR and dietary change only during the study period.

Our study has several limitations, including the biases that are typically associated with non-comparative observational study designs. In addition, the study enrolled patients were already receiving gliclazide MR at stable doses for 90 days prior to the inclusion visit. This suggests that the study drug was well tolerated in these patients. Other relevant biases include underreporting of adverse events and hypoglycemic episodes, particularly those that were self-reported in patients' diaries, as well as recall bias. At visit V0, patients were advised about changes required to the timing of their gliclazide MR dose during the month of Ramadan according to current IDF-DAR guidelines at the discretion of the treating physician.

Results of our study show real-life evidences gathered by the investigators according to their standard clinical practice. Treatment adherence to gliclazide MR during Ramadan in Indonesia is high (98.8%). Majority of patients (92.4%) who attended the inclusion visit (V0) also attended the end-of study visit (V1), and only a few data were missing considering the observational nature of the study.

CONCLUSION

In Indonesian population, gliclazide MR either as monotherapy or combinations with other OADs has been shown to be an effective, safe and

well-tolerated treatment in patients with T2DM who perform fasting during Ramadan with a consistently low incidence of hypoglycemia, whilst maintaining glycemic control.

ACKNOWLEDGMENTS

The study was supported by Servier (Servier Affaires Medicales). We acknowledge the kind contribution of all the investigators, staff, and patients at the participating sites from the DIA-RAMADAN Study. The following are also DIA-RAMADAN study investigators in Indonesia: Benny Santosa, Bowo Pramono, Budi Santoso, Eva Nia M, Fatimah Eliana, FX Suharnadi, Himawan Sanusi, Ida Ayu Khsanti, Laksmi Sasiarini, M Robikhul, Rulli Rosandi, Sony Wibisono.

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The Validity and Reliability of the Indonesian Version of the Chronic Liver Disease Questionnaire (CLDQ) in Measuring Quality of Life in Patients with Liver Cirrhosis

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ABSTRACT

Background: *Liver cirrhosis remains the major cause of liver-related morbidity and mortality around the world. Cirrhosis also negatively affects health-related quality of life. Quality of life evaluation in cirrhosis treatment is often overlooked, despite its importance compared to traditional outcome. One of the specific tools to measure quality of life in cirrhosis patient is the Chronic Liver Disease Questionnaire (CLDQ). Although this tool has been widely used in many countries, no studies have been conducted on its validity and reliability in the Indonesian language. This study aimed to assess the validity and reliability of the Indonesian version of CLDQ using appropriate methods.* ***Methods:*** *This is a cross-sectional study conducted at Hepatobiliary outpatient clinic in Dr. Cipto Mangunkusumo National General Hospital (RSCM), from April-May 2021. The CLDQ was first translated into the Indonesian language and subsequent pretest was performed on 10 people, resulting in the final Indonesian version of the CLDQ. The final version was later tested in the main study with larger number of subjects (52 people). Validity was assessed using construct and external validity tests, while reliability was tested using internal consistency and test-retest methods.* ***Results:*** *The Indonesian version of CLDQ had a good construct validity (r 0.613-0.917), moderate external validity (54.1%), strong correlations between CLDQ and SF-36, good internal consistency (Cronbach-Alpha ≥ 0.7), and good test-retest reliability (ICC > 0.7).* ***Conclusion:*** *The Indonesian version of CLDQ is valid and reliable in measuring the quality of life of liver cirrhosis patients in Indonesia.*

Keywords: *chronic liver disease questionnaire, liver cirrhosis, quality of life, validity, reliability.*

INTRODUCTION

Liver cirrhosis, or frequently simply called cirrhosis, is a disease which remains a major cause of liver-related morbidity and mortality around the world.[1-4] In 2017, cirrhosis caused 1.32 million deaths worldwide (2.4%), a 1.9% increase from 1990 data.[4] Data from the USA showed that cirrhosis ranked 12 in all causes of mortality in 2007.[1] In Indonesia, the epidemiology data of cirrhosis remains lacking. According to one study conducted in a public hospital in Indonesia, the average prevalence of

cirrhosis is 3.5% out of all patients admitted to the Internal Medicine ward.[5]

Liver cirrhosis is irreversible in nature. Therefore, therapies for cirrhosis patients are targeted at palliative aspects instead of curative.[6] The goal of palliative treatment is to increase the quality of life of patients as cirrhosis disrupts the patients' health-related quality of life (HRQoL).[7] The HRQoL terminology resides below the wider quality of life (QoL) terminology, and it is an important multidimensional concept in patient's perspective including physical health, mental health, and social welfare.[8,9] The evaluation of HRQoL in cirrhosis treatment is frequently overlooked, due to the fact that cirrhosis treatment tend to focus only on the clinical aspects or traditional outcome. However, the HRQoL aspects tend to be more important for the patient personally as it pertains to aspects they value, such as the emotional and lifestyle aspects.[10]

There are various generic and specific tools to measure HRQoL in cirrhosis patients. The most widely-used generic tool is short form-36 (SF-36), while the most frequently-used specific tool is the chronic liver disease questionnaire (CLDQ). The CLDQ is widely used in many countries is because it is simple, easy to use, with fewer questions, related closely with severity of chronic liver disease, and it is the first questionnaire which specifically evaluates HRQoL in chronic liver diseases, including cirrhosis.[10,11]

Data about HRQoL in cirrhosis patients in Indonesia is limited, especially data on HRQoL measurements using CLDQ as the evaluation tool. Two studies conducted in two cities in Indonesia (Yogyakarta and Medan) have used CLDQ to evaluate the quality of life in liver cirrhosis patients; however, they have not conducted and reported the validity and reliability of the questionnaire, which raises a question on its translation and adaptation from the original language (English).[12,13] Although the validity and reliability of local versions of CLDQ has already been tested in many countries, the validity and reliability of the native Indonesian version needs to undergo the same testing process, due to the fact that the sociodemographic and clinical characteristics of cirrhosis patients differs from the original CLDQ study.[14] Therefore, this study is conducted to test the validity and reliability of the Indonesian version of CLDQ using appropriate methods according to the questionnaire validation guidelines. The resulting questionnaire could be used to evaluate HRQoL of Indonesian liver cirrhosis patients.

METHODS

This is a cross sectional study conducted in the Hepatobiliary Outpatient Clinic in Dr. Cipto Mangunkusumo National General Hospital (RSCM) from April-May 2021. The study was divided into two periods. The first period was pretest to test the prefinal version of CLDQ that has been translated into the Indonesian language with limited number of subjects (10 people), and the second period was the main research to test thevalidity and reliability of the final Indonesian version of CLDQ with a larger number of subjects (52 people), as determined using sample size formula for correlation tests. The characteristics of subjects in the first period differs from that of the second period. The methods was adapted from a study by Beaton DE, et al.[15] that was also used by several validity and reliability studies. The CLDQ has 29 questions and divided into six domains including: abdominal (AB), fatigue (FA), systemic (SY), activity (AC), emotion (EM), and worry (WO).

Before conducting the pretest, the authors requested permission from the original authors of CLDQ to be translated and validated into Indonesian language. The original English version of CLDQ was then translated and adapted with the help of four certified and experienced translators. Two Indonesian speaker translators (one with medical background and one with none) were assigned for the forward translation (English to Indonesian) and the other two were English speaker translators who were assigned for the backward translation (Indonesian to English). Every time the translator finished, the authors analyzed, synthesized, and discussed the translation results with all of the translators. After forward translation is completed, the pre-final Indonesian version of CLDQ was generated and pre-tested to 10 respondents who

matched the inclusion and exclusion criteria. The respondents' feedback from pre-test were included and the questionnaire was subsequently backward translated, resulting in the final Indonesian version CLDQ final version. The final version was assessed for its validity and reliability on 52 respondents, who also fulfilled the pre-determined criteria. Questionnaires were distributed using consecutive sampling methods. The validity of the Indonesian version of CLDQ was assessed using the construct and external validity tests, while its reliability was assessed using internal consistency and test-retest approach. The study flowchart is presented in the **Figure 1**.

Data were processed and analyzed using statistical package for social sciences (SPSS) for Windows version 21.0. Construct validity was analyzed using multitrait- multimethod analysis approach, and external validity was analyzed by conducting correlation test between CLDQ and other questionnaire. In this study, the SF-36 as a generic questionnaire was used due to several reasons: SF-36 is widely used in other validity and reliability studies in the other countries, it is the only questionnaire that has been translated and validated in Indonesian language to measure the quality of life in liver cirrhosis, and there are no other specific questionnaire besides CLDQ that has been translated and validated in Indonesian language. Internal consistency was assessed using Cronbach- Alpha coefficient and test-retest approach was used to calculate intraclass correlation coefficient with one-week interval.

Ethics

Ethical approval for this study was obtained from the Medical Research Ethics Committee of the Faculty of Medicine Universitas Indonesia, Dr. Cipto Mangunkusumo National Central General Hospital number: KET- 134/UN2. F1/ETIK/PPM.00.02/2021. All subjects were provided detailed information about the study, and have signed the informed consent form voluntarily. All subjects' information was kept confidential.

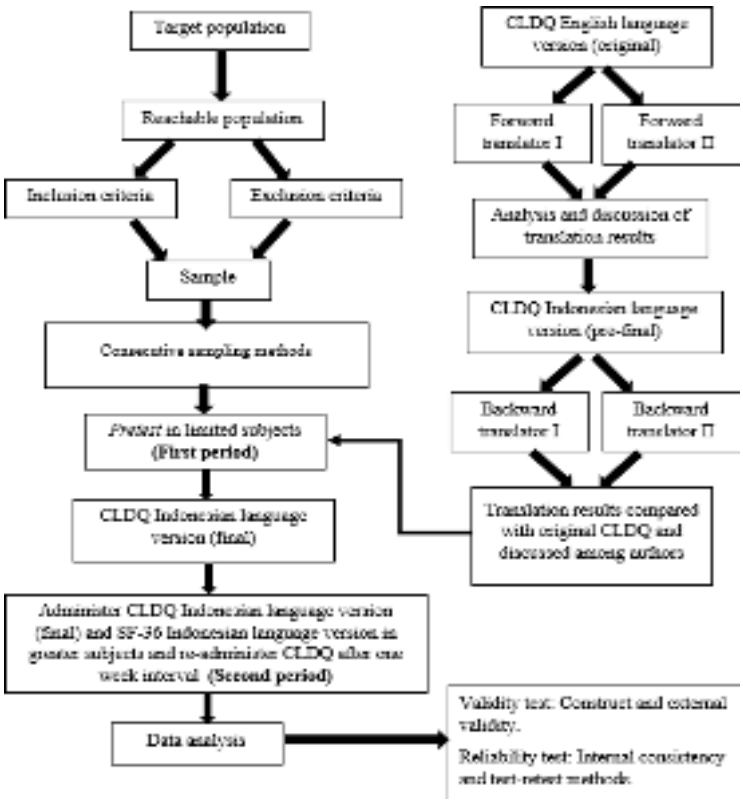

Figure 1. Study flowchart.

RESULTS

Fifty-two subjects were recruited from the Hepatobiliary Outpatient Clinic in Cipto Mangunkusumo Hospital (CMH) from April-May 2021, to test the final Indonesian version of CLDQ. **Table 1** shows the subjects' characteristics.

Table 1. Characteristics of study subjects.

Variables	N=52
Gender, n (%)	
Male	37 (71.2)
Female	15 (28.8)
Age, average (SD)	53 (SD 10.18)
Marital status, n (%)	
Married	45 (86.5)
Single	2 (3.8)
Divorced/widow/widower	5 (9.6)
Educational background	
Elementary school	9 (17.3)
Middle school (junior and senior high)	30 (57.7)
University	13 (25.0)
Ethnicity, n (%)	
Javanese	23 (44.2)
Sundanese	6 (11.53)
Batavia	8 (15.38)
Padang	5 (9.61)
Bataknese	10 (19.23)
Occupation, n (%)	
Unemployed	21 (40.38)
Private sector employee	12 (23.07)
Public sector employee	4 (7.69)
Others	13 (25)
Income per month, n (%)	
< Rp 5,000,000.00	44 (84.61)
Rp 5,000,000.00-Rp 10,000,000.00	6 (11.53)
> Rp 10,000,000.00	2 (3.84)
Time since diagnosis of cirrhosis, n (%)	
<5 years	38 (73.1)
5-10 years	11 (21.2)
>10 years	3 (5.8)
Child-Pugh score, n (%)	
A	33 (63.5)
B	16 (30.8)
C	3 (5.8)
History or current ascites,	
Yes	9 (17.3)
No	43 (82.7)
History of hepatic encephalopathy	
Yes	0 (0.0)
No	52 (100.0)
History of esophageal varices ligation	
Yes	25 (48.07)
No	27 (51.92)
Hepatitis B or C serological marker	
HBsAg (-), Anti-HCV (-)	3 (5.8)
HBsAg (-), Anti-HCV (+)	19 (36.5)
HBsAg (+), Anti-HCV (-)	29 (55.8)
HBsAg (+), Anti-HCV (+)	1 (19)

The first result of the study was the translation and adaptation process of the CLDQ. The first translation, which was a forward translation from English to Indonesian language, did not produce any significant problems. Although there were discrepancies in some questions of the questionnaire between the two forward translators, such as bodily pain translated into *nyeri pada bagian tubuh* by the first translator, and *nyeri pada tubuh* by the second translator, the authors managed to synthesize, analyze, and discuss with both of the translators and chose the best words, phrases, grammars, and sentences that truly represent the meaning of the questions being asked without compromising the practicality and effectiveness of the sentences. This is done in order to prevent confusion of the subjects.

The similar finding was also found in the backward translation. After the backward translators gave the authors their translation results, the authors synthesize, analyze, and compared them with the original English language of CLDQ. There were no significant differences between both of the translation results and the original CLDQ, although there were slight differences in some word choices, but not compromising the meaning of the original questions. The backward translators are not familiar with (blinded) the original language of CLDQ.

The prefinal CLDQ version was produced after the aforementioned process, and small amount of subjects could fill in the questionnaire without significant difficulties. There were no subjects confused with the questions being asked in the questionnaire. The average time of the subjects filled in the questionnaire was relatively short, which was 5.3 minutes. The process of filling in the prefinal CLDQ version was termed the pretest process, and the results was considered to make the final CLDQ version that was used in larger amount of subjects to test for validity and reliability.

The main results of the study were the validity and reliability of the final Indonesian version of CLDQ. The questionnaire's construct validity was assessed using multitrait-multimethod analysis, with good validity defined as good correlation between scores of each CLDQ questions with their own domains, which is also known as convergent validity. On the other hand, items with good construct validity must also have weaker correlation with other domains they do not belong to, known as discriminant validity. Good correlation is defined as having r 0.3-0.6 (moderate) and r > 0.6 (strong), while r < 0.3 is defined as weak correlation. Our analysis found that 93.1% of all CLDQ questions showed strong correlations (r > 0.6) with their own domains and 6.9% had moderate correlations (r 0.3-0.6), while most of the questions had weaker correlations with other domains. **Table 2** shows the construct validity CLDQ.

The external validity of the questionnaire was tested using Spearman correlation test between CLDQ (six domains) and SF-36 (eight domains). Our analysis found that there were 54.1% good correlations (8.3% strong correlations with r 0.6-0.79 and 45.8% moderate correlations with r 04-0.59). The rest of the correlations were found to be weak and very weak. **Table 3** shows the external validity of the questionnaire.

To test for the reliability of the questionnaire, internal consistency was assessed by measuring Cronbach-Alpha in each of the CLDQ domains and the overall questionnaire, with the good internal consistency defined as having Cronbach-Alpha ≥ 0.7. The test-retest method on the other hand, was tested by having respondents complete

Table 2. Construct validity of CLDQ in Indonesian language

	CLDQ Domains					
CLDQ Question Numbers	**AB**	**FA**	**SY**	**AC**	**EM**	**WO**
1	**0,877***	0,449	0,522	0,543	0,484	0,375
5	**0,898***	0,449	0,522	0,543	0,484	0,375
17	**0,917***	0,551	0,485	0,658	0,576	0,431
2	0,579	**0,769***	0,454	0,529	0,553	0,362
4	0,329	**0,774***	0,388	0,387	0,438	0,406
8	0,499	**0,848***	0,479	0,501	0,543	0,448
11	0,520	**0,848***	0,470	0,558	0,618	0,504
13	0,285	**0,688***	0,430	0,375	0,236	0,228
3	0,729	0,507	**0,613***	0,577	0,522	0,231
6	0,305	0,294	**0,615***	0,463	0,374	0,090
21	0,302	0,299	**0,695***	0,353	0,386	0,246
23	0,267	0,352	**0,659***	0,213	0,340	0,438
27	0,149	0,317	**0,554***	0,232	0,227	0,176
7	0,570	0,489	0,533	**0,846***	0,461	0,415
9	0,568	0,630	0,463	**0,857***	0,513	0,264
14	0,604	0,417	0,508	**0,850***	0,588	0,338
10	0,410	0,566	0,571	0,399	**0,817***	0,620
12	0,508	0,495	0,501	0,700	**0,789***	0,600
15	0,377	0,496	0,332	0,515	**0,593***	0,459
16	0,473	0,323	0,415	0,461	**0,719***	0,345
19	0,557	0,640	0,563	0,494	**0,864***	0,733
20	0,552	0,380	0,431	0,501	**0,751***	0,382
24	0,362	0,333	0,565	0,470	**0,626***	0,419
26	0,309	0,495	0,453	0,376	**0,741***	0,471
18	0,300	0,410	0,344	0,308	0,568	**0,850***
22	0,345	0,484	0,344	0,265	0,569	**0,874***
25	0,304	0,429	0,351	0,395	0,619	**0,852***
28	0,483	0,459	0,343	0,365	0,590	**0,788***
29	0,353	0,323	0,334	0,349	0,461	**0,644***

*Bolded numbers have p < 0.05, with all numbers in the table being r value (correlation). Abbreviations: CLDQ: chronic liver disease questionnaire, AB: abdominal, FA: fatigue, SY: systemic, AC: activity, EM: emotion, WO: worry.

Table 3. External validity of CLDQ in Indonesian language.

	SF-36							
Domains	**PF**	**MH**	**EV**	**SF**	**RLP**	**RLE**	**BP**	**GH**
CLDQ:								
AB	0,344*	0,630*	**0,544***	0,099	0,343*	0,446*	0,564*	0,487*
FA	0,490*	0,487*	**0,583**	0,187	0,440*	**0,509***	0,270	0,180
SY	**0,515***	0,340*	0,401*	0,032	0,366*	0,503*	0,298*	0,092
AC	0,461*	0,539*	**0,505***	0,162	0,509*	**0,520***	0,387*	0,332*
EM	0,395*	0,682*	**0,645***	0,381*	0,431*	**0,611***	0,304*	0,306*
WO	0,507*	0,526*	**0,406***	0,185	0,303*	0,457*	0,108	0,398*

*Numbers with asterisks have $p < 0.05$ and bolded numbers have moderate (r 0.4-0.59) and strong (r 0.6-0.79) correlations. All numbers in the table represents r value (correlation). Abbreviations: CLDQ: chronic liver disease questionnaire, AB: abdominal, FA: fatigue, SY: systemic, AC: activity, EM: emotion, WO: worry, SF-36: short form- 36, PF: physical functioning, MH: mental health, EV: energy/vitality, SF: social functioning, RLP: role limitation due to physical health, RLE: role limitation due to emotional problem, BP: bodily pain, GH: general health.

the questionnaire two times with a one week interval. The correlation between the test and retest scores was measured using intraclass correlation coefficient (ICC) between domains scores and overall questionnaire scores. The analysis showed that the Cronbach- Alpha of all the domains in the Indonesian version of CLDQ and overall CLDQ were good, with $\alpha \geq 0.7$. The ICC of all domains and overall questionnaire also has good and very good correlation (good and very good correlation are defined as having ICC of 0.61-0.8 and > 0.8, respectively). Table 4 below presents the reliability test result for the questionnaire.

DISCUSSION

The subjects of this study showed some similarities in characteristics with subjects in the other validity and reliability studies, such as the majority gender in this study being men (71.2%) which is similar with studies in Thailand (63.5%), China (75.4%), India (85.2%), Singapore (68.2%), Germany (53%), Greece (65%), Italy (63.9%), Persia (64.5%), Serbia (54.4%), and Spain (70.5%).[16-25] The average age of the subjects in this study was 53 ± 10.18 years old which does not differ significantly from studies in Thailand (51.6 ± 8.9 years), Germany (52.7 ± 13.9 years), Serbia (53.8 ± 12.9 years), and Spain (55.25 ± 12.83 years).[16,20,24,25] Subjects in this study were mostly married (86.5%) and this is similar to studies in Thailand (81.3%), China (86.9%), and Singapore (85.5%).[15,16,18] The educational background in this study was dominated by middle school graduates (57.7%), which is similar to studies in Singapore (59.1%) and China (64%). It is important to note that the subjects educational background provided no hindrance towards understanding the CLDQ questions because the sentences are simple and easy to understand.[16,18] Other sociodemographic characteristics do not have similarities with other countries, such as: ethnicity, unemployment status, and low income defined as less than Rp 5,000,000.00 per month.

Table 4. Reliability of CLDQ in Indonesian language

CLDQ Domains	Cronbach-Alpha	ICC (95% CI)
Abdominal (AB)	0.927	0.864 (0.774-0.919)
Fatigue (FA)	0.861	0.757 (0.611-0.853)
Systemic (SY)	0.884	0.793 (0.665-0.876)
Activity (AC)	0.858	0.752 (0.604-0.850)
Emotion (EM)	0.937	0.881 (0.802-0.930)
Worry (WO)	0.909	0.834 (0.727-0.901)
Overall CLDQ	0.947	0.900 (0.832-0.941)

As for the clinical characteristics of the subjects, the majority of subjects in this study had been diagnosed with liver cirrhosis for less than 5 years (73.1%), with Child- Pugh A stage (63.5%). The majority also did not have ascites (82.7%), nor had they experienced hepatic encephalopathy (100%). The results are similar with studies in Thailand, Singapore, and Greece, which conducted their sampling in the outpatient clinic, therefore making the clinical characteristics of the patients similar. The

majority of patients had stable and compensated liver cirrhosis profiles.[16,19,21] In terms of history of esophageal varices ligation, there was no significant difference between respondents, which could be seen in every stage of cirrhosis, although was commonly found from Child-Pugh B cirrhosis. Most of the respondents in this study (55.8%) had positive HBsAg serological marker without positive anti-HCV, and this is similar with other studies in Singapore (78.9%) and Greece (44.5%). This is likely due to the high prevalence of Hepatitis B infection in Indonesia, Singapore, and Greece.[19,21]

The construct validity of the questionnaire showed that all of the CLDQ questions had moderate and strong correlations with their own domains (6.9% and 93.1% had moderate (r 0.3-0.6) and strong (r > 0.6) correlations, respectively), indicating a good convergent validity. The majority of the CLDQ questions also had weaker correlations with other domains they do not belong to, indicating that the questionnaire had good discriminant validity. The results of the current study demonstrated better validity with an r ranging from 0.554-09.917, than a study from Singapore, with r of 0.43-0.84.[19] The difference might be due to difference in subjects recruited for the validity test, where the study from Singapore recruited all chronic liver disease patients and not just patients with cirrhosis, in contrast to the only-cirrhosis patients recruited in this study. With good convergent and discriminant validity, it could be concluded that the Indonesian version of CLDQ has good construct validity.

The second validity testing method was the external validity. Our analysis revealed that there were 54.1% moderate and strong correlations between the CLDQ and the SF-36 domains, while the rest of the correlations were weak and very weak. This result differs from that of two studies from Singapore and India, with 66.6% moderate correlations and no strong correlation in the Singaporean study, and 70.8% moderate correlations and 20.8% strong correlations in the Indian study.[18,19] The difference might be due to different types of HRQoL measurement between CLDQ and SF-36. CLDQ domains are more specific to measure the HRQoL in liver cirrhosis patients than SF-36 domains which are more generic. Consequently, there were questions with no correlations between the CLDQ and SF-36 domains. For example, the AB domain in CLDQ focuses more on problems related to the abdominal area, and did correlate with the SF domain in SF-36, which focuses more on the social functions disrupted by the disease, such as: activity with friends, family, or society. However, the number of moderate and strong correlations in the Indonesian version of CLDQ remains above 50%, indicating a good external validity. With good construct and external validity, it can be concluded that the Indonesian CLDQ is valid for measuring the health-related quality of life in Indonesian patients with liver cirrhosis.

For reliability testing, the current study used internal consistency and test-retest methods. The Cronbach-Alpha in all of CLDQ domains were in the range of 0.858-0.937, while overall Cronbach-Alpha was 0.947. The Cronbach-Alpha in this study is similar with studies from Thailand (0.93-0.94), Greece (0.74-0.94), Japan (0.809-0.971), and Sweden (0.75-0.96).[16,21,26,27] The fact that this study revealed a good Cronbach-Alpha (>0.7) indicates that the Indonesian version CLDQ has a good internal consistency, meaning that there was a good correlation between one CLDQ questions and the other.

The test-retest method analysis with Spearman correlation found that the ICC of the Indonesian version of CLDQ was in the range of 0.752-0.864, and the overall ICC was 0.9. The results were similar with studies in Germany (0.78-0.86), India (0.74-0.92), Spain (0.727-0.903), and Sweden (0.73-0.88).[18,20,25,27] However, this study used a different interval of retest with the other studies: one week compared to 3-8 days in Germany, two weeks in India, Spain, and Sweden. The reason this study used one week as an interval for retest was because in one week it is expected that no significant changes has occurred in the subjects that could affect the study results, and not too short that the subjects may still remember their own answers. With a good ICC of more than 0.7, the Indonesian CLDQ has good test-retest results, indicating a good correlation between the same domain and overall CLDQ scores in the first day (test) and the eighth day

(retest). The fact that the internal consistency and test-retest reliability is good, indicates that that the Indonesian version of CLDQ is reliable in measuring the health-related quality of life in Indonesian patients with liver cirrhosis.

The strength of this study lies in the fact that it revealed good validity and reliability of the Indonesian version of the CLDQ in measuring the quality of life of Indonesian liver cirrhosis patients. The study has followed the established questionnaire validation guidelines used by other validity and reliability studies. This study is also the first in Indonesia to conduct a validity and reliability test towards specific HRQoL questionnaire in liver cirrhosis patients. The limitation of this study is the feasibility problem of the CLDQ question number 29 when it is applied in Indonesian population. The question asks about the perception of patients regarding the liver availability for transplant. Although all subjects could answer the question without any problem and understood the question very well, the availability of liver donor remains low in Indonesia. This explains the low rate of liver transplant in our country.

CONCLUSION

The Indonesian version of CLDQ has good validity and reliability and can be used to measure the quality of life in liver cirrhosis patients in Indonesia.

AUTHOR CONTRIBUTION

All authors contributed equally in this study from idea and study design, data collection, data analysis, and article writing and revision.

FUNDING STATEMENT

The work is not a grant study and was funded personally by all authors.

CONFLICT OF INTEREST

The authors declare no conflict of interest in this study.

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Correlation of Moxifloxacin Concentration, C-Reactive Protein, and Inflammatory Cytokines on QTc Interval in Rifampicin-Resistant Tuberculosis Patients Treated with Shorter Regimens

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ABSTRACT

Background: *Drug-resistant tuberculosis (DR-TB) is a global health concern. QTc prolongation is a serious adverse effect in DR-TB patients receiving a shorter regimen. This study aimed to evaluate the correlation of moxifloxacin concentration, CRP, and inflammatory cytokines with QTc interval in DR-TB patients treated with a shorter regimen.* ***Methods:*** *This study was performed in 2 groups of rifampicin-resistant (RR-TB) patients receiving shorter regimens. Correlation for all variables was analyzed.* ***Results****: CRP, IL-1β, and QTc baseline showed significant differences between 45 RR-TB patients on intensive phase and continuation phase with p-value of <0.001, 0.040, and <0.001, respectively. TNF-α and IL-6 between RR-TB patients on intensive phase and continuation phase showed no significant difference with p=0.530 and 0.477, respectively. CRP, TNF-α, IL-1 β, and IL-6 did not correlate with QTc interval in intensive phase (p=0.226, 0.281, 0.509, and 0.886, respectively), and also in continuation phase (0.805, 0.865, 0.406, 0.586, respectively). At 2 hours after taking the 48th-dose, moxifloxacin concentration did not correlate with QTc interval, both in intensive phase (p=0.576) and in continuation phase (p=0.691). At 1 hour before taking the 72nd-hour dose, moxifloxacin concentration also did not correlate with QTc interval in intensive phase (p=0.531) and continuation phase (p=0.209).* ***Conclusion:*** *Moxifloxacin concentration, CRP, and inflammatory cytokines did not correlate with QTc interval in RR-TB patients treated with shorter regimens. The use of moxifloxacin is safe but should be routinely monitored and considered the presence of other risk factors for QTc prolongation in RR-TB patients who received shorter regimens.*

Keywords: *Drug-resistant Tuberculosis, shorter treatment regimen, QTc interval.*

INTRODUCTION

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* strains resistant to anti-TB drugs is becoming a global health concern with an increasing number of cases. *World Health Organization* (WHO) reported 465,000 cases of Drug-Resistant Tuberculosis (DR-TB) in 2020 with a treatment success rate of 57%. Indonesia currently ranks 5th for countries with high DR-TB cases with a treatment success rate of less than 50%, due to the high mortality rate and loss to follow-up.[1] In 2016, the WHO recommended a standardized shorter regimen off 9-12 months to treat Multidrug-Resistant/Rifampicin-Resistant (MDR/RR-TB) patients with a specific inclusion criteria.[2] Indonesia started to implement the shorter regimen in 2017.[3] However, not all MDR/RR-TB patients were treated with shorter regimens until the end of treatment and 16/224 (7%) of patients switched their regimens from shorter regimen to individual regimens due to the presence of prolonged QT.[4] Another study reported the incidence of increased QTc interval of >30 ms in 21/98 (21.4%) and >60 ms in 10/98 (10.2%) of DR-TB patients who received shorter regimens.[5] Interval QT prolongation is a serious adverse effect and can potentially cause *Torsade de Pointes* (TdP) and sudden cardiac death.[6] Moxifloxacin is one of the components of shorter regimen and is often criticized for its higher risk of QTc interval prolongation and TdP.[4,6] According to the national program, moxifloxacin was given in 400 mg, 600 mg, or 800 mg dosages based on body weight.[3]

Inflammatory activation due to systemic inflammation was indicated as a new potential cause of acquired long QT syndrome via cytokine-mediated changes in cardiomyocyte ion channels.[7] Impaired expression and or function of several cardiac ion channels was affected by systemically or locally released inflammatory cytokines (mainly TNF-α, IL-1, and IL-6), resulting in a decrease of K+ currents and or an increase of ICaL. Cardiac or systemic inflammation promotes QTc-interval prolongation via cytokine-mediated effects, and this may increase sudden cardiac death risk.[8]

C-reactive protein (CRP) is one of the acute phase proteins that increases during systemic inflammation.[9,10] It is also commonly used as a prognostic marker in TB.[11] Elevated CRP serum level is a strong independent predictor of heart disease and cardiovascular disease in asymptomatic individuals.[9,10] Xie et al. (2015) suggested that CRP may directly or indirectly influence QTc interval via influencing the expression of K+ channel interaction protein 2 (KChIP2) and formation of transient outward potassium current (Ito.f) density of cardiomyocytes.[12]

Prolongation of QTc interval is usually asymptomatic and requires routine electrocardiography (ECG) monitoring during treatment using QT-prolonging drug.[2,13] Hence, it is very important to thoroughly assess DR-TB patients before attributing QTc prolongation solely due to anti-TB drugs.[14] Although several studies have reported QT prolongation in DR-TB, the correlation of inflammatory markers and QTc interval is still rarely being studied. In this study, we aimed to evaluate the correlation of moxifloxacin concentration, CRP, and inflammatory cytokines with QTc interval in DR-TB patients treated with shorter regimen.

METHODS

An observational analytic study with consecutive sampling was conducted from September 2019 to February 2020 in Dr. Soetomo Hospital Surabaya, one of East Indonesia TB referral hospitals. Study subjects were RR pulmonary TB patients based on the GeneXpert examinations with age 18 to 65 years old who will start the intensive phase and who are on the continuation phase of shorter treatment regimens. RR-TB patients with baseline QTc >500 ms, potassium <3.5 mmol/L, magnesium <1.7 mmol/L, calcium <8.5 mmol/L, creatinine clearance <30 cc/m, a*spartate aminotransferase* - alanine *aminotransferase* (AST-*ALT) >5x upper limit normal (ULN), body mass index (BMI) <18 kg/m^2,* on anti-arrhythmia therapy, anti-depressant therapy, with bradycardia, anti-fungal treatment (azoles), erythromycin therapy, and phenytoin therapy were excluded from this study.

The respondents were given an explanation of the research and publications to be carried out. All respondents information is kept confidential and only used for research purposes. After getting an explanation, the respondent is allowed to refuse the study or resign in the middle of the study. The respondents gave their written consent and permission for publication of the letters and to participate in the research. We confirm that all the research meets the ethical guidelines and in accordance with the Declaration of Helsinki.

Ethics

An informed consent was signed by all participants and the ethics committee of Dr. Soetomo Hospital with ethical clearance number 1444/KEPK/VIII/2019 on August 23rd, 2019.

Operational Definition

Rifampicin-resistant tuberculosis (RR-TB) was defined as the results of *Mycobacterium tuberculosis* detected with rifampicin resistance based on GeneXpert MTB/RIF.[15] RR-TB patients in intensive phase were defined as RR-TB patients who are eligible for shorter regimens and will start intensive phase of treatment. RR-TB patients in continuation phase were defined as RR-TB patients who have completed the intensive phase (4-6 months), i.e. those who have sputum smear conversion after the 4th, 5th, or 6th month. Shorter regimens were as recommended by the WHO in 2016 and the national program in 2019, consisted of 4-6 Km – Mfx – Eto(Pro) – HHigh Dose – Cfz – E – Z / 5 Mfx – Cfz – E – Z for 9-11 months.[2,16] Electrocardiography (ECG) was defined as a 12-lead surface heart recording using an ECG machine. The QT interval is that portion of the ECG that begins at the start of the QRS complex and ends at the termination of the T wave. The QTc referred to the corrected QT interval using the Fredericia formula.[14] The changes of QTc (ΔQTc) referred to the difference between the QT interval at baseline and the QT interval at 2 hours after taking the 48th-hour dose, and 1 hour before taking the 72nd-hour dose.

Concentration of Moxifloxacin

Blood samples were collected and put into heparin tubes at 2 hours after taking the 48th-hour dose and 1 hour before taking the 72nd-hour dose. Blood samples were centrifuged and the plasma was stored in the deep freezer with a temperature of -80^0 C. The moxifloxacin concentration was measured by a validated method using High-Performance Liquid Chromatography (HPLC). The separation of moxifloxacin from the plasma matrix using protein precipitation, followed by measurements using the Waters HPLC Alliance e2695 with a detector of Waters 2998 Photodiode Array (PDA). 240 µL of acetonitrile solution (100%) was added to the 200 µl of plasma sample. The sample was then vortexed for 1 minute and centrifuged at a speed of 10,000 g for 5 minutes. A total of 200 µl of supernatant was put into the vial and injected into the HPLC with an injection volume of 10 µl. Separation using a Sunfire™ C18 column (4.6 x 100 mm, 5 µm; Waters, Ireland). The mobile phase consisted of 0.4% TEA in aquabides with a pH of ±3 and 100% of acetonitrile (75%:25% (v/v)). The flow rate is 1 ml/min and the PDA detector was set at a wavelength of 296 nm. Accuracy for standard concentration curves is between 95.5% to 103.4%, depends on the standard concentration level. The coefficient of variation for intra- and inter-assay was less than 7.2% for the range from 0.204 to 10,200µg / mL. The lowest limit value which can be quantified was 0.204 µg/mL.

Measurement of CRP Levels

Venous blood samples from each subject were collected into heparin tubes. Serum was separated by centrifugation at 3,000 rpm for 5 minutes and stored at 4^0 C for 24 hours for the analysis.[9] CRP levels were determined by an immunoturbidimetric assay using SIEMENS Dimension clinical chemistry system for quantitative determination of CRP in serum and plasma. This instrument automatically calculates and prints the concentration of CRP in [mg/L] mg/dL. Analytical measurement range was 0.5-250.0 mg/L or 0.05-25.00 mg/dL.

Measurement of Inflammatory Cytokines Levels

Samples of venous blood with an amount of 5 cc were taken from each patient and put into EDTA serum tubes. All samples were stored in a deep freezer with a temperature of -80^0 C. After

the samples had sufficient amounts, all samples were put at room temperature for 2 hours or at 4^0 C for a night. The samples were centrifuged for 15 minutes to separate the blood plasma and serum. The cytokines levels were measured using the ELISA method with a kit of Elabsiences.

QTc Interval Measurement

QT interval was measured automatically using ECG machine merc BLT E30 (Guangdong Biolight Meditech, Germany, 2017) at baseline before treatment, 2 hours after taking the 48th-hour dose, and 1 hour before taking the 72nd-hour dose. Heart rate-corrected QT (QTc) interval was calculated using Fredericia formula,[14] manually by cardiologists.

Data Analysis and Ethical Statement

The data obtained in this study were presented as tables and graphics. Data were analyzed using SPSS 21.0 software (IBM Corp., Armonk, NY, USA). P-value <0.05 was considered as significant statistically. This study was conducted in accordance with the Declaration of Helsinki. An informed consent was signed by all participants. This study was approved by the ethics committee of Dr. Soetomo Hospital with ethical clearance number 1444/KEPK/VIII/2019 on August 23rd, 2019.

RESULTS

This study included 29 RR-TB patients on intensive phase and 16 RR-TB patients on continuation phase of shorter regimens. The clinical symptoms found in this study were cough, fever, chest pain, haemoptysis, weight loss, night sweats, dyspnea at rest, and dyspnea during activity. **Table 1** showed that the clinical symptoms of RR-TB patients improved in continuation phase, but there is no significant difference between the reported symptoms in intensive phase and continuation phase. Albumin, CRP, IL-1β, QTc baseline, and QTc at 2 hours after the 48th dose showed significant differences between RR-TB patients on intensive phase and continuation phase with p = 0.002, <0.001, 0.040, <0.001, and 0.026, respectively. While TNF-α, IL-6, moxifloxacin concentration at 2 hours after the 48th dose, moxifloxacin concentration and QTc at 1 hour before 72nd dose between RR-TB patients on intensive phase and continuation phase showed no significant difference with p = 0.530, 0.477, 0.686, 0.610, and 0.325. This was presented in **Table 1**.

Table 2 showed that CRP, TNF-α, IL-1β, and IL-6 did not correlate with QTc interval in intensive phase with p = 0.226, 0.281, 0.509, and 0.886, respectively. CRP, TNF-α, IL-1β, and IL-6 also did not correlate with QTc interval in continuation phase with p = 0.805, 0.865, 0.406, and 0.586, respectively. This result indicated that inflammatory markers could not predict QTc interval in our study.

At 2 hours after the 48th dose, it was known that moxifloxacin concentration did not correlate with QTc interval and ΔQTc, both in intensive phase (p = 0.576 and 0.415) and continuation phase (p = 0.691 and 0.353). At 1 hour before the 72nd- hour dose, moxifloxacin concentration also did not correlate with QTc interval and ΔQTc in intensive phase with p = 0.531 and 0.813, and in continuation phase with p = 0.209 and 0.464, as presented in **Table 3**.

Scatter plot in **Figure 1** showed that the distribution of CRP, TNF-α, IL-1β, and IL-6 did not correlate with QTc interval. Levels of CRP, TNF-α, IL-1β, and IL-6 are overlapping between intensive and continuation phases, while QTc interval showed an increased in continuation phase.

The distribution of moxifloxacin concentration and QTc interval at 2 hours after taking the 48th-hour dose and 1 hour before taking the 72nd-hour dose did not form a specific pattern as presented in **Figure 2**. This scatter plot showed that moxifloxacin concentration did not correlate with QTc interval, as the results of correlation analysis in **Table 3**.

DISCUSSION

Multidrug-Resistant/Rifampicin-Resistant TB (MDR/RR-TB) is an emerging threat to TB control, with clinical presentation of patients with MDR/RR-TB being identical to that of patients with drug-susceptible disease.[17] All patients with RR-TB in this study were symptomatic, most commonly with cough (66.7% in intensive phase and 33.3% in continuation phase), other symptoms including fever, chest pain,

Table 1. Characteristics of Study Subjects

Characteristics	RR-TB on Start of Intensive Phase (N=29)	RR-TB on Start of Continuation Phase (N=16)	P-value
Age (years)*	37 (18-62)	44 (19-56)	0.569
Sex**			0.673
Women	13 (59%)	9 (41%)	
Men	16 (70%)	7 (30%)	
BMI (m/kg^2)*	20.4 (18.03-28.65)	19.06 (18.26-27.68)	0.530
Diabetes mellitus**	14 (73.7%)	5 (26.3%)	0.429
Cough**	28 (66.7%)	14 (33.3%)	0.285
Fever**	12 (75%)	4 (25%)	0.272
Chest pain**	6 (100%)	0 (0%)	0.075
Haemoptysis**	9 (64.3%)	5 (35.7%)	1.000
Weight loss**	14 (60.9%)	9 (39.1%)	0.608
Night sweats**	10 (71.4%)	4 (28.6%)	0.738
Dyspnea at rest**	3 (60%)	2 (40%)	1.000
Dyspnea during activity**	7 (70%)	3 (30%)	1.000
Potassium (mmol/l)***	4.3 ± 0.45	3.96 ± 0.4	0.019
Calcium mg/dl)***	9.03 ±0.46	8.67 ± 0.2	0.001
Albumin***	3.43 ± 0.28	3.65 ± 0.14	0.002
CRP (mg/dl)*	1.5 (0.2-10.9)	0.15 (0.1-0.6)	<0.001
TNF-α (pg/mL)*	6.8 (0.13-36.22)	4.79 (0-43.34)	0.530
IL-1β (pg/mL)*	20.13 (2.23-708.7)	7.42 (0.6-113.47)	0.040
IL-6 (pg/mL)*	43.17 (10.14-1076)	40.61 (4.47-113.99)	0.477
QTc Baseline(ms)***	417.28 ± 31.2	455.94 ± 16.6	<0.001
Moxifloxacin **			0.727
600	17 (60.8%)	11 (39.2%)	
800	12 (70.6%)	5 (29.4%)	
Moxy Conc (48+2) (µg/mL)***	4.3 ± 2.32	4.61 ± 2.54	0.686
QTc 48+2 (ms)***	444.38 ± 31.25	467.94 ± 35.7	0.026
ΔQTc (48+2)-Baseline (ms)*	20 ((-17) – (81))	2.5 ((-44) – (115))	0.036
Moxy Conc (72-1) (µg/mL)*	1.01 (0.01 – 3.27)	0.91 (0.01 – 1.61)	0.610
QTc 72-1 (ms)*	448 (386-518)	447 (428-524)	0.325
ΔQTc (48+2) - (72-1) (ms)*	0 ((-75) – (60))	7.5 ((-77) – (52))	0.122

* Median (min-max) using Mann-Whitney Test; ** Chi-square; ***Mean ± Standard Deviation using T-test; BMI = Body Mass Index.

Table 2. Correlation Analysis at Baseline using Pearson or Spearman-rho Test

Intensive phase	QTc baseline		Continuation phase	QTc baseline	
	R	P		R	P
CRP (mg/dL)	-0.232	0.226	CRP (mg/dL)	0.067	0.805
TNF-α (pg/mL)	0.207	0.281	TNF-α (pg/mL)	0.046	0.865
IL-1β (pg/mL)	0.128	0.509	IL-1β (pg/mL)	-0.223	0.406
IL-6 (pg/mL)	-0.028	0.886	IL-6 (pg/mL)	-0.147	0.586

R: Correlation Coefficient; P: Sig. (2-tailed).

Table 3. Correlation Analysis at 2 Hours after the 48th Dose and at 1 Hour before the 72nd- Hour Dose

			Moxi Conc at 48+2			Moxi Conc at 72-1
Intensive phase	**QTc at 48+2**	**R**	**-0.108**	**QTc at 72-1**	**R**	**0.121**
		P	**0.576**		**P**	**0.531**
	ΔQTc ((48+2) – Baseline))	**R**	**-0.157**	**ΔQTc ((48+2) – (72-1))**	**R**	**-0.046**
		P	**0.415**		**P**	**0.813**
Continuation phase	**QTc at 48+2**	**R**	**0.108**	**QTc at 72-1**	**R**	**-0.332**
		P	**0.691**		**P**	**0.209**
	ΔQTc ((48+2) – Baseline))	**R**	**0.249**	**ΔQTc ((48+2) – (72-1))**	**R**	**-0.197**
		P	**0.353**		**P**	**0.464**

Correlation Analysis using Pearson or Spearman-rho Test; R: Correlation Coefficient; P: Sig. (2-tailed).

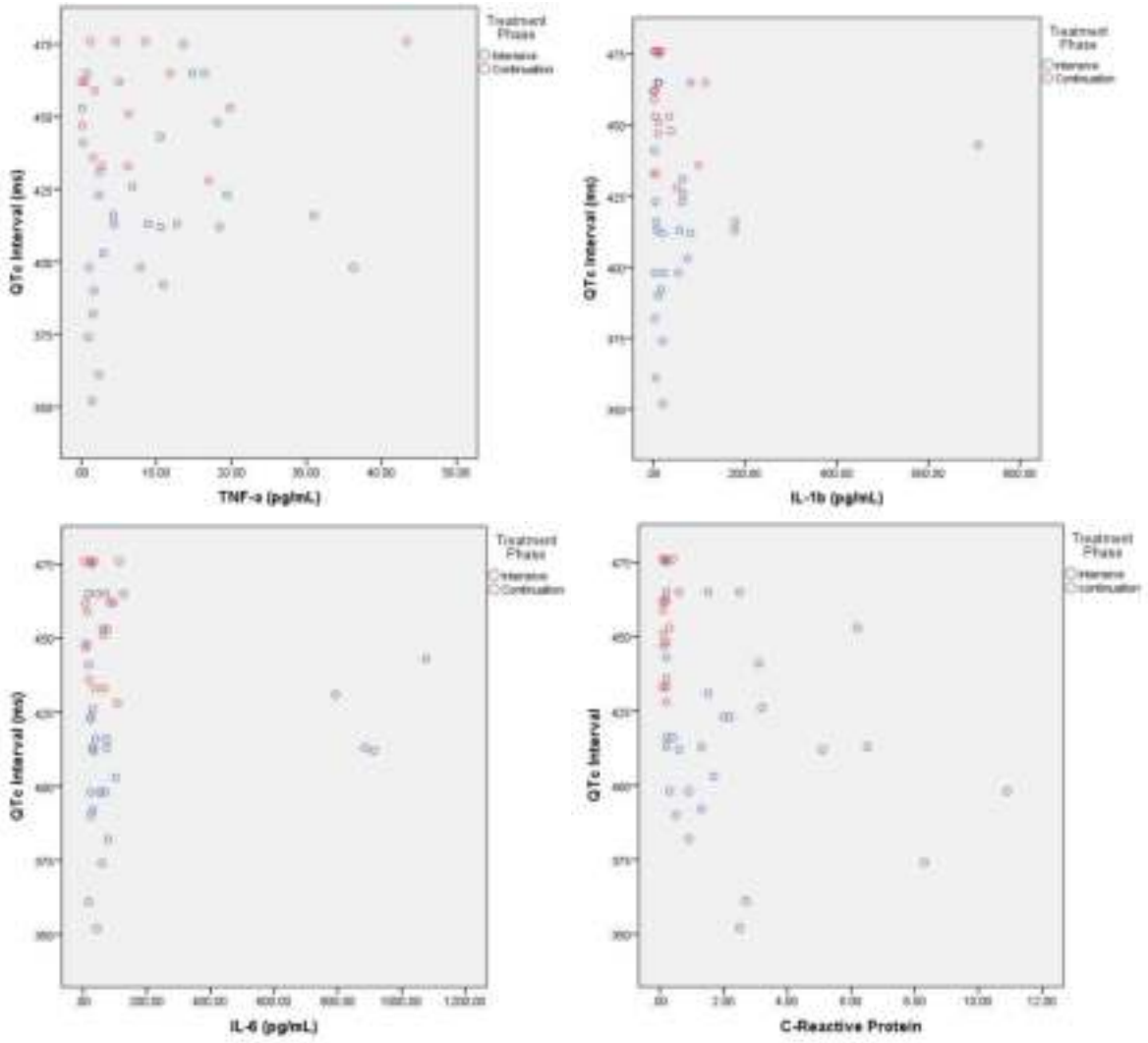

Figure 1. Scatter Plot of Inflammatory Cytokines (TNF-α, IL-1β, and IL-6), C-Reactive Protein, and Baseline of QTc Interval in RR-TB Patients.

haemoptysis, weight loss, night sweats, dyspnea at rest, and dyspnea during activity. A study in 93 MDR-TB patients by Brode et al. (2015) also reported productive cough as the most common symptoms in MDR-TB, followed by weight loss, malaise, fever, haemoptysis, night sweats, and chest pain.[18] The symptoms were more often reported in intensive phase, then improved in continuation phase (**Table 1**), as the intensive phase of RR-TB treatment aimed to significantly decrease the bacillary burden. The improved symptoms may results from the decreased bacillary burden and the decreased inflammation (inflammation caused by *Mycobacterium tuberculosis* infection) after intensive phase of treatment.

Interval QTc prolongation is a serious effect and is often reported in DR-TB patients treated with shorter regimens. Moxifloxacin is considered as a QT-prolonging drug and is often criticized to cause QTc prolongation in DR-TB patients.[1,6] Moreover, QT prolongation related to inflammatory factors also has been widely reported, as has been known that inflammation occurs as a response to injury, lipid peroxidation, and infection, including TB infection.[26]

In this study, RR-TB patients on intensive phase of shorter regimen have a higher level of CRP, TNF-α, IL-1β, and IL-6 levels, compared to those in continuation phase. CRP and IL-1β, and QTc baseline were significantly different between RR-TB patients on intensive phase and continuation phase with p-value of <0.001, 0.040, and <0.001, respectively. While TNF-α and IL-6

between RR-TB patients on intensive phase and continuation phase showed no significant difference with p = 0.530 and 0.477, respectively (**Table 1**). A higher level of inflammatory biomarkers in intensive phase showed that the inflammation due to *Mycobacterium tuberculosis* infection was still high because the patients have just received DR-TB treatment, while patients on continuation phase have been treated for a few months and have experienced sputum conversion which indicated decreased inflammation in lung tissue. Pulmonary TB infection ellicits an inflammatory process in lung tissue, which is correlated with CRP levels changes,[27] and induction of inflammatory cytokines to regulate immune system.[25] This immune process depends on Th1-cell activity, including TNF-α. IL-1β directly kills *Mycobacterium tuberculosis* in macrophages. IL-6 is a requirement in host resistance to infection. IFN-γ , TNF-α , IL-12.[28]

At baseline examination, QTc interval in continuation phase was found higher than intensive phase (**Table 1**), while correlation analysis in **Table 2** showed that CRP, TNF-α, IL-1β, and IL-6 did not correlate with QTc interval in intensive phase and continuation phase. This was different from previous studies that reported a correlation between inflammation marker and QTc prolongation. CRP levels were found higher and correlated with QTc prolongation in hypertensive and rheumatoid arthritis patients.[21-23] Other studies reported increased TNF-α in elderly general population, elevated IL-6 levels in patients who experienced TdP, and higher levels of IL-1β in patients with connective tissue diseases, all being risk factors for long QTc intervals.

The correlation between CRP and cardiovascular risk is through systemic inflammation. Inflammatory cytokines such as TNF-α, IL-6, and IL-1 act directly on cardiomyocyte ion channels expression and function, and may represent a risk factor for QTc prolongation.[7] Another study found that IL-6 negatively affected cardiomyocyte ion channel function and increased risk for QT prolongation, suggesting that patients with high levels of IL-6 should receive routine ECG and counseling if other QTc prolonging risk factors are present. Systemic inflammation promotes QTc-interval prolongation via cytokine-mediated effects. Released inflammatory cytokines are able to directly affect the expression and/or function of several cardiac ion channels, resulting in a decrease of K+ current and/or an increase of calcium current. While in this present study, it was shown that inflammation due to DR-TB infection did not correlate with QTc interval (**Table 2**).

Another factor was considered for acquiring QTc prolongation, and moxifloxacin as a component in the standardized shorter regimen was suspected to induce QTc prolongation. The mechanism of drug-induced QT prolongation is due to blockage of the human ether a-go-go gene (hERG) that is responsible for the inward potassium rectifier (IKr) repolarizing current. [31-33] Our study showed that at 2 hours after the 48th dose, moxifloxacin concentration did not correlate with QTc interval, both in intensive and continuation phases. At 1 hour before the 72nd- hour dose, moxifloxacin also did not affect QTc interval in intensive and continuation phases (**Table 3**). Yoon et al. (2017) also revealed the safe use of moxifloxacin on QTc changes in DR-TB patients. Moxifloxacin at the dosage of 400, 600, or 800 mg does not correlate with the QTc interval.

Moxifloxacin is relatively safe, and the prolongation caused by moxifloxacin is considered minimal or moderate, but should be carefully monitored when other risk factors are present. QT prolongation is usually asymptomatic and requires routine ECG monitoring during QT drug use, to ensure the safe use of moxifloxacin and prevent serious adverse effects which can be life-threatening.

CONCLUSION

Our study found that moxifloxacin concentration, CRP, and inflammatory cytokines did not correlate with QTc interval in DR-TB patients treated with shorter regimens. The use of moxifloxacin is safe but should be routinely monitored and considered the presence of other risk factors for QTc prolongation in DR-TB patients received shorter regimens.

ACKNOWLEDGMENTS

The authors would like to thank Mrs. Atika, M.Sc., who helped us in statistical analysis.

CONFLICT OF INTERESTS

The authors report no conflicts of interest in this work.

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Palliative Screening Tools to Identify Palliative Care Consultation at Tertiary Hospital

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ABSTRACT

***Background*:** *The need of palliative care is increasing, but it is not all achievable. It is necessary to identify palliative patients in order to provide the proper care according to the needs of the patients. Cipto Mangunkusumo Hospital has been making the identification using a palliative-patient screening questionnaire, but no performance assessment has been carried out on the screening tool. This study aimed to evaluate the performance of the screening-tool questionnaire used on palliative-care patients at Cipto Mangunkusumo Hospital in order to assess the need of palliative-care consultation and to find out the optimal cut-off point of palliative care screening tools.* ***Methods*:** *The design of this study is cross-sectional and was conducted at Cipto Mangunkusumo National Central Public Hospital in July – October 2019. The sampling was collected by consecutive sampling. The reliability test was performed by the intraclass correlation coefficient (ICC). The internal consistency was measured by the Cronbach's-Alpha coefficient. The criterion-validity test was run by an evaluation using the Pearson test.* ***Results*:** *There were 64 subjects collected, the largest age group was 51-70 years (50%). Cancer was the main disease found in most of the subjects (56 people / 87.5%). The most common comorbidity was kidney disease (11 people). The most common palliative score distribution was 6 (15 people). The average score was 7.51. The mortality rate at the hospital was 51.6%, 33 patients from a total of 64 patients. From the palliative score distribution curve, the AUC value was 0.687 with a 95% CI (0.557-0.818). The optimal cut-off point was 8. All patients were palliative according to expert opinion based on WHO criteria.* ***Conclusion*:** *The performance of this tool is sufficient to screen palliative patients in a terminal and complex condition, but requires improvements to screen for patients who need early palliative care. The optimal cut-off point to determine the limit of consultation on palliative patients is found at score 8.*

***Keywords*:** *palliative care, performance, screening questionnaire, optimal cut-off point.*

INTRODUCTION

Palliative care is an approach of care which aims to prevent and reduce various types of pain – physical, psychological, social, or spiritual – suffered by patients with life-threatening diseases.[1] The number of patients who are at risk of developing such conditions, such as patients with cardiovascular disease, cancer, chronic obstructive pulmonary disease, AIDS, and diabetes, continues to increase every year.[1] There are more than 20 million people worldwide who need palliative care in the last years of their lives, but there are only 14% of them who receive palliative care.[2]

A study reports that palliative-care units require fewer resources and shorter patient-care duration than the general care; therefore, they are more cost effective.[3,4] Another report suggests that palliative care results in the same survival level as the general care, improvement in symptoms, and more satisfaction in the patients and their families.[5]

The identification of palliative patients using screening tools can increase the number of patients referred to palliative care.[6,7] These tools can be used on various care backgrounds and patients, such as in the Emergency Unit, the Intensive Care Unit (ICU), cancer patients, geriatric patients, etc. Nevertheless, given the varying patient characteristics in each health facility, further evidence-based validity and standardization are required for the use of palliative-care assessment tools in every health care facility. Cipto Mangunkusumo National Central Public Hospital (CMH) has been developing screening tools adapted from other screening tools with modifications since 2015, but until now its performance has not been assessed. The aim of the research is to develop a more reliable and valid palliative-patient screening tool for use at CMH.

METHODS

The research method used in this study is a cross-sectional study. The research was conducted by collecting data from CMH medical records and started from July to October 2019 until the required data were met. The sampling technique was carried out using the consecutive sampling method.

Research inclusion criteria include: Adult patients, over 18 years of age with progressive chronic disease; recipients of palliative team consultation; and, patient with complete data in the medical records. The exclusion criteria in this research include patiens with incomplete medical records. The researchers also sought opinion from two experts to assess whether a patient was categorized as palliative/non-palliative or terminal/non-terminal.

The subjects of this research were doctors and nurses who filled out the medical records of inpatients. The researchers informed the research objectives and asked the consent to conduct the research.

The research data processing was carried out using the SPSS24.0 computer program. The normality test was run using the Shapiro-Wilk method. If the data distribution is normal (p=>0.05), a parametric test will be conducted using the Pearson correlation test. If the distribution is not normal (p=0.05), then a non-parametric test will be conducted using the Spearman correlation test. The internal consistency was assessed by calculating the Cronbach's-Alpha coefficient.

Ethics

This research was granted clearance from the Committee for Medical Research Ethics, Faculty of Medicine – Universitas Indonesia (Panitia Etik Penelitian Kedokteran Fakultas Kedokteran Universitas Indonesia) Number: KET-828/UN2. F1/ETIK/PPM.00.02/2019.

RESULTS

There were 64 palliative patients.Three patients were excluded because of incompleted data. Patient data were consulted to the palliative team consisting of 2 experts in the palliative field, which results in 64 palliative patients, as shown in **Figure 1**.

The overall research subjects were dominated by the age group of 51-70 years old (50%), while the age group of 18-30 years old had the lowest number of participants, i.e. 35 male patients (54.69%) and 29 female patients (45.31%). Cancer was the primary disease found in most of the patients (56 people – 87.5%), followed by patients

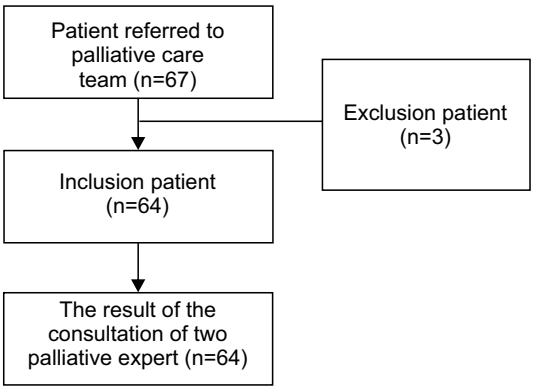

Figure 1. Study flow diagram.

who suffer from a chronic heart disease (4 people – 6.3%), advanced COPD (1 person – 1.6%), and 3 people were consulted with other main diagnoses, namely ARDS (2 people) and hepatic cirrhosis (1 person), as shown in **Table 1**.

Table 1. Characteristics of Research Subjects

Characteristics	N=64
Age, n (%)	
18-30	5 (7.8)
31-50	16 (25.0)
51-70	32 (50.0)
>70	11 (17.2)
Sex, n (%)	
Male	35 (54.7)
Female	29 (45.3)
Screener, n (%)	
Doctors	61 (95.3)
Nurses	3 (4.7)
Primary Diseases	
Cancer (Recurrent/metastases)	56 (87.5)
Advanced COPD	1 (1.6)
Stroke	0
Chronic Kidney Disease	0
Coronary Heart Disease	4 (6.3)
HIV/AIDS	0
Congenital Disease	0
Comorbid Diseases	
Chronic Heart Disease	7 (10.9)
Moderate Kidney Disease	11 (17.19)
Moderate COPD	4 (6.3)
Congestive Heart Failure	6 (9.4)
Other Conditions/Complications	15 (23.4)
Functional	
Fully active, able to perform activities without obstacles.	1 (1.6)
There are obstacles in strenuous activities but able to walk and perform light activities, such as house chores and light office work	1 (1.6)
Able to walk, perform self-care activities, but not able to perform all activities more than 50% of waking hours	3 (4.7)
Able to perform limited self-care activities, spend more time in bed or wheelchair, more than 50% of waking hours	24 (37.5)
Not able to perform self-care activities; spend most of the time in bed. Harsh condition / disable.	35 (54.6)
Other criteria	
Will not undergo curative treatment	44 (68.7)
In severe-disease conditions and choose not to continue with the therapy	16 (25.0)
Untreated pain more than 48 hours	12 (18.7)
Have uncontrollable symptoms (e.g. nausea and vomiting)	14 (21.9)
Have a psychosocial and spiritual condition that needs attention	10 (15.6)
Frequent visit to the Emergency Unit / hospitalized (> 1x/ month for the same diagnosis)	12 (18.7)
More than once of the same diagnosis within 30 days	13 (20.3)
Undergo long treatment without any significant progress (10 days).	19 (29.7)
Undergo long treatment at the ICU without any progress (> 2 weeks)	5 (7.8)

Gastrointestinal cancer was the most found disease (31.3%), while infection was the most frequent comorbid disease (17.2%). There were 23.4% patients treated due to infection. There were 8 people (12.5%) suffering from depression, and 21 people (32.8%) with malnutrition. The most used painkiller was opiate (28.1%), while the use of paracetamol was 10.9%. One patient (1.6%) received psychopharmacotherapy and psychotherapy. The highest Palliative Performance Score was 40% (15 patients).

Distribution of Palliative Score of Screening Tools at Cipto Mangunkusumo Hospital

Table 2 describes the distribution of the palliative score collected during the research. The average score is 7.51. The mortalities of the patients at the hospital were 33 patients (51.6%) out of the total 64 patients.

Table 2. Distribution of Palliative Score and Mortalities at the Hospital

Palliative Score	Live (n=30)	Die (n=34)
4	2 (6.7)	0 (0.0)
5	1 (3.3)	2 (5.9)
6	10 (33.3)	5 (14.7)
7	6 (20.0)	4 (11.8)
8	6 (20.0)	8 (23.5)
9	3 (10.0)	8 (23.5)
10	2 (6.7)	6 (17.6)
11	0 (0.0)	1 (2.9)

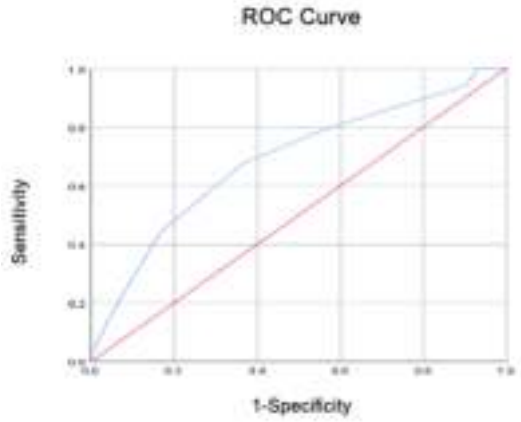

Figure 2. Distribution of Palliative Score

From the palliative score distribution curve, it is found that this tool is able to distinguish life and death, with an AUC value of 0.687 and 95% IK (0.557-0.818) with moderate correlation.

Table 3. Sensitivity and Specificity

Score	Sensitivity	Specificity
4	1.000	0.00
5	1.000	0.07
6	0.939	0.10
7	0.758	0.43
8	0.636	0.63
9	0.424	0.83
10	0.212	0.93
11	0.029	1.00

The optimal cut-off point to determine the limit of consultation on palliative patients is found at score 8.

Validity and Reliability

Based on the validity test that has been completed, 4 domains have varying validity, while the reliability test has a Cronbach's Alpha value of 0.560 from 4 domains, which means it has low reliability (<0.70). The Corrected Item – Total Correlation value shows that domain III and IV are above 0.3.

Table 4. Corrected Item – Total Correlation Value

	Average Scale when Items Deleted	Variation Scale when Items Deleted	Corrected Item Total Correlation	Cronbach's Alpha when Items Deleted
Domain I	12.92	9.184	0.224	0.588
Domain II	14.38	9.889	0.250	0.630
Domain III	12.64	8.520	0.356	0.540
Domain IV	12.78	6.110	0.438	0.425

Table 5. Validity and Reliability of Each Domain

Domain	r	p
Domain I	0.289	0.021
Domain II	0.175	0.167
Domain III	0.425	<0.001
Domain IV	0.736	<0.001

The criteria domain has the best correlation compared to the other domains, while the comorbidity domain has no correlation.

Expert Opinion

From the **Table 6**, it is indicated that all patients are palliative patients and more than 95% are patients in a terminal condition.

Table 6. Results of Consultations with Experts in the Palliative Field

	Researcher 1	Researcher 2
Palliative	64 (100)	64 (100)
Non-palliative	0 (0)	0 (0)
Terminal	62 (96.9)	61 (95.3)
Non-terminal	2 (3.1)	3 (4.7)

DISCUSSION

The participants involved in this research were dominated by those aged 51-70 years old, while those aged 18-30 years old were in a group with the lowest number of participants (7.8 %).

This shows that in general, the patients who receive palliative care by the palliative team at CMH are elderly, which is in accordance with the research at CMH on 300 palliative patients between 2016 and 2018 where 43.7% of the patients were aged 50-70 years old.[8] Data from WHO in 2011 indicates that 20.4 million people need palliative care, 69% of whom are aged above 60 years old, 25% are aged 15-59 years old.

Cancer is the most common condition found in the participants involved in this research. Early and continuous palliative care should be given to cancer patients.[8,9] Different types of cancer play a role in the difference of the improvement in the quality of life of the patients but do not affect the patient's survival rate.[8]

The characteristics of other primary diseases that are commonly found in this research are acute heart failure and advance COPD. CHF and COPD are the two main causes of chronic conditions. CHF, particularly, is the main cause

of death worldwide while COPD is projected to increase to be the third highest cause by 2030.[4]

In this research, there were 12 patients with untreated pain for more than 48 hours. The incidence of pain in palliative, progressive patients and patients with cancer was high, with 90% of them suffering from advanced-stage cancer. [10] This untreated pain was experienced by 43% of patients with obstacles from receiving treatments is commonly found in Asia.[11] Morphine is an effective analgesic used in the pain treatment for patients with cancer.[12]

Pain was not the most common complaint in this research because CMH demonstrates good adequacy in dealing with pain, in accordance with the research conducted at CMH on 258 patients who consulted for palliative care in 2016-2018, 175 (61,4%) patients complained of pain, and it was found that 87,5% of them received adequate pain treatment.[13]

Performance of Palliative Screening Tools at CMH

Based on the validity test, 4 domains had varying validity. The r of domains of primary disease, comorbid disease, and other criteria is more than 0.2641; therefore, domains I, III, and IV are valid. The domain of other criteria has the highest correlation that is 0.736, while the domain of comorbid disease has no correlation.

According to Fabrigar *et al*., the low correlation value indicates that there is only a small variation as a result of the sample being too homogenous, so it is possible to fail in identifying the number of factors that actually exist.[14] The consistency of the measuring instruments measures what needs or should be measured. The reliability test in this research has a Cronbach's Alpha value of 0.560 from 4 domains.

Other reliability results are seen by showing the value of *alpha if deleted item* and the value of *corrected item-total correlation*. If the value of *corrected item-total correlation* $\geq$ 0.3, it is evident that the items contained in the subscale measure the constructs in the subscale.[15] In this research, the domains of functional status and other criteria has the value of *corrected item-total correlation* $\geq$ 0.3, which is 0.356 and 0.438. Therefore, it can be stated that such items are able to measure patients that should be consulted to the palliative team. The validity and reliability in this research is satisfactory, but improvements are required in the domains of primary and comorbid disease.

The identification process is a stage in overcoming multi-layered and complex problems. These problems may reduce the level of success in early palliative care within the hospital.[15] The effectiveness of palliative care is indicated by patients who have been identified with positive quality of life towards the end of treatments.[16] The sooner a patient is detected in need of palliative care, the sooner the needs of the treatments fulfilled, which will impact the patient's quality of life. Patients who need early identification are no longer patients who are expected to die, but patients who are at risk of deteriorating conditions and terminal conditions are more likely to receive proactive assessment and treatment plans from health workers. As a tool in assessing a patient's condition, palliative-care screening tools must have assessment contents which are valid, applicable, easy to understand, and acceptable to the users, health workers and patients, as well as easy to analyze and interpret.

In this research, the optimal cut-off point of the palliative score is 8. However, as a screening tool, the cut-off point of 6 can also be considered. In this research, the cut-off point is higher than the original score, which is 8. A report in Taiwan using a similar screening tool shows that the AUC value for all cut-off points is 0.84 – 0.88. Based on the Youden's index, the optimal cut-off point value for 14 days are 2.16 Therefore, palliative care in Taiwan can be given earlier due to the lower cut-off point, which is 2. This is in line with the suggestion that palliative care should be initiated earlier.

Several influencing factors to the cut-off point are the knowledge and skills of experts in filling out the screening tools, the type of hospital, whether it is primary, secondary, tertiary, the distribution of patient characteristic, and hospital policy.

The medical personnel knowledge on when to consult with the palliative team also plays an important role, where there has to be conformity on when to consult and the understanding on palliative self-care.

Hospital policy greatly influences the coverage of palliative services. With good support from hospital management, it is expected that a policy on palliative care will be regulated and led with clear governance, leadership, and management/operation that can guide the implementation of palliative care at a hospital, so they are able to provide comprehensive and holistic palliative services. This also applies to the screening of palliative patients.

This palliative screening tools is suitable to be applied at hospitals that treat patients with terminal conditions, at tertiary hospitals. However, it is not necessarily applicable to primary and secondary health services.

Suggestion for further studies are: research with larger samples and a more even distribution of primary and comorbid diseases, dissemination of information to health workers on when to consult with the palliative team and on the screening tools, and further development of this screening tool.

Study Limitation

Even this is the first study in Indonesia to assess the performance of palliative screening, limitations include:

- The patients admitted to the hospital were already in severe/terminal conditions.
- This research took samples from a tertiary hospital, where the majority of the patients were patients with complex and terminal conditions; therefore, it is necessary to develop other tools which are able to reach all levels of health services.
- There was an uneven distribution of primary and comorbid diseases. There were no samples from patients diagnosed with HIV/AIDS, stroke, chronic kidney disease, or congenital abnormalities.
- The absence of proper standards in assessing palliative-need services can also result in the differences in consultation time with the palliative team.
- There are differences in the perception and the understanding of officers in filling out the palliative-screening sheet.

CONCLUSION

This palliative screening tool is sufficient to assess palliative patients in terminal and complex conditions, while to assess patients who need early palliative care, it still requires improvements. The optimal cut-off point to refer to the palliative team in this research was 8.

ACKNOWLEDGMENTS

I would like to thank to Dean of Faculty of Medicine and Rector of Universitas Indonesia for support and grant, PITTA A No:NKB-0400/UN2.R3.1/HKP.05.00/2019

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One Year Survival of Extrahepatic Cholangiocarcinoma Patients Who Did Not Undergo Curative Resection and Paliative Chemotherapy and Its Associated Factors

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ABSTRACT

Background: *Extrahepatic cholangiocarcinoma is rare but fatal. Patients who come are usually already in the advanced stage that can not undergo curative resection and chemotherapy also seems to be very rarely done. The survival rate and its associated factors in Indonesia are unknown. This study aimed to identify 1-year survival of patients with extrahepatic cholangiocarcinoma without curative resection and palliative chemotherapy and its associated factors.* ***Methods:*** *This is a cross-sectional study using medical records of extrahepatic cholangiocarcinoma (perihilar and distal) inpatient and outpatient patients at Cipto Mangunkusumo Hospital, Jakarta from January 2015 to March 2020, reviewed retrospectively. The following factors were analyzed in terms of mortality: metastasis, sepsis, hypoalbuminemia, serum bilirubin level, serum CA 19-9 level, billiary drainage, neutrophyl lympocyte ratio (NLR) and comorbid factors.* ***Results:*** *115 out of 144 patients were enrolled in this study with male proportion of 50.4%, and proportion of patients aged 65 years or above was 71.3%. 1 year survival rate was 10 % and median survival was 3 months (CI 95% 2.388-3.612)Multivariate analysis showed that only sepsis, unsuccessful or no prior biliary drainage and total bilirubin >19.8 mg/dl were independent predictors of mortality.* ***Conclusion:*** *1 year survival of extrahepatic cholangiocarcinoma without curative resection and paliative chemotherapy was 10 %.Sepsis, unsuccessful or no prior bilirary drainage, and total biirubin >19.8 mg/dl are factors significantly associated with shortened survival in malignant obstructive jaundice patients.*

Keywords: *Survival, extrahepatic cholangiocarcinoma, curative resection, paliative chemotherapy, mortality-related factors*

INTRODUCTION

Cholangiocarcinomas are malignancies that arise from the biliary tract epithelia. Patients with cholangiocarcinoma usually present at late stages of the disease, and early symptoms might be nonspecific. Therefore, these cancers remain difficult to diagnose and treat and their prognosis is generally poor. Approximately half of untreated patients die within 3–4 months.[1]

Until now there is no data on the prevalence of cholangiocarcinoma published in Indonesia, but in daily practice extrahepatic cholangiocarcinoma cases are more common, and patients who come usually undergo biliary drainage therapy. Patients who come are usually already in the advanced stage that could not undergo curative resection. Chemotherapy also seems to be very rarely done, as patients usually come with poor performance status.

Several overseas studies have been conducted to try to identify factors related to the survival of people with cholangiocarcinoma, especially extrahepatic cholangiocarcinoma. Metastasis[2], sepsis[3], comorbid[4], failure of drainage therapy[3], hypoalbuminemia[5], hyperbilirubinemia[5], high CA 19-9[6] and high Neutrophyl Lympocyte Ratio (NLR).[7]

Until now there has been no comprehensive research on the survival of extrahepatic cholangiocarcinoma patients in Indonesia and the factors that affect it. There has also been no research on the survival of extrahepatic cholangiocarcinoma sufferers who do not undergo resection and palliative chemotherapy. So the purpose of this study is to find out the survival of one year of extrahepatic cholangiocarcinoma sufferers who do not undergo curative resection and palliative chemotherapy and factors that affect the mortality of 1 year.

METHODS

This is a cross-sectional study which was retrieved using total sampling technique by tracing medical records and electronic health record of the extrahepatic biliary cancer (perihilar and distal) patients aged 18 years or older who had been hospitalized in Cipto Mangunkusumo Hospital in Jakarta from January 2015 to March 2020. We include patients without curative resection and paliative chemotherapy. This study has been approved by The Ethics Committee of the Faculty of Medicine, University of Indonesia, with registry number KET-31/UN2.F1/ETIK/PPM.00.02/2021.

The subjects characteristics are divided into two, clinical characteristics and characteristics by treatment. Clinical characteristics contain age, gender, symptoms, onset of symptoms to diagnosis, tumor location, risk factors for cholangiocarcinoma, bilirubin levels, CA levels 19-9, Albumin levels, NLR values, presence of cholangitis, sepsis, comorbidity, and metastasis.

Characteristics based on therapy are: resectability, drainage type, drainage failure, drainage failure based on drainage method, failed drainage based on tumor location, ERCP stent type, ERCP complications, and PTBD complications

Eight potential prognostic factors were studied: metastasis, comorbidity, sepsis, unsuccessful or no history of biliary drainage, albumin levels, bilirubin levels, CA 19-9 levels and neutrophyl Lympocyte Ratio. All malignant tumors found in the extrahepatic biliary duct (perihilar and distal) were included in the study after confirmation through Computed Tomography (CT) scan and/or MRI-MRCP (Magnetic Resonance Imaging-Magnetic Resoncance Cholangiopancreatography and/or Endoscopic Retrograde Cholangiopancreatography (ERCP) and/or Endoscopic Ultrasound (EUS) with or without biopsy confirmation.

The presence of comorbidities in the subjects was identified based on the total score of Charlson comorbidity index as documented on their medical records. Sepsis was identified according to Sepsis-3 criteria with the quick SOFA score ≥ 2.

Laboratory parameters include albumin, bilirubin and CA 19-9, and were measured on the first day of admission. Albumin levels below 3.4 g/dl was defined as hypoalbuminemia. High bilirubin was defined if bilirubin ≥19.8 mg/dl. High CA 19-9 levels was defined as levels ≥300 U/ml.

Biliary drainage had been performed through PTBD (Percutaneous Transhepatic Biliary Drainage) and ERCP. Drainage procedure

was considered success if a minimal 2 mg/dl bilirubin serum decreased after 2 to 5 days post drainage. Patients were divided into two groups: first group with history of successful biliary drainage procedure and the second group with unsuccessful or no history of biliary drainage.

We evaluated outcomes of mortality and the time of death of the observed subjects (time to event), which were determined since the first visit to hospital. Follow up for assessment of survival was done by phone calls. If the patient was unreachable via phone call, we did a home visit to the patients' registered adress.

Data analysis was performed using SPSS version 23.0 for univariate, and multivariate analyses. The level of significance used in our study was $\alpha = 0.05$. Variables were considered significant when the p value < 0.05. Cumulative one year survival was measured from the date of diagnosis of extrahepatic cholangiocarcinoma to the event and calculated by the methods of Kaplan-Meier, which was followed by Cox proportional hazard regression. The variables were then included into a multivariate model when the p value < 0.25.

RESULTS

Within the period of the study, we found 154 adult patients aged >18 years with extrahepatic cholangiocarcinoma. As many as 29 subjects were excluded due to data loss in their medical records; 10 were excluded for having curative resection (5 patients) and paliative chemotherpay (5 patients) therefore, we were left with 115 subjects with characteristics as shown in **Table 1**.

Table 1. Clinical characteristics of subjects

Clinical characteristic	Frequency, n (%) N=115
Age	
- ≥65	82(71.3)
- <65	33(28.7)
Sex	
- Male	58(50.4)
- Female	57(49.6)
Risk Factors,	
- Hepatitis B	8(6.9)
- Hepatitis C	1(0.8)
- Cirrhosis	5(4.3)
- Choledochal cyst	1(0.8)
Symptoms	
- Icterus	112 (97.4)
- Abdominal Pain	57 (49.6)
- Weight loss	46 (40.0)
- Itching	12 (10.4)
Onset from symptom to diagnosis (median)	2 months (0-12)
Tumor location	
- Perihilar	92 (80.0)
- Distal	23 (20.0)
Cholangitis	
- Yes	59 (51.3)
- No	56 (48.7)
Sepsis	
- Yes	31 (27.0)
- No	84 (73.0)
Comorbidity (CCI index)	
- ≥ 2	13 (11.3)
- 0-1	102 (88.7)
Metastasis	
- Yes	64 (55.7)
- No	51 (44.3)
Total bilirubin	
- >10 mg/dl	104 (90.4)
- ≤10 mg/dl	11 (9.6)
Total bilirubin	
- >19.8 mg/dl	71 (61.7)
- ≤19.8 mg/dl	44 (38.3)
CA 19-9	
- >300 IU/ml	60 (52.2)
- ≤300 IU/ml	55 (47.8)
Albumin	
- <3.4 g/dl	100 (87.7)
- ≥3.4 g/dl	15 (12.3)
Neutrophyl/Lymphocyte Ratio (NLR)	
- >7.45	54 (47.0)
- ≤7.45	61 (53.0)
Neutrophyl/Lymphocyte Ratio (NLR)	68 (59.1)
- >5.5	47 (40.9)
- ≤5.5	
Pathology Results	
- Adenocarcinoma	23 (20.0)
- Atipical malignancy suspicion	4 (3.4)
- Atypical	22 (19.1)
- Dysplasia	2 (1.7)
- Malignant	3 (2.6)
- No malignant cell	26 (22.6)
- Not examined	35 (30.6)

Table 2. Clinical characteristic by treatment.

Treatment characteristic	Frequency, n(%) N = 115
Resectability	
- Unresectable	75(65.2)
- Resectable (not resected)	40(34.8)
Biliary drainage	
- Success	81(70.4)
- Not success/no drainage	34(29.6)

Drainage type	
- ERCP	92 (80.0)
- PTBD	16 (13.9)
- No drainage	7 (6.1)
Failed drainage by drainage type	
- ERCP(n=92)	23/92 (25.0)
- PTBD(n=16)	6/16 (37.5)
Failed drainage by tumor location	
- Perihilar (n=92)	21/92 (22.8)
- Distal (n=23)	8/23 (34.7)
ERCP stent type (n=92)	
- Plastic	64/92(69.6)
- Metal	26/92(28.3)
- NBD (*nasobiliary drainage*)	2/92(2.1)
ERCP complications, (n=92)	
- Pancreatitis	12/92 (13.0)
- Post sphincterotomy bleeding	2/92 (2.0)
- Perforation (Non fatal)	1/92 (1.0)
- Cholangitis (Fatal)	1/92 (1.0)
PTBD complication, (n=16)	
- Perforation (fatal)	1/16(6.2)
- Leakage	1/16(6.2)

The proportion of survival in patients with extrahepatic cholangiocarcinoma based on observation of month 3, 6, 9, and 12 was 35%, 23%, 10%, and 10%, respectively as can be seen in **Table 2**; therefore, we found that the proportion of 1 year mortality in patients with extrahepatic cholangiocarcinoma who did not undergo curative resection and paliative chemotherapy was 90%. By the Kaplan-Meier curve in **Figure 1**, showed that median survival time (which was the time when 50% of study subjects survived) was 3 months (CI 95% 2.388-3.612).

Bivariate analysis, which is presented in **Table 3**, was performed to evaluate factors that affect the survival of subjects with extrahepatic cholangiocarcinoma. The analysis was done using Cox regression analysis. The degree of association was presented in the form of hazard ratio (HR). Seven variables, which were included as candidates in the multivariate analysis using Cox Proportional Hazard Model were variables with p value < 0.25 in the bivariate analysis. Those factors were metastasis, unsuccessful biliary drainage or no drainage, comorbidity, sepsis, high bilirubin, low albumin and high NLR.

Table 3. Proportion of survival in patients with extrahepatic cholangiocarcinoma who did not undergo curative resection and paliative chemotherapy.

Survival at month	Cumulative survival
0	0.50
3	0.35
6	0.23
9	0.10
12	0.10

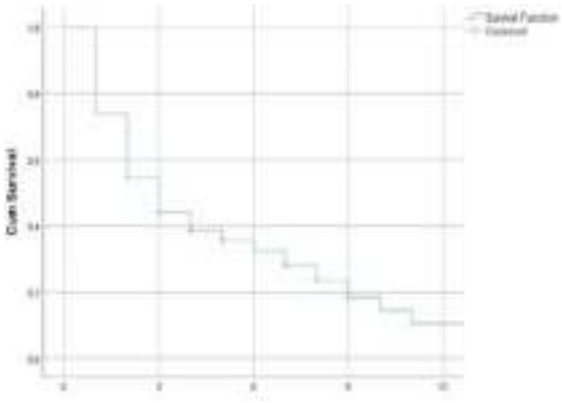

Figure 1. The Kaplan-Meier curve on 1-year survival of patients with extrahepatic cholangiocarcinoma who did not undergo curative resection and paliative chemotherapy.

Tabel 4. Univariate analysis on factors affecting 1-year survival in patients with extrahepatic cholangiocarcinoma who did not undergo curative resection and paliative chemotherapy.

Variables	Mortality		Person Time	Incidence Rate (10^{-3})	HR (IK95%)	P
	No	Yes				
Failed biliary drainage, n (%)						
- Yes	1 (2.9)	33 (97.1)	86	38.37		
- No	20 (24.7)	61 (75.3)	411	14.84	2.377 (1.535-3.680)	**<0.001**
Sepsis, n (%)						
- Yes	2 (6.5)	29 (93.5)	69	42.03		
- No	19 (22.6)	65 (77.4)	428	15.18	2.497 (1.585-3.932)	**<0.001**
Comorbidity (CCI index) , n (%)						
- 0≥2	0 (0.0)	13 (100.0)	37	35.14		
- 0-1	21 (20.6)	81 (79.4)	460	17.61	1.859 (1.027-3.365)	**0.040**

Metastasis						
- Yes	7 (10.9)	57 (89.1)	231	24.67		
- No	14 (27.5)	37 (72.5)	266	13.91	1.653 (1.085-2.517)	**0.019**
Total bilirubin,n (%)						
- >19,8 mg/dl	13 (12.5)	91 (87.5)	99	27.73		
- ≤ 19,8 mg/dl	8 (72.7)	3(27.3)	398	10.81	2.369 (1.505-3.729)	**<0.001**
CA 19-9 , n (%)						
- >300 IU/ml	12 (20.0)	48 (80.0)	232	20.68		
- ≤300 IU/ml	9 (16.4)	46 (83.6)	265	17.35	1.113 (0.742-1.671)	0.605
Albumin, n(%)						
- <3,4 g/dl	16(16.0)	84(84.0)	108	21.82		
- ≥3,4 g/dl	5(35.7)	9(64.3)	385	8.33	2.428(1.201-4.907)	**0.014**
Neutrophyl/Lymphocyte Ratio (NLR) , n (%)						
- >5,5	6 (8.8)	62 (91.2)	246	25.20		
- ≤5,5	15 (31.9)	32 (68.1)	251	12.75	1.844(1.19-2.848)	0.006

Tabel 5. Multivariate analysis on factors affecting 1-year survival in patients with extrahepatic cholangiocarcinoma who did not undergo curative resection and paliative chemotherapy.

Variables	HR (CI 95%)	P
Sepsis	1.879 (1.171-3.014)	**0.009**
Total Bilirubin >19.8 mg/dl	1.972 (1.248-3.117)	**0.004**
Unsuccessful Biliary Drainage/no drainage	1.807 (1.150-2.842)	**0.010**

Meaningful variables in multivariate analysis are sepsis, bilirubin levels > 19.8 mg/dl and failed or unattributed biliary drainage. Shown in **Table 4** are the hazard ratio (HR) with a confidence interval (IK) of 95% of each meaningful prognosis factor.

DISCUSSION

In this study there were 58 patients (50.4%) that were male. The most common age group was ≥ 65 years old with as many as 82 people (71.3%), and the median age of the subjects being 58 years (29-86) years. It is similar with a study by Ruiz et al in Spain, where the population of extrahepatic cholangiocarcinoma consists of 34 (50%) males. However, the average age in their study was higher at 73.4±11.5 years.[4]In a study in Korea by Park et al, the proportion of male extrahepatic cholangiocarcinoma patients was 67%, with mean age of 62±10.1.[2] In research in China by Wang et al in patients with extrahepatic cholangiocarcinoma, proportion of men were 65%, with a higher mean age of 68.9±11.158.[8]

In this study, the proportion of perihilar cholangiocarcinoma (Klatskin tumor) was greater, which is 92 (80%) compared to distal 23 (20%). This is approximately the same as the proportion of incidence of cholangiocarcinoma in general where perihilar cholangiocarcinoma is the most common type of tumor that is 60% cases, 30% cases of distal cholangiocarcinoma and 6-10% cases of intrahepatic cholangiocarcinoma.[9]

In this study, strong risk factors were hepatitis B 8 (6.9%), hepatitis C 1 (0.8%), cirrhosis 5 (4.3%), and choledochal cyst 2 (1.7%). Some other risk factors were diabetes 12 (10.4%) and alcohol 1 (0.8%). This is more or less similar to the population of cholangiocarcinoma sufferers in study by Yusoff inMalaysia.[10]

In this study, based on the criteria of resectability via imaging, it was found that as many as 40 (34.8%) were suitable for resection.[11] This is similar to the study that approximately one-third of patients can be resected during diagnosis.[9] But only 5 patients did undergo resection for curative purpose. The reason for this were many, such as refusing surgery, not coming for further evaluation, poor performance status, and preoperative restaging.

In multivariate analysis bilirubin levels ≥ 19.8 mg / dl, failed / not performed biliary drainage and sepsis was found as an independent prognostic factor.

In our study, the proportion of patients with high bilirubin levels (>10 mg /dl) was 104 out of 115 patients (90.4%). Median value of bilirubin

was 22 mg / dl (ranging from 5,3 mg / dl to 53.40 mg / dl) which was higher than other studies.[8, 12]

High bilirubin levels caused by biliary obstruction will cause impaired liver function, disrupt endotoxin cleansing, cause coagulation system disorders, immune system and gastrointestinal intestinal barrier. Endotoxins in normal liver conditions are produced in small amounts and then through the portal vein it enters the liver and are inactivated by the liver reticuloendotelial system. Increased levels of endotoxins in biliary obstruction conditions plus impaired liver function will cause a condition named Systemic Inflammatory Response Syndrome (SIRS) which will cause sepsis and then multiple organ failure. So if these high bilirubin levels were not treated with good drainage, it tends to cause infection, sepsis and death.[13-15]

Under normal conditions the bile fluid is in a sterile state. However, tumor obstruction can lead to bacterial growth and colonization in approximately 25% of patients.[16] Li, et al[17] in China found the proportion of patients with bile duct obstruction due to solid tumors experiencing biliary tract infection was 21 % while in study by Gaspersz et al[18] in Netherland, there are 45% patients with perihilar cholangiocarcinoma experiencing biliary tract infection before the procedure of biliary drainage could be carried out. In our study, 59 patients (51.3%) had acute cholangitis at admission and 31 (27%) had sepsis. While sepsis was one of independent predictive factor in our study. The tendency to develop biliary tract infection in our population may be due to several reasons, namely delays in diagnosis (median from onset to diagnosis was 2 month), and higher bilirubin levels in our study indicating that there has been a long obstruction.

From the results obtained above, the role of biliary drainage becomes very important to improve survival that can be caused by hyperbilirubinemia and sepsis. From our study unsuccessful biliary drainage became one of independent predictive factor for mortality. Biliary drainage in cases of biliary obstruction due to malignancy is considered an important palliative therapy because it can reduce symptoms caused by hyperbilirubinemia, thus allowing patients to undergo surgery, chemotherapy, radiotherapy and local therapy against tumors so as to increase survival in patients with malignant etiology.[19] Research by Kurniawan et al., with the same drainage success criteria, obtained biliary drainage that failed or not performed will increase the risk of death. Other studies by Brountzos et al[20], Zhang,et al[19], with different drainage success criteria also found similar results.

In this study, the 1-year survival rate of extrahepatic cholangiocarcinoma patients who did not undergo curative resection and palliative chemotherapy was 10%, with a median survival of 3 months It is more or less similar to research in Malaysia by Yusoff et al.[10]

In this study there were only 5 patients who performed resection with curative purposes and 5 patients who received palliative chemotherapy during this study, which were excluded in this study. In study by Yusoff et al in Malaysia, there were more patient to be resected and these might be due to the median duration from symptom to diagnosis being more early compared to our study which was 30 days, making curative resection more likely to be achieved.[10]

This study, is the first study in Indonesia that examines the survival of extrahepatic cholangiocarcinoma patients (perihilar and distal) and is also the first research in to study the population of extrahepatic cholangiocarcinoma undergoing supportive therapy only, giving us data on extrahepatic cholangiocarcinoma prognosis if no procedures that can improve survival (curative resection and palliative chemotherapy) were done and the positive benefit of biliary drainage in this setting.

Limitations of this study include the possibility of information bias due to retrospective design. There were very little histopathological data, so the accuracy of diagnosis in extrahepatic cholangiocarcinoma in this study became less accurate, for in our study the positive result of malignancy from pathology were only 20%. However, this was due to the diagnostic procedure of cholangiocarcinoma extrahepatic examination that is commonly done such as bile duct brushing through ERCP and cytology examination of bile fluid aspiration through

PTBD has a low sensitivity although the specificity is very high.[9]

CONCLUSION

One year survival of patients with extrahepatic cholangiocarcinoma in our study was 10 % and sepsis, unsuccessful or no biliary drainage and total bilirubin >19.8 mg/dl were the three independent prognostic factors for mortality.

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Urease Levels and Gastritis Stage in Dyspeptic Patients

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ABSTRACT

Background: *Dyspepsia is a frequent main symptom of inpatients and outpatients scenario in Indonesia. However, the number of endoscopy facilities are still low, thus the use of non-invasive method to detect gastritis is necessary. We measured the relationship between urease levels and the stage of gastritis in dyspeptic adult patients.* ***Methods:*** *A cross-sectional study included outpatient dyspepsia patient from November 2018 to February 2019. We examined ^{14C}-Urea Breath Test (UBT) and determined the stage of gastritis based on the Updated Sydney System classification.* ***Results:*** *The urease level of acute and chronic gastritis positive patients were higher than negative patients ($p = 0.001$, $r = 0.353$; $p < 0.0001$, $r = 0.433$, respectively). The AUC value of ^{14C}-UBT to detect acute, chronic, and atrophic gastritis are 0.889, 0.632 and 0.544, respectively. The best cut-off points of ^{14C}-UBT to predict acute gastritis was ≥26.50δ‰ with sensitivity and specificity being 88.89% and 63.95%, respectively. Whereas the best cut-off points for chronic gastritis was ≥34.50δ‰ with 82.89% sensitivity, 63.16% specificity. As for atrophic gastritis, it showed very low AUC value, hence it is not a sufficient test modality to predict atrophic gastritis cases.* ***Conclusion:*** *^{14C}-UBT is sufficient for predicting acute or chronic gastritis but not for atrophic gastritis.*

Keywords**:** *Dyspepsia, gastritis severity, urea breath test, cancer.*

INTRODUCTION

Dyspepsia is the most common gastrointestinal symptom in clinical practice.[1] Approximately 44.7% patients with dyspepsia had gastritis or duodenitis diagnosed by endoscopic examination in Indonesia.[2] Dyspepsia might be caused by two factors, infection and non-infection. Infection is mostly caused by *Helicobacter pylori*, whereas non-infection might be caused by stress, diet habits, hormonal factors and other functional factors.[3] Detection of *H. pylori* infection could be performed by many ways, such as histological examination, stool antigen test, anti *H. pylori* antibody and urea breath test (UBT).[4]

Gastritis, especially atrophic gastritis is the common contributing factor for gastric cancer.[5] Inflammation of the gastric mucosa may cause loss of glands that will eventually be replaced by intestinal-type epithelial cells, which is considered as a low-grade dysplasia.[6] This dysplastic tissue then become intestinal type gastric cancer as the end result of progressive changes in the gastric mucosa.[7] Mechanism of gastritis induced by urease enzyme activity remains unclear. Urea and urease may increase mucosal damage due to increased ammonia level in the gastric mucosa.[8] A study in mice given ammonia showed an increase in the number of inflammatory cells induced by chronic gastritis, suggesting a significant relationship between ammonia levels and gastritis.[9] Another study in patients with dyspepsia confirmed that ammonia levels were significantly associated with the severity of gastritis.[10] In addition, peptic ulcer patients had significantly higher urease level than patients without peptic ulcers.[11] It is suspected that there are urease-producing bacteria, including pathogens other than *H. pylori* which cause chronic gastritis in areas with low prevalence of *H. pylori* such as Indonesia. UBT is a non-invasive method to detect *H. pylori* which relied on the fact that *H. pylori* secretes urease enzyme which converts urea into ammonia and carbon dioxide.[12,13] The UBT is a reliable method to detect *H. pylori* and performed based on the ability of *H. pylori* to break down urea, which is absorbed from the stomach and eliminated in exhalation.[14] If the isotope is detected in the breath, the test is positive, suggesting *H. pylori* presence in the stomach.[15] The amount of urease activity, detected by value from UBT may reflect the *H. pylori* bacterial load in the stomach.[16] Indeed, UBT is mainly used for detecting *H. pylori*, but since there are other bacteria that has urease activity, such as *Proteus mirabilis*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Staphylococcus aureus*).[17] However, the end point of urease activity is producing ammonia, a toxic substance for the stomach. Therefore, UBT may have potential usage and become a non-invasive alternative diagnostic modality to detect urease-related gastritis.

Indonesia is a multi-ethnic country with over 267 million people living in more than seventeen thousand islands with regional disparities in health service quality.[18] Dyspepsia and gastritis are included in the top 10 diseases and is common in inpatients and outpatients clinics of Indonesia. However, the number of endoscopy experts in Indonesia is lacking and the number of endoscopy centers is still low.[19] Recently, ^{14}C-UBT, a non-invasive method with simple, less expensive, accurate and easy handling is massively used in clinical practice. This study aimed to determine relationship between urease levels with the severity of gastritis in dyspeptic patients.

METHODS

We conducted a cross sectional study from November 2018 to February 2019 in Dr. Soetomo Teaching Hospital, Surabaya, Indonesia. Ninety five dyspeptic patients aged 18 to 70 years old were included in this study. We excluded patients receiving antibiotics and bismuth drugs 4 weeks prior to examination, proton pump inhibitor 2 weeks prior to examination, patients with history of gastric surgery, bleeding gastrointestinal tract within 4 weeks, impaired kidney diseases, liver cirrhosis, diabetes mellitus, gut malignancy, history of smoking and alcohol consumption, history of NSAID consumption and patients with endoscopy contraindication. We collected demographics data and dietary habits by questionnaire.

One day before endoscopy, all patients were examined by ^{14}C-UBT (Heliprobe, Stockholm,

Sweden) using ^{14}C-urea (250 uCi, Amersham) reconstituted with 25 ml of sterile distilled water. Subjects were fasted for at least six hours prior to the test. They removed false teeth (if present), and cleansed their mouth with antiseptic solution such as thymol, salol, menthol, saccharin, fuchsin, water and ethanol. A baseline breath sample was collected and identified as time 0. Then, they swallowed 5 uCi of ^{14}C-urea dissolved in 20 ml of water. Breath samples were collected at 5, 10, 15, 20 and 30 minutes. Patients were instructed to blow through tubing attached to a safety trap into a scintillation vial containing 2.5 ml of 400 mM Hyamine (Sigma) in methanol with 15 mg/l thymolphthalein (blue alkaline color). They had to blow until the solution became colorless indicating the collection of 1 mmol of CO_2. Once the breath samples had been collected, scintillation fluid (10 ml-5.5 g PPO/0.2g POPOP of 2:1 v/v Toluene/Triton-X) was added to the vial; counting proceeded for 5 minutes per vial, and the results were expressed as cpm/mmol CO_2. Counting efficiency of the Beckman LS 10^{0}C was 93%.

Endoscopy and biopsy were performed on the next day. Experienced endoscopists collected single biopsy samples from corpus and antrum of the gaster for histological examination. Patients with evidence of activity or inflammation in the antrum or corpus upon histological examination were considered positive for gastritis. The severity of gastritis is determined by histological examination based on the updated Sydney system classification.[20] Informed consent was obtained from all participants, and the protocol was approved by the Ethics Committee of Dr. Soetomo Teaching Hospital (Surabaya, Indonesia).

Statistical Analysis

Statistical Analysis is done using the SPSS statistical software package version 23 (SPSS, Inc., Chicago, IL, USA). Correlation analysis used Spearman's Signed Rank Test because the distribution data was abnormal. Correlation coefficient considered with r and significant analysis with *P* value was <0.05. In addition, to determine the cut-off point of UBT examination we used Receiver Operating Characteristic (ROC) analysis for showing area under curve (AUC) then we calculated the sensitivity and specificity from the determined cut-off point.

RESULTS

Demographical Characteristics of Patients

The total study population was 95 consecutive dyspeptic patients (52 female and 43 male; age range 20-65 years). Female patients had a higher proportion of chronic and atrophic gastritis (4/52, 7.7%; and 15/52, 28.8%, respectively, **Table 1**), however statistically insignificant (p = 0.130). Age group of >60 years old had a more acute gastritis than other age groups (6/21, 28.6%, p = 0.018). Christian (5/20, 25.0%) and Buddhist (1/3, 33.3%) patients had higher association with acute gastritis (p = 0.038). However, there was no association between marital status, job, income, education and ethnics with prevalence of gastritis (all p >0.05).

The amount of resident 1–4 people had higher proportion in acute and chronic gastritis (7/71, 9.9%, p = 0.049 and 15/71, 21.1%, p =

Table 1. Demographical Characteristic of Respondents

Demographical Characteristic	n	Acute Gastritis	Chronic Gastritis	Atrophic Gastritis
Sex				
Male	43	5 (11.6)	6 (14.0)	9 (20.9)
Female	52	4 (7.7)	13 (25.0)	15 (28.8)
Age				
20-29 years old	4	0 (0.0)	1 (25)	2 (50.0)*
30-39 years old	9	0 (0.0)	0 (0.0)	0 (0.0)
40-49 years old	31	2 (6.5)	5 (16.1)	4 (12.9)
50-59 years old	30	1 (3.3)	5 (16.1)	10 (33.3)
>60 years old	21	6 (28.6)*	8 (38.1)	8 (38.1)
Marital Status				
Married	87	9 (10.3)	18 (20.7)	21 (24.1)
Single	8	0 (0.0)	1 (12.5)	3 (37.5)

Job				
Civil Servant	5	0 (0.0)	0 (0.0)	1 (20.0)
Housewife	35	2 (5.7)	7 (20.0)	9 (25.7)
Employee	42	5 (11.9)	8 (19.0)	11 (26.2)
Doctor	1	0 (0.0)	0 (0.0)	0 (0.0)
Teacher	2	0 (0.0)	1 (50.0)	0 (0.0)
Student	2	0 (0.0)	0 (0.0)	0 (0.0)
Retired	2	0 (0.0)	1 (50.0)	0 (0.0)
Farmer	6	2 (2.1)	2 (33.3)	3 (50.0)
Income				
Under Minimum Regional Income**	69	6 (8.7)	15 (21.7)	16 (23.2)
Upper Minimum Regional Income**	26	3 (11.5)	4 (15.4)	8 (30.8)
Religion				
Buddhism	3	1 (33.3)*	1 (33.3)	1 (33.3)
Hindu	2	0 (0.0)	0 (0.0)	0 (0.0)
Moeslim	65	3 (4.6)	10 (15.4)	13 (20.0)
Catholic	5	0 (0.0)	1 (20.0)	2 (40.0)
Christian	20	5 (25.0)	7 (35.0)	8 (40.0)
Education				
Not educated	1	0 (0.0)	0 (0.0)	0 (0.0)
Elementary school	9	1 (11.1)	2 (22.2)	2 (22.2)
Junior high school	13	2 (15.4)	5 (38.5)	7 (53.8)
Senior high school	43	2 (4.7)	7 (16.3)	8 (18.6)
Diploma	2	0 (0.0)	0 (0.0)	1 (50.0)
Bachelor	25	4 (16.0)	5 (20.0)	6 (24.0)
Master	2	0 (0.0)	0 (0.0)	0 (0.0)
Ethnic				
Ambon	2	0 (0.0)	0 (0.0)	1 (50.0)
Bataknese	22	5 (22.7)	6 (27.3)	7 (31.8)
Javanese	49	2 (4.1)	9 (18.4)	10 (20.4)
Madura	4	0 (0.0)	0 (0.0)	1 (25.0)
Sunda	1	0 (0.0)	0 (0.0)	0 (0.0)
Tioghoa	11	2 (18.2)	4 (36.4)	5 (45.5)
Alas	1	0 (0.0)	0 (0.0)	0 (0.0)
Balinese	3	0 (0.0)	0 (0.0)	0 (0.0)
Padang	1	0 (0.0)	0 (0.0)	0 (0.0)
Pak Pak	1	0 (0.0)	0 (0.0)	0 (0.0)

* p <0.05 with chi-square analysis
** USD 272 currency on March 2020

0.031, respectively, **Table 2**), but only tended in atrophic gastritis (19/71, 26.8%, p = 0.094). The frequency of eating with hand had association with acute, chronic and atrophic gastritis (p = 0.026, p = 0.045 and p = 0.036, respectively). Smokers had higher prevalence of acute gastritis than non-smokers (5/22, 22.7% vs. 4/73, 5.5%, p = 0.015). Source of water, alcohol drinker, hand washing after toilet use and before eating did not influence prevalence of gastritis (all p >0.05).

Among 95 subjects, 19 (26.3%) frequently consumed analgesics and had association with acute gastritis (p = 0.005, **Table 3**). In addition, anxiolytic users had a higher acute gastritis rather than non-users (5/26, 19.2% vs. 4/69, 5.8%, p = 0.045). The most six common symptoms in acute, chronic and atrophic gastritis were epigastric pain (9/92, 9.8%; 14/92, 20.3%; 23/92, 24.2%, respectively), easy to feel full when consuming food or drink (8/64, 12.5%; 16/64, 25.0%; 17/64, 26.6%, respectively), nausea (6/64, 9.4%; 12/64, 18.8%; 15/64, 23.4%, respectively), feeling bloated (6/69, 8.7%; 14/69, 20.3%; 16/69, 23.2%, respectively), heart burn (4/46, 8.7%; 8/46, 17.4%; 13/46, 28.3%, respectively) and vomiting (7/72, 9.7%; 14/72, 19.4%; 16/72, 22.2%, respectively), but there was no significant association between all symptoms with gastritis (all p >0.05).

There were three most comon diseases from endoscopy including erosive gastritis (20/95, 21.1%), gastroesophageal reflux disease (18/95, 18.9%) and superficial gastritis (13/95, 13.7%). The prevalence of *H. pylori*-positive subjects in this study was very low (4/48, 8.3%). When we used the cut-off point of UBT from manual

Table 2. Health Behavior of Subjects

Health Behavior	n	Acute Gastritis	Chronic Gastritis	Atrophic Gastritis
Resident in One House				
1 – 4 people	71	7 (9.9)*	15 (21.1)*	19 (26.8)
5 and more people	24	2 (8.3)	4 (16.7)	5 (20.8)
Source of Water				
Well	8	0 (0.0)	0 (0.0)	1 (12.5)
New Mineral Water	14	1 (7.1)	1 (7.1)	3 (21.4)
Refill Mineral Water	48	4 (8.3)	11 (22.9)	14 (29.2)
Boiled Water	25	4 (16.0)	7 (28.0)	6 (24.0)
Hand Wash After Toilet				
Never	1	0 (0.0)	0 (0.0)	0 (0.0)
Rarely	6	0 (0.0)	1 (16.7)	2 (33.3)
Sometimes	9	1 (11.1)	3 (33.3)	3 (33.3)
Often	25	2 (8.0)	2 (8.0)	6 (24.0)
Always	54	6 (11.1)	13 (24.1)	13 (24.1)
Hand Wash Before Eat				
Never	1	0 (0.0)	0 (0.0)	0 (0.0)
Rarely	3	0 (0.0)	0 (0.0)	1 (33.3)
Sometimes	13	2 (15.4)	3 (23.1)	3 (23.1)
Often	35	3 (8.6)	5 (14.3)	10 (28.6)
Always	43	4 (9.3)	11 (25.6)	10 (23.3)
Eating with Hand				
Never	7	1 (14.3)	1 (14.3)	2 (28.5)
Rarely	24	3 (12.5)	8 (33.3)	7 (29.2)
Sometimes	31	0 (0.0)	2 (6.5)	6 (19.4)
Often	20	1 (5.0)	3 (15.0)	4 (20.0)
Always	13	4 (30.8)*	5 (38.5)*	5 (38.5)*
Smoking				
Yes	22	5 (22.7)*	5 (22.7)	4 (18.2)
No	73	4 (5.5)	14 (19.2)	20 (27.4)
Alcohol				
Yes	21	4 (19.0)	5 (23.8)	5 (23.8)
No	74	5 (6.8)	14 (18.9)	19 (25.7)

** p <0.05 with chi-square analysis*

Table 3. Medical Status of Subjects

Medical Status	n	Acute Gastritis	Chronic Gastritis	Atrophic Gastritis
Symptom				
Bloated				
Yes	69	6 (8.7)	14 (20.3)	16 (23.2)
No	26	3 (11.5)	5 (19.2)	8 (30.8)
Epigastric pain				
Yes	92	9 (9.8)	18 (19.6)	23 (24.2)
No	3	0 (0.0)	1 (33.3)	1 (33.3)
Heart Burn				
Yes	46	4 (8.7)	8 (17.4)	13 (28.3)
No	49	5 (10.2)	11 (22.4)	11 (22.4)
Nausea				
Yes	64	6 (9.4)	12 (18.8)	15 (23.4)
No	31	3 (9.7)	7 (22.6)	9 (29.0)
Vomiting				
Yes	23	2 (8.7)	5 (21.7)	8 (34.8)
No	72	7 (9.7)	14 (19.4)	16 (22.2)
Easy to fill				
Yes	64	8 (12.5)	16 (25.0)	17 (26.6)
No	31	1 (3.2)	3 (9.7)	7 (22.6)
Proton Pump Inhibitor				
Yes	4	1 (25.0)	2 (50.0)	1 (25.0)
No	91	8 (8.8)	17 (18.7)	23 (25.3)
Antibiotics				
Yes	11	2 (18.2)	4 (36.4)	2 (18.2)
No	84	7 (8.3)	15 (17.9)	22 (26.2)

Analgesic				
Yes	19	5 (26.3)*	5 (26.3)	4 (21.1)
No	76	4 (5.3)*	14 (18.4)	20 (26.3)
Anti-anxiety				
Yes	26	5 (19.2)*	6 (23.1)	7 (26.9)
No	69	4 (5.8)*	13 (18.8)	17 (24.6)

** p <0.05 with chi-square analysis*

instruction (50.00), there was no correlation between diseases and positivity of *H. pylori*.

Urease Levels and Stage of Gastritis

Based on the gastritis stage, we observed a significant trend of increasing UBT level with both degree of acute and chronic antral gastritis (r= 0.366 and r= 0.404, respectively; both P < 0.001) (**Figure 1**). However, we could not find correlation between degree of both atrophic gastritis and intestinal metaplasia in antrum. As for in the corpus, we only could find a significant correlation between corporal atrophy and UBT level (r= 0.270, P=0.036). The others histological parameter (acute gastritis, chronic gastritis and intestinal metaplasia) did not show a significant association (all P > 0.05).

We validated the accuracy of ^{14}C-UBT to predict acute gastritis. Acute gastritis is expressed as a neutrophil infiltration ≥1 on the gastric mucosa. The AUC of the urea levels compared with acute gastritis with AUC score was 0.889 (95% CI = 0.729 – 0.950) (**Figure**

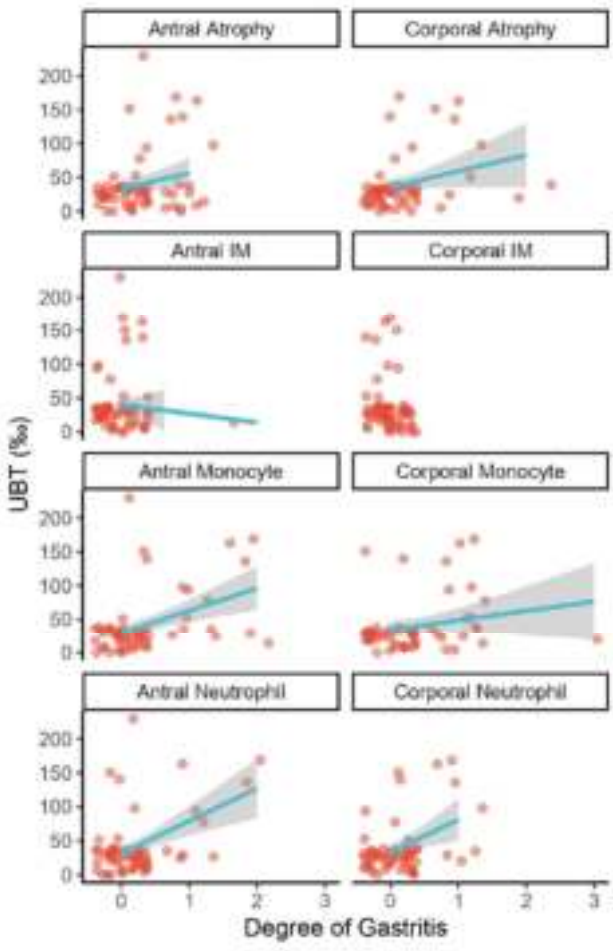

Figure 1. Association between urease levels with the degree of gastritis in each stage.

2). The best cut-off point was ≥26.50 δ‰ with sensitivity of 88.89%, specificity of 63.95%, positive predictive value (PPV) of 71.15%, negative predictive value (NPV) of 85.20%, positive likelihood ratio of 2.47, negative likelihood ratio of 0.17 and accuracy of 76.42%.

In addition, we also determine the performance of ^{14}C-UBT for detecting chronic gastritis. UBT level yielded an AUC score of 0.632 (95% CI = 0.592 – 0.883) (**Figure 3**). The best cut-off point was ≥34.50 δ‰ with sensitivity, specificity, PPV, NPV, positive likelihood ratio and negative likelihood ratio being 82.89%, 63.16%, 78.69%, 69.23%, 3.69, and 0.44, respectively with overall 73.03% accuracy.

The validation examination for atrophic gastritis showed a very low AUC score of 0.544 (95% CI = 0.396 – 692). Therefore, it is not sufficient for determining the best cut-off. As for the accuracy of the ^{14}C UBT for intestinal metaplasia was not measured because there were only 2 positive cases.

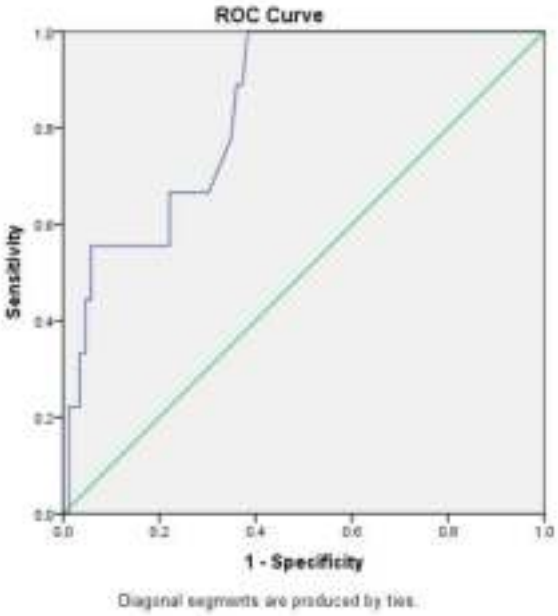

Figure 2. The urea levels compared with acute gastritis with AUC score.

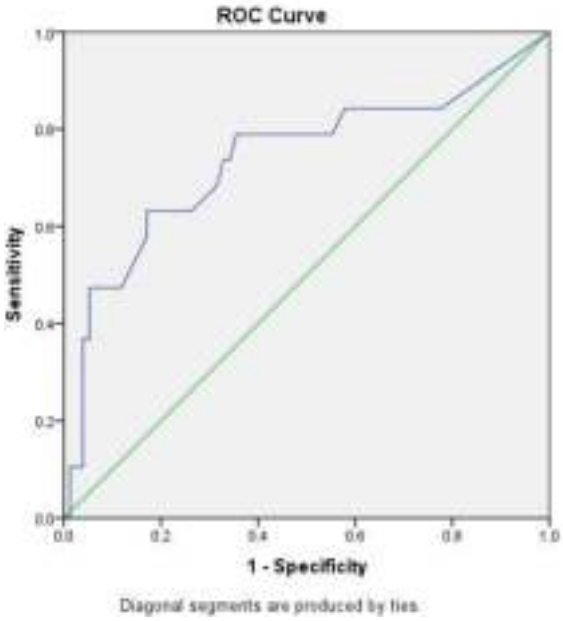

Figure 3. Urease activity level compared to chronic gastritis resulted AUC score.

DISCUSSION

We confirmed the accuracy of ^{14}C-UBT to predict severity of gastritis but not for atrophic gastritis. The cut-off point ^{14}C-UBT to measure acute and chronic gastritis were higher than atrophic gastritis. This result is in agreement with a previous study which showed that the UBT value was correlated to gastric cancer and was significantly lower than that for gastritis, duodenal ulcer, or gastric ulcer in *H. pylori*-positive patients.[21,22] They also found a low UBT value were associated with the risk of gastric cancer, similar with this study where the cut-off points in atrophic was lower than acute or chronic gastritis.[16]

Urease level has better sensitivity in acute and chronic gastritis than atrophic gastritis due to the difference in *H. pylori* colonization bacterial load. Extensive gastric mucosal atrophy may decrease colonization by *H. pylori* and produce a low UBT value.[4,23] In addition, UBT value is mainly influenced by *H. pylori* colonization which lead to increasing neutrophil infiltration, therefore it contribute to the higher association between acute gastritis rather than the atrophic gastritis.[24,25] However, Indonesia has a low prevalence of *H. pylori* infection.[26] In this study, we also confirmed that

the prevalence of *H. pylori* infection was very low, suggesting in Indonesian cases generally the bacteria do not have a major influence on clinical outcomes[27], especially in ethnic groups with low prevalence of *H. pylori*. Therefore, due to dyspepsia and gastritis being one of the top 10 diseases in Indonesia, non-*H. pylori* urease-producing bacteria might a major role causing gastritis. Non-*H. pylori* bacteria such as non-*H. pylori Helicobacter spp., Mycobacterium spp., Staphylococcus spp, Proteus mirabilis*, *Citrobacter freundii*, *Klebsiella pneumoniae*, and *Enterobacter cloacae*. could produce urease enzyme.[17,25]

Sufficient sensitivity but low specificity of ^{14}C-UBT for determining acute gastritis, suggests that ^{14}C-UBT has sufficient ability to screen for acute gastritis, but low specificity indicates UBT test was not a good diagnostic method.[28,29] Thus, other modalities, either invasive or non-invasive to determine gastritis is necessary. There were options for non-invasive diagnostic method that had potential determining the gastritis status, such as serum pepsinogen level. Serum pepsinogen had been well recognized as a non-invasive screening option for early stages of gastric cancer. Combination of serum pepsinogen and anti-*H. pylori* antibody, Miki and co-workers had established a stratification method for gastric cancer risk.[30] That method had been applied and showed promising results in several populations, including in Indonesia.[31,32] Therefore, serum pepsinogen might be still become the best non-invasive methods to measure severity of gastritis, especially in Indonesia.[33]

Urease exposure can cause an inflammatory reaction by producing reactive oxygen species and inducing the expression of inducible NO-synthesizing enzyme.[34] Urease can also give a toxic effect indirectly by producing ammonia, a product of urea hydrolysis.[16] The presence of ammonia in the stomach can cause hypoxia in gastric tissue by increasing intracellular and intra mitochondrial pH. Ammonia also interferes with the activity of tricarboxylic acid which can reduce ATP synthesis so that it interferes with cell migration and cell proliferation which can inhibit repair of the gastric epithelium. This activity causes the activation of the danger associated molecular pattern (DAMP) that recognized by the pattern recognition receptor and activate monocytes and neutrophils and the recruitment of inflammatory cells, such as IL-1, IL-8 and TNF-αv.[35] In addition to inducing the release of proinflammatory cytokines, ammonia can also enter the G cell nucleus easily and bind the gene-regulating gastrin unit so that it can activate expression and enhance gastrin formation.[36] That mechanism might be a responsible way explaining observed gastritis in the high ammonia individuals.

Based on demographic characteristics, age group of >60 years old had higher acute gastritis prevalence than other age groups because ageing reductes of mucous cells in the gastric mucosa of elderly, which is associated with a decreasing prostaglandin concentration.[26] The research finding also stated smokers had higher prevalence of acute gastritis than non-smokers, and it is in agreement with other studies.[15] Smokers have higher cases in gastritis because the gaster produce higher amounts of acid than in non-smokers. Female patients were found to have higher prevalence of chronic and atrophic gastritis, but it is statistically insignificant. Some authors support a small contribution of sex differences where there is predominance in *H. pylori* related outcomes in males, including gastric cancer.

There were several limitations in this current study, first it had a very low sample number and was only collected in one center. In addition, there was no healthy individuals that were included in the population. Therefore, interpretation warrants caution since it may not represent the whole Indonesian population. Our study did not have any information regarding *H. pylori* status which might be considered as a main factor affecting the value of ^{14}C-UBT, since urease is currently believed to mainly come from bacterial infection; therefore, the association between UBT and gastritis might be affected by *H. pylori*. This condition is needed a careful consideration.

CONCLUSION

Our study showed UBT has a sufficient potential for predicting acute and chronic antral

gastritis with a good value of sensitivity. As for other gastritis parameters, UBT showed not a good choice for predicting those stages. The UBT mainly used for determining *H. pylori* infection; therefore, the involvement of *H. pylori* infection in the development of gastritis still need to be carefully considered.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interests.

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Major Ocular Abnormalities Among Hemodialysis Patients in Indonesia

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ABSTRACT

Background: *This study aimed to identify the major ocular abnormality findings (i.e., cataract and conjunctival and corneal calcification (CCC)) among hemodialysis (HD) patients and their risk factors.* ***Methods:*** *A single institute-based cross-sectional study in Indonesia. Demographic data, medical histories, and complete ocular examinations were collected. For two major ocular abnormalities found, a generalized estimating equation was incorporated in a logistic regression model to assess the relationship with their risk factors.* ***Results:*** *We analyzed 318 eyes (159 individuals), of which 54.7% male and 45.3% female. The mean age was 51.6±11.3 years. The mean HD period was 3.5±3.2 years. Hypertension and diabetes mellitus (DM) was found in 81.1% and 34.6%, respectively. The major ocular abnormalities found were cataract (206 eyes; 64.78% (95% CI 59.53-70.03)), followed by CCC (135 eyes; 42.45% (95 % CI 37.02-47.88)). In a multivariate model, higher education (odds ratio (OR) 0.17; 95% CI 0.04-0.74), hypertension (OR 0.15; 95% CI 0.03-0.79), DM (OR 10.49; 95% CI 1.57-70.06), Systolic Blood Pressure (SBP) 120-129 mmHg (OR 0.05; 95% CI 0.003-0.69), SBP ≥140 mmHg (OR 0.05; 95% CI 0.004-0.67), Diastolic Blood Pressure (DBP) 80-89 mmHg (OR 7.44; 95% CI 1.13-48.73), and DBP ≥90 mmHg (OR 48.47; 95% CI 3.4-692.03) showed significant association with cataract. Meanwhile, there was no significant association between CCC and any predictor.* ***Conclusion:*** *Cataract and CCC were found to be the major ocular abnormalities among HD patients in this study, with DM and higher DBP as the risk factors for cataract. This finding supports recommendations for integrated regular eye screening in HD patients.*

Keywords**:** *hemodialysis, ocular abnormality, ocular manifestation, chronic kidney disease, health-system*

INTRODUCTION

It has been estimated that 2.16 million people in Asia will need renal replacement therapy (RRT) by 2030.[1] This increasing trend of the prevalence and incidence of end-stage renal disease (ESRD) in patients was seen in

a study in Indonesia a few years ago.[2] Several studies have demonstrated ocular manifestations among patients with chronic kidney disease (CKD),[3–5] and ESRD undergoing hemodialysis (HD).[6–9] The increased frequency of ocular abnormality in the HD population may perhaps be explained by genetic factors, socioeconomic disparities, difficult access to treatment, and poor diabetes and hypertension management in some individuals.[10]

Some of the most common ocular abnormalities found in CKD or HD patients include corneal and conjunctival calcification (CCC),[11] that are strongly associated with vascular calcification,[12] cataract,[3,7,11] and diabetic retinopathy, and are possibly reversible in their most curable stage if diagnosed early.[13,14] Rim et al. also showed that patients who began HD were more likely to undergo cataract surgery,[15] however the Beijing eye study demonstrated that CKD was not significantly associated with any major ocular diseases.[16] Due to the tremendous implications of any visual impairment,[6,17] morbidity,[7] or social capacity of dialytic patients, an interdisciplinary approach and eye screening are necessary to prevent further complication.[3,6,7] Since the patients may not complain of any visual problem until the vision is lost,[14] the dialysis community also needs to be aware of the high incidence rate of visual loss.[18]

In Indonesia, limited studies and data are provided related to the pattern of ocular abnormalities in HD patients, which is a vulnerable subject with high-risk ocular pathology and proves a barrier in getting access to the ophthalmology unit. This study aims to identify the major ocular abnormalities found among regular HD patients and find their risk factors. Furthermore, to highlight the importance of having a standard protocol and an integrated periodic ocular screening among HD patients, we hypothesized that the prevalence of ocular abnormalities would be relatively high and that some ocular abnormalities would be associated with certain risk factors.

METHODS

This cross-sectional study was conducted at Hemodialysis (HD) Unit, Dr. Soetomo General Academic Hospital (DSGAH), Surabaya, Indonesia, in May-June 2019. This study was supported by the Universitas Airlangga Research Grant. An ethical clearance approval was obtained from the institutional review board of DSGAH (*Komisi Etik Peneliti Kesehatan Rumah Sakit Dr. Soetomo* No.1155/KEPK/V/2019). All subjects underwent regular HD, twice per week at the HD unit. Before subject recruitment, written informed consent was obtained from all the participants. All the procedures performed in this study were in accordance with the ethical standards of the institutional research committee and the Declaration of Helsinki.

The inclusion criteria consisted of all patients who underwent regular HD in HD unit, DSGAH, and were willing to join the research, while the exclusion criteria included physical weakness during the examination, history of malignancy, and underwent renal transplantation during the study. Demographic profiles and medical histories including age, gender, education, occupation, HD period (years), etiology (hypertension, diabetes mellitus), body mass index (BMI), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were collected. Body weight, SBP, and DBP were measured before the HD session started. The complete ocular examination started with the best-corrected visual acuity (BCVA) examination by logarithmic (LogMAR) visual acuity chart, ETDRS, and was followed by intraocular pressure (IOP) measurement. The biomicroscopic examination was performed using slit-lamp biomicroscopy and fundus examination by well-trained ophthalmologists.

Study Variables

As for lens-related findings, we classified them into normal lens, immature cataract, mature cataract, and post cataract surgery. Immature cataract was defined as lens opacity associated with a mild to moderate degree of visual impairment, while mature cataract was defined as lens opacity associated with severe to blindness degree of visual impairment. Post cataract surgery findings, classified into pseudophakic and aphakic, were considered as cataract in the analysis. Conjunctival and corneal calcification (CCC) was graded according to the method described by Porter and Crombie in 1973.[19]

Age, sex, education, occupation, HD period, hypertension, diabetes mellitus, BMI, SBP, and DBP were considered as predictors. Age was classified into 4 groups (less than 40, 40-49, 50-59, and ≥60) and sex was classified into female and male. Education was classified as ≤9 years and >9 years of basic compulsory education, whereas occupation was classified as unemployed/retired or employed. HD period was classified into 4 groups (<1, 1-5, 6-10, >10 years), based on average life expectancy and the survival rate on dialysis.[20–22] Dichotomous variables were employed for hypertension and diabetes mellitus according to the history taken from patients' medical records. Body mass index (BMI) was classified into 4 groups based on WHO's classification (underweight, normal, overweight, and obese).[23] Systolic blood pressure (normal, elevated, grade 1, grade 2) and diastolic blood pressure (normal elevated, grade 1, grade 2) were classified according to American College of Cardiology/ American Heart Association Clinical Practice Guidelines, 2017.[24]

Statistical Analysis

For statistical analysis, both eyes of each patient were taken into account. Stata version 15 (StataCorp, College Station, TX, USA) was used to perform all the statistical analyses, p values <0.05 were considered statistically significant. The results were reported as a percentage for categorical variables and as mean and standard deviation (SD) with a range for the quantitative variables. For the purpose of analysis, a binary variable was employed for cataract and CCC. Pseudophakic eyes and aphakic eyes were considered as cataracts, while grading for CCC was simplified to grade 0 indicating no calcium deposits and grade 1 indicating calcium deposits found in conjunctiva only or both conjunctiva and cornea.

A generalized estimating equation model was incorporated in the logistic regression models to assess the relationship between ocular abnormalities (cataract and CCC) and their risk factors in HD patients, adjusting for the inter-eye correlation. Crude and adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated to investigate the association between the abnormalities of and predictors for each eye.

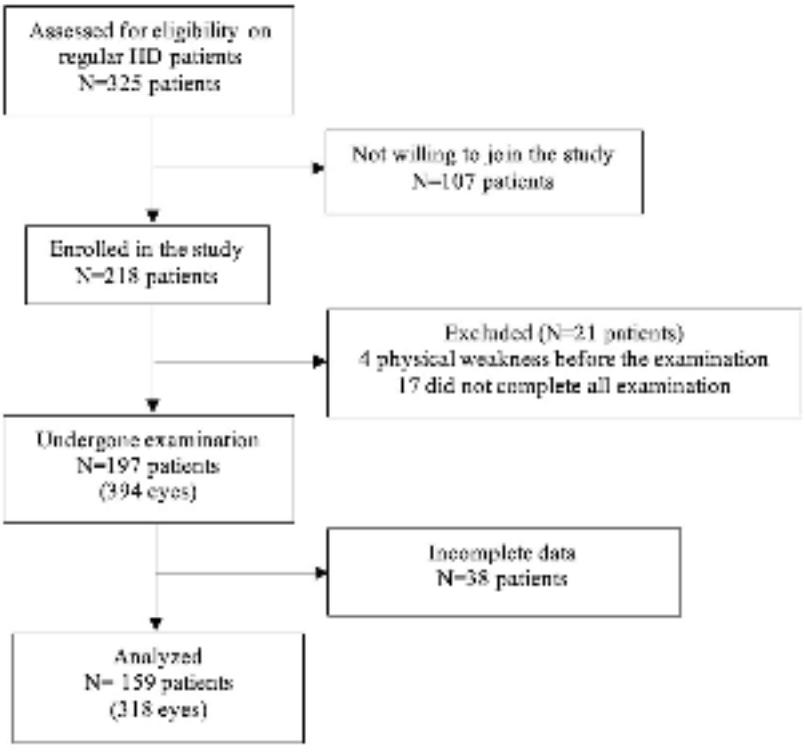

Figure 1. Flowchart of enrolment of HD patients.

RESULTS

This study registered 218 individuals. Eye examinations were performed for 197 patients (394 eyes), but 38 individuals were excluded from the analysis due to missing variables (**Figure 1**). Of 159 patients, 87 (54.7%) were male and 72 (45.3%) were female. The mean age was 51.6±11.3 (range: 21-82) years, with the most prevalent age group being 50-59 years (57 subjects; 35.9%). We found 53 (33.4%) subjects underwent ≤9 years basic compulsory education in Indonesia and the rest (66.6%) had achieved >9 years basic education. Most of the patients were unemployed or retired (89; 56%) (**Table 1**).

Table 1. Demographic Characteristics of Hemodialysis Patients (N=159)

Characteristic	Value
Age (years)	
Mean±SD	51.6 ± 11.3
Median (Range)	52 (21-82)
Age Category (years), n (%)	
<40	26 (16.3)
40-49	40 (25.2)
50-59	57 (35.9)
≥60	36 (22.6)
Sex (n,%)	
Male	87 (54.7)
Female	72 (45.3)
Basic Compulsory Education (n,%)	
≤9 years	53 (33.4)
>9 years	106 (66.6)
Occupation (n,%)	
Unemployed/retired	89 (56.0)
Employed/work	70 (44.0)

Tablel 2. Systemic Characteristic of Hemodialysis Patients (N=159)

Characteristic	Value
HD Period (years)	
Mean±SD	3.5 ± 3.2
Median (Range)	2.5 (0.08-14)
HD Period Category (n,%)	
<1 year	52 (32.7)
1-5 years	73 (45.9)
6-10 years	26 (16.4)
>10 years	8 (5.0)
Hypertension Etiology (n,%)	
Yes	129 (81.1)
No	30 (18.9)
Diabetes Mellitus Etiology (n,%)	
Yes	55 (34.6)
No	104 (65.4)
Body Mass Index (BMI)	
Mean±SD	23.1±4.2
Median (Range)	22.5 (13.9-40.8)
BMI Category (n,%)	
Underweight <18.5	17 (10.7)
Normal 18.5-22.9	72 (45.3)
Overweight 23-24.9	31 (19.5)
Obese ≥25	39 (24.5)
Systolic Blood Pressure (mmHg)	
Mean±SD	144.3±22.2
Median (Range)	140 (95-210)
Systolic Blood Pressure Category (n,%)	
Normal (<120)	17 (10.7)
Elevated (120-129)	16 (10.1)
Grade 1(130-139)	19 (11.9)
Grade 2 (≥140)	107 (67.3)
Diastolic Blood Pressure (mmHg)	
Mean±SD	82±10.5
Median (Range)	80 (45-121)
Diastolic Blood Pressure Category (n,%)	
Normal-Elevated (<80)	32 (20.1)
Grade 1 (80-89)	62 (39.0)
Grade 2 (≥90)	65 (40.9)

From the systemic characteristics of the patients, the mean HD period was found to be 3.5±3.2 (range: 0.08-14) years. As many as 73 (45.9%) patients underwent HD for 1-5 years (**Table 2**). Hypertension was found in 129 cases (81.1% (95% CI 75.05-87.21)), while DM was found in 55 cases (34.6% (95% CI 37.19-41.98)). The mean BMI was 23.1±4.2, with the most prevalent BMI (72; 45.3%) considered normal BMI. The mean SBP was 144.3±22.2 (95-210) mmHg and the mean DBP was 82±10.5 (45-121) mmHg. Most of the patients were found with SBP grade 2 (107; 67.3%) and DBP grade 2 (65; 40.9%) (**Table 2**). The two major ocular abnormalities found in this study were cataract (206 eyes; 64.78% (95% CI 59.53-70.03)), which consisted of immature cataracts (164; 51.57%), mature cataracts (15; 4.72%) and pseudophakic/aphakic eyes (27; 8.5%). CCC (135 eyes; 42.5% (95% CI 37.02-47.88)) consisted of eyes with conjunctival deposit only (74; 23.3%) and both conjunctival and corneal deposit (61; 19.2%) (**Table 3**).

Table 3. Ocular Characteristic Findings in Hemodialysis Patients (N=318 Eyes)

Characteristic	Value
BCVA (LogMAR)	
Mean±SD	0.46±0.71
Median (Range)	0.20 (0.00-3.00)
Frequencies for Conjunctival and Corneal Calcification (n,%)	
No deposits	183 (57.5)
Conjunctival deposit only	74 (23.3)
Conjunctival and corneal deposit	61 (19.2)
Frequencies for Cataract (n,%)	
No cataract	112 (35.2)
Immature cataract	164 (51.6)
Mature cataract	15 (4.7)
Pseudophakic and aphakic	27 (8.5)
Frequencies for Posteriors Segment Findings (n,%)	
Normal	110 (34.6)
Non-Proliverative Diabetic Retinopathy	28 (8.8)
Proliverative Diabetic Retinopathy	21 (6.6)
Hypertensive Retinopathy grade 0-2	73 (23)
Hypertensive Retinopathy grade 3-4	10 (3.1)
Central Retinal Vein Occlusion	2 (0.6)
Others (macular abnormality)	18 (5.7)
Difficult to evaluate (media opacity)	56 (17.6)

Table 4 shows the measure of association between cataract and predictors. In a simple logistic regression model, there was no statistically significant association between cataract and any predictor, except for diabetes mellitus. However, after adjusting for all other variables, higher education level (adjusted OR 0.17; 95% CI 0.04-0.74; P-value: 0.02), hypertension (adjusted OR 0.15; 95% CI 0.03-0.79; P-value:0.03), diabetes mellitus (adjusted OR 10.49; 95% CI 1.57-70.06; P-value:0.02), elevated blood pressure vs. normal (adjusted OR 0.05; 95% CI 0.003-0.69; P-value:0.03), SBP grade 2 vs. normal (adjusted OR 0.05; 95% CI 0.004-0.67; P-value:0.02), DBP grade 1 vs. normal (adjusted OR 7.44; 95% CI 1.13-48.73; P-value:0.04), and DBP grade 2 vs. normal (adjusted OR 48.47; 95% CI 3.4-692.03; P-value:<0.001) showed significant association with cataract. No statistically significant association between CCC and any predictor was observed (**Table 5**).

Table 4. The Measure of Association Between Cataract and Predictors

Variables	Cataract			
	Crude		*Adjusted	
	OR (95% CI)	P	OR (95% CI)	P
Age (years)				
<40	Reference			
40-49	2.03 (0.20-20.76)	0.55	1.73 (0.19-15.44)	0.62
50-59	3.50 (0.41-29.88)	0.25	2.92 (0.44-19.53)	0.27
60	1.87 (0.18-19.48)	0.60	0.54 (0.04-8.26)	0.66
Sex				
Female	Reference			
Male	0.64 (0.21-1.89)	0.42	0.89 (0.26-3.07)	0.86
Education				
9-years basic education	Reference			
Higher education	0.43 (0.14-1.28)	0.13	0.17 (0.04-0.74)	0.02
Occupation				
Unemployed	Reference			
Employed	0.86 (0.29-2.61)	0.79	0.99 (0.23-4.29)	0.99
HD Period (Years)				
<1	Reference			
1-5	4.73 (0.93-24.17)	0.06	20.19 (0.57-716.65)	0.09
6-10	2.81 (0.35-22.27)	0.33	6.41 (0.28-146.48)	0.25
>10	4.81 (0.37-62.98)	0.23	33.69 (0.34-3376.79)	0.14
Hypertension				
No	Reference			
Yes	0.35 (0.11-1.14)	0.08	0.15 (0.03-0.79)	0.03

Diabetes Mellitus				
No	Reference			
Yes	4.33 (1.37-13.68)	0.01	10.49 (1.57-70.06)	0.02
Body Mass Index (BMI)				
Underweight	Reference			
Normal (18.5-22.9)	1.32 (0.15-11.92)	0.80	1.08 (0.11-10.53)	0.95
Overweight (23-24.9)	1.10 (0.09-13.24)	0.94	1.37 (0.12-15.04)	0.79
Obese (≥ 25)	2.35 (0.26-21.49)	0.45	4.97 (0.47-52.71)	0.18
Systolic Blood Pressure (mmHg)				
Normal	Reference			
Elevated 120-129	0.31 (0.03-3.38)	0.34	0.05 (0.003-0.69)	0.03
Grade 1: 130-139	0.40 (0.06-2.85)	0.36	0.18 (0.03-1.28)	0.09
Grade 2: ≥ 140	0.38 (0.09-1.58)	0.18	0.05 (0.004-0.67)	0.02
Diastolic Blood Pressure (mmHg)				
Normal	Reference			
Grade 1: 80-89	1.32 (0.25-7.04)	0.75	7.44 (1.13-48.73)	0.04
Grade 2: ≥ 90	1.67 (0.32-8.60)	0.54	48.47 (3.39-692.03)	<0.01

*Adjusted: adjusted for all variables

Table 5. The Measure of Association Between Conjunctival and Corneal Calcification (CCC) and Predictors.

Variables	Conjunctival and Corneal Calcification (CCC)			
	Crude		*Adjusted	
	OR (95% CI)	P	OR (95% CI)	P
Age (years)				
<40	Reference			
40-49	2.02 (0.77-5.31)	0.16	1.86 (0.69-4.98)	0.22
50-59	1.31 (0.52-3.32)	0.57	1.23 (0.45-3.33)	0.68
60	0.87 (0.30-2.48)	0.79	0.79 (0.25-2.51)	0.69
Sex				
Female	Reference			
Male	1.24 (0.67-2.28)	0.49	0.99 (0.44-2.22)	0.98
Education				
9-years basic education	Reference			
Higher education	1.19 (0.62-2.29)	0.60	0.86 (0.42-1.76)	0.68
Occupation				
Unemployed	Reference			
Employed	1.65 (0.90-3.03)	0.11	1.44 (0.62-3.33)	0.39
HD Period (Years)				
<1	Reference			
1-5	1.30 (0.66-2.59)	0.45	1.27 (0.59-2.68)	0.54
6-10	0.88 (0.34-2.28)	0.80	0.75 (0.28-1.99)	0.56
>10	1.54 (0.35-6.80)	0.57	1.47 (0.29-7.26)	0.64
Hypertension				
No	Reference			
Yes	1.04 (0.47-2.29)	0.92	1.11 (0.46-2.66)	0.82
Diabetes Mellitus				
No	Reference			
Yes	0.91 (0.48-1.72)	0.77	0.91 (0.43-1.93)	0.79
Body Mass Index (BMI)				
Underweight	Reference			
Normal (18.5-22.9)	1.43 (0.48-4.26)	0.53	1.33 (0.38-4.56)	0.65
Overweight (23-24.9)	1.16 (0.34-3.92)	0.81	1.03 (0.26-3.99)	0.97
Obese (≥ 25)	1.57 (0.50-4.99)	0.44	1.53 (0.41-5.74)	0.53

Systolic Blood Pressure (mmHg)				
Normal	Reference			
Elevated 120-129	0.75 (0.19-3.01)	0.68	0.87 (0.19-4.06)	0.86
Grade 1: 130-139	1.29 (0.34-4.84)	0.71	1.66 (0.41-6.79)	0.48
Grade 2: ≥ 140	1.08 (0.38-3.03)	0.89	1.15 (0.33-3.97)	0.82
Diastolic Blood Pressure (mmHg)				
Normal	Reference			
Grade 1: 80-89	0.74 (0.32-1.73)	0.49	0.70 (0.25-1.93)	0.49
Grade 2: ≥ 90	0.86 (0.37-1.99)	0.72	0.82 (0.27-2.45)	0.72

*Adjusted: adjusted for all variables

DISCUSSION

Cataract and CCC were found to be the major ocular abnormalities in HD patients in this study. Among all predictors, diabetes and DBP showed to be the major contributors to cataract. However, none of the predictors showed a significant relationship with CCC. The odds ratios in the DBP categories showed a dose-response relationship in which DBP grade 1 was 7.4 times more likely to have the odds of having cataract; higher DBP (≥90 mmHg) showed 48.5 times greater odds of having cataract as compared to normal DBP (<80 mmHg). Further, DM was found to be associated with 10.5 times higher odds of having cataract as compared to subjects with no DM. Higher education level (>9 years of basic compulsory education) showed a protective effect against cataract in this study. Despite its significance, we assume that the association between cataract and higher education level in this study could be due to a chance finding and not necessarily causal relationship.

Furthermore , hypertension and SBP showed unexpected results that were protective against cataract. This may be due to the selection bias and blood pressure error measurement based on single measurement. On the other hand, age, gender, occupation, HD period, and BMI showed no significant relationship with cataract. This may indicate that unlike the general population, renal impairment itself along with certain related treatments increase the risk of cataract and corneoscleral calcification.[25,26] Moreover, chronic kidney disease is also an independent risk factor.[27] Further, the relationship between BMI and the risk of cataract is controversial across observational studies.[28] HD period and BMI in this study demonstrated no significant relationship with cataract or CCC, which may be due to different etiology and levels of severity of the disease, different levels of phosphate calcium, and varying impact of the treatment on each patient. The non-significant association between CCC and any predictor needs to be explored further.

The high prevalence of ocular abnormalities among HD patients based on several studies [3,29,30] has been revealed in this study. The prevalence of cataract in HD patients varies between 11.1%-61.4%.[4,6,7,11,29] Increased cataracts in CKD patients may represent common risk factors, including age, smoking, high blood pressure, diabetes, dyslipidemia, and obesity.[27] Wang et al., found the increase of prevalence of cataract in CKD patients after controlling for age, hypertension, and diabetes mellitus in a large cross-sectional population-based study in Taiwan.[31] The prevalence of CCC in HD patients among cross-sectional studies varies between 1.4%-32.2%.[4,6,7] Moreover, it is possible for individuals with severe kidney disease and ESRD to develop metastatic calcification on the eyelid margins, conjunctival tissue, and cornea.[27] Kianersi et al. found that the duration of dialysis has a significant association with conjunctival calcification but not with corneal calcification or cataract.[7] While a longitudinal study by Hsiao et al. demonstrated that HD period was associated with the degree of calcification.[12]

Hypertension, followed by diabetes mellitus, was the most prevalent of HD etiologies in this study, which showed that there has been an epidemiological transition in the etiology of ESRD in Indonesia, where glomerulonephritis

was the most prevalent etiology, approximately 10 years ago.[2] Based on previous studies, the most prevalent renal disorders with ocular consequences are hypertension and diabetes.[7,25,26,29] The presence of diabetes and high blood pressure were found to be associated with a four-fold increase in cataract.[32] Furthermore, another previous study showed that SBP but not DBP or hypertension were associated with the occurrence of cataract.[33] This finding was inconsistent with our findings in that both SBP and DBP showed significant association with cataract. However, the error measurement of blood pressure may have impacted the association.

Cataract is a multifactorial disease. There are several risk factors for cataracts, including diabetes mellitus,[34–36] hypertension,[37] systemic corticosteroid usage, smoking as well as UV radiation exposure.[36] The risk of cataract formation is significantly enhanced in a population with hypertension,[37] due to the elevation of inflammatory cytokines detected in hypertension patients.[38,39] Due to a wide range of outcomes in different research, it is unclear if CKD contributes to the development or progression of cataract.[25] Imbalances of the fluid and electrolytes equilibrium,[8] underlying comorbidities in individuals with renal failure,[8,40] and the treatment being administered such as steroid,[26] may enhance the risk of ocular problems in patients. Possible explanations for the development of cataract in HD patients include accumulation of toxic metabolites and calcium deposits in the lens,[11,27] oxidative stress, inflammation and vitamin D deficiency.[27] On the other hand, the pathogenesis for ocular calcifications is not entirely known. Some studies explained its association to secondary hyperparathyroidism,[11] higher levels of serum Ca-P,[6,12,40] serum copper, cystatin, intact parathyroid hormone, and vitamin D.[40]

Patients on HD are vulnerable subjects with high-risk ocular abnormalities, yet face difficulties in getting access to eye treatment and services. This study was conducted in a HD unit and the patients were able to decide the best timing for their eye screening according to their convenience, before or after the HD session. The majority of these patients found this setting helpful, even though most of them do not acknowledge the necessity of an eye care examination and do not consider eye screening as a top priority for HD patients. The results of this study emphasize that regular eye examination is needed for early diagnosis, evaluating the existence and prevention of HD-related eye manifestations. Further, to obtain better data to support integrated health services to conduct a thorough eye examination in HD unit and to provide clinical guidelines for standard ophthalmology screening among HD patients.

There are several limitations to this study. First, we could not assume the causal relationship between the outcome and any predictor due to the cross-sectional nature of the study. Second, the patients had ocular examinations at different times (before or after HD session), depending on their willingness and the strength of their body to undergo thorough ocular examination. This may have impacted the result of some eye measurements despite that some studies found no significant influence between pre- and post-HD sessions. Third, cataract was determined when opacification was evident and was not graded in detail, as only immature and mature cataract were obtained. Finally, certain data from electronic medical records were unavailable. Ideally, to corroborate the findings, a longitudinal study with other centers is needed to assess the incidence and risk factors of ocular diseases in the HD population. Further, performance knowledge and awareness studies toward ocular abnormalities among HD patients will be beneficial.

CONCLUSION

High prevalence of ocular abnormalities among HD patients were revealed in this study. Cataract and CCC were found to be the major ocular abnormalities in HD patients and are potentially reversible if addressed early. Hence, integrated health services involving ophthalmology and HD unit should be pursued. In addition, awareness among HD patients regarding potential ocular abnormalities related to renal disease, especially with diabetes mellitus, hypertension, and poor metabolic

control, should be raised. Furthermore, the importance of detailed eye screening despite all limitations among HD patients should be emphasized to prevent further complications and perform necessary treatment before the occurrence of visual loss.

FUNDING

This study was supported by Universitas Airlangga Research Grant (Grant SK Rektor Unair No. 1408/UN3/2019).

ACKNOWLEDGMENTS

The authors would like to thank DiORSS (Diabetic Ocular Renal Surabaya Study) team, Vitreo-retinal Division-Dept. of Ophthalmology Faculty of Medicine Universitas Airlangga/ RSUD. Dr. Soetomo, dr. Togar E. S, Sp.M, dr. Denisa Rosati, dr. Made Satya, dr. Amelia RK, dr. Ifan R. Lukmana, SpM and Ophthalmology Instrument's Suppliers in Surabaya : PT Pancaraya Krisnamandiri (Autoref keratometer type accuref k-90030, Shinnippon); PT Berjaya Mandiri (Houvitaz NCT, HNT-7000 non-contact tonometer); PT Triastri Meditama (slit-lamp biomicroscopy vision 66 type); PT Topsindo Megah Utama (3D OCT, DRI-OCT Triton, Topcon Incorporation), for their contributions to this study.

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Identification of The Immune Subtype Among Muscle-invasive Bladder Cancer Patients by Multiple Datasets

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ABSTRACT

Background: *Immunotherapies including PD-1/PD-L1 antibodies have been approved for the treatment of Muscle-invasive Bladder Cancer (MIBC) patients. However, immunotherapies could only be beneficial for about 20% MIBC patients. Thus, identification of the immune subtype is becoming increasingly important. This study aimed to explore the immune subtype by analyzing the gene expression profiles.* ***Methods****: A total of 6 datasets including (GSE13507, GSE31684, GSE32548, GSE32894, GSE69795, and TCGA-BLCA) were downloaded. The gene expression profiles from different datasets were combined since the batch effects were removed. We performed unsupervised clustering analysis to identify the immune subtype by the combined gene expression profiles. The tumor-infiltration levels of 22 immune cells, immune scores, and tumor purity were calculated, and the survival analysis was performed to investigate the prognosis difference between immune subtypes. The enriched pathways for each immune subtype were obtained.* ***Results:*** *We identified four novel immune subtypes (referred to S1, S2, S3, and S4) among MIBC patients. We found that S1 was enriched in immune scores had the best prognosis. In contrast, S3 was poor in immune scores and had the worst prognosis. Subtype S1, S2, S3, and S4 were enriched in immune-related pathways, extracellular matrix-related pathways, metabolism-related pathways, and cancer-related pathways, respectively.* ***Conclusion:*** *The current study suggests that the immune subtypes based on gene expression profiles could contribute to select the appropriate MIBC patient for immunotherapies.*

Keywords: *Molecular subtype, Immunotherapy, MIBC, Immunotype, TMB, Bioinformatics.*

INTRODUCTION

Bladder cancer (BC) is the most common genitourinary cancer of the urinary tract.[1,2]. A quarter of BLCA patients have muscle-invasive bladder cancer (MIBC), which has a higher risk of metastasis, or cancer cells migrating to regional pelvic lymph nodes and/or visceral regions, making the disease incurable.[3] Muscle-invasive bladder cancer (NMIBC) and non-muscle-invasive bladder cancer (NIBC) are the two kinds of BC (MIBC). Around a quarter of BC patients will develop MIBC, and more than half of MIBC patients will experience relapse and metastasis.[4] Radial cystectomy (RC) plus neoadjuvant cisplatin-based chemotherapy (NAC) is the standard first-line multimodal treatment for MIBC patients, however roughly 60% of MIBC patients do not exhibit a significant therapeutic response.[5] Furthermore, because of its toxicity, many people are unable or unwilling to accept cisplatin treatment.[6] The five-year survival rate for MIBC patients is as low as 50%.[7] There is also an urgent need for new treatment drugs. Immunotherapies, particularly immune checkpoint blockade (PD-1/PD-L1), have recently been licensed, improving the prognosis of MIBC patients significantly.[8] The practical use of immunotherapy, however, may be limited because only 20% of MIBC patients respond to treatment.[9] Tumor-infiltrating T cells.[10] PD-L1/PD-1 levels,[11] highly microsatellite instability (MSI-H),[12] tumor mutational burden (TMB),[13] and intestinal microbiota.[14] have all been found to be good indicators of immunotherapy efficacy. These potential markers were frequently unstable because numerous genes and pathways were involved in tumor immune evasion.[15] In the Checkmate025 research, for example, responses to Nivolumab (PD-1 antibody) exhibited no correlation with PD-L1 level, and patients with a high level of PD-L1 had a worse prognosis.[15] As a result, immunological subtypes established by clustering samples based on big genes from many datasets could be a good predictor of immunotherapy success.

According to multiple research,[16] patients with high tumor PD-L1 levels had better treatment response rates and lived longer. TIL density, especially CD8+ T cells, is a strong positive prognostic indicator, and immunotherapy works in part by reactivating a preexisting tumor immune response.[17] TMB stands for the amount of somatic mutations per million bases,[18] and tumor cells with a high TMB are more likely to generate neoantigens, which can be identified by T cells and trigger an anti-tumor response.[19] In 22 different tumor types, attempts to identify PD-1 antibody responders by combining TMB and tumor-infiltrating T cells have recently been published.[20] Apart from these biomarkers, other studies have advocated molecular subtype as a distinct technique for identifying immunotherapy candidates.[21–23] Based on RNA expression profiling, individuals with MIBC can be categorized into luminal and basal subtypes, with the basal subtype being more connected with the epithelial-mesenchymal transition (EMT), immune-related pathways, and worse prognosis than the luminal subtype.[24–26] However, more study is needed to confirm the role of molecular subtypes in predicting the therapeutic response of MIBC patients to immunotherapy.

In the age of precision immunotherapy, it's crucial to create an immunotype model that can predict immunotherapy response rates and identify mediators that are key determinants. Models and biomarkers could be utilized to influence immunotherapy response, adapt cancer treatment, cut costs, and avoid immune-related side effects.

In the current study, 683 samples from six separate cohorts were used to generate immunological subgroups. S1 was shown to have the best prognosis of the four immunological subtypes studied. Subtypes S1, S2, S3, and S4 were all enriched in immune-related, extracellular matrix-related, metabolism-related, and cancer-related pathways. Overall, our findings may aid researchers in better understanding the diversity of MIBC patients and identifying those who will benefit from immunotherapy.

METHODS

The expression matrix and clinical information of 6 bladder cancer datasets including GSE13507 (62 MIBC and 103 NMIBC samples),[27] GSE31684 (66 MIBC and 27 NMIBC

samples),[28] GSE32548 (38 MIBC samples and 93 NMIBC samples),[29] GSE32894 (93 MIBC and 215 NMIBC samples),[30] GSE69795 (20 MIBC samples and 18 NMIBC samples),[31] and TCGA-BLCA (404 MIBC and 4 NMIBC samples)[32] were downloaded. By using the SVA software[33] on the information from NMIBC and MIBC, these 6 datasets were merged into a single dataset, and batch effects were removed. Batch effects in datasets were detected using principal component analysis (PCA).

Identification of Immune Subtypes

The gene list and 736 immune-related genes were obtained from the Gene Expression Omnibus (GEO) under the entry 'GPL25507'. The 'ConsensusClusterPlus' program[34] used MIBC expression profiles of immune-related genes to identify the immunological subtype. The K-means technique was used to produce consensus clustering with 1,000 re-samplings.

Survival Analysis and Calculation of Immune Cell Proportions

To estimate survival distributions for each subtype, the overall survival data from these six datasets were merged, and Kaplan–Meier survival curves were displayed. Using the survival package in R, we did a log-rank test to see if differences between immune subtypes were significant. The CIBERSORT algorithm was used with 1000 permutations to compute immune cell proportions (such as B cells, dendritic cells, macrophages, neutrophils, NK cells, CD4+ T cells, and CD8+ T cells) against each sample. Using the estimate package, the ESTIMATE method[36] was used to determine immune scores, stromal scores, and tumor purity, and the Kruskal–Wallis test was chosen to compare the differences.

Functional Enrichment Analysis of Immune Subtypes

Subtype-specific pathways were discovered for each subtype by comparing samples from that subtype to the remaining samples using the GSEA approach. False discovery rate (FDR) 0.05 was used as the limit for subtype-specific pathways. The 'fGSEA' program was used to analyze differentially expressed genes among diffuse glioma subtypes using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.

RESULTS

Removing the Batch Effects among Datasets

The "sva" program was used to normalize and remove batch effects from six datasets: GSE13507, GSE31684, GSE32548, GSE32894, GSE69795, and TCGA-BLCA. Before the batch effect was abolished, MIBC samples were mixed with NMIBC samples, and samples from other datasets were clearly segregated (**Figures 1A-B**). On the other hand, the PCA plot demonstrated that MIBC samples were segregated from NMIBC samples, and samples from different datasets were mixed (**Figures 1C-D**). The batch effects in six datasets were removed as a result of these findings. After batch effects were removed, the "sva" software produced the combined expression profiles of these six datasets. MIBC samples from the integrated expression profiles (a total of 683 samples) were kept for further study.

Identification of the MIBC Immune Subtypes

MIBC immune subtypes were identified using an expression matrix of 736 immune-related genes derived from merged expression data. To identify the distinct subtypes (K = 2, 3, 4, 5, and 6) among 683 MIBC samples, we used the 'ConsensusClusterPlus' program. Based on the CDF curves and Delta plots, the optimal division (k = 4) was chosen as the optimal number of clusters (**Figure 2A-B**). The heatmap's boundary remained pretty clear-cut at K = 4 (**Figure 2C**), indicating that the sample cluster was stable and robust. **Table 1** summarizes the distribution of immune subtypes among datasets.

We discovered substantial prognostic differences among the identified immunological subtypes using the previously described classification (log-rank test, p= 0.012, **Figure 2D**). Subtype 1 (S1) patients had a longer median survival time (67.3 months) than subtype 2 (S2) patients (35.9 months), subtype 3 (S3) patients (30.9 months), and subtype 4 (S4) patients (median survival: 30.9 months) (median survival: 16.9 months). Overall, we discovered four MIBC immunological subgroups that were linked to

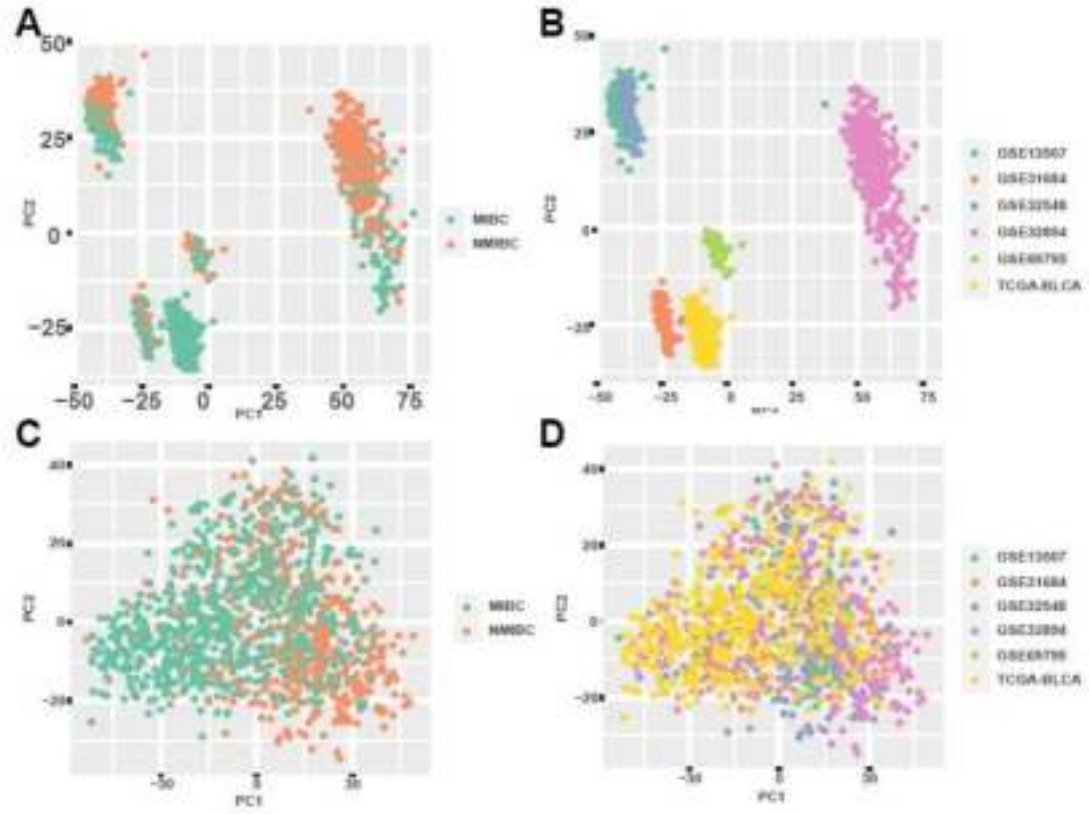


Figure 1. The normalization and batch effect removal from six datasets. (**a**) PCA plot illustrated the cluster of the samples by NMIBC/MIBC before batch effect removal. (**b**) PCA plot illustrated the cluster of the samples by datasets before batch effect removal. (**c**) PCA plot illustrated the cluster of the samples by NMIBC/MIBC after batch effect removal. (**d**) PCA plot illustrated the cluster of the samples by datasets after batch effect removal.

clinical outcomes based on gene expression profiles.

Correlation of MIBC Immune Subtypes with Tumor-infiltrating Immune Cells

The CIBERSORT technique was used to calculate tumor-infiltrating immune cells, and it revealed variances in immune cells among MIBC immune subtypes (**Figure 3**). (1) CD8 T cells, M1 macrophages, M2 macrophages, Monocytes, and Memory CD4 T cells were all greater in S1 samples. (2) In naive B cells and M0 macrophages, S2 samples were greater. (3) Resting NK cells, naive T cells, and Eosinophils were all greater in S3 samples. (4) In resting dendritic cells, active Mast cells, and neutrophils, S4 samples were greater.

Correlation of MIBC Immune Subtypes with Immune Scores and Molecular Subtypes

The immune subtypes' immunological scores, stromal scores, and tumor purity were calculated using the ESTIMATE technique. Immune and stromal scores were found to be

Table 1. The distribution of immune subtypes among datasets.

Subtype / Dataset	S1 N=149	S2 N=198	S3 N=195	S4 N=141
GSE13507	3 (2.01%)	20 (10.1%)	31 (15.9%)	8 (5.67%)
GSE31684	12 (8.05%)	18 (9.09%)	24 (12.3%)	12 (8.51%)
GSE32548	6 (4.03%)	13 (6.57%)	14 (7.18%)	5 (3.55%)
GSE32894	28 (18.8%)	21 (10.6%)	15 (7.69%)	29 (20.6%)
GSE69795	0 (0.00%)	8 (4.04%)	9 (4.62%)	3 (2.13%)
TCGA-BLCA	100 (67.1%)	118 (59.6%)	102 (52.3%)	84 (59.6%)

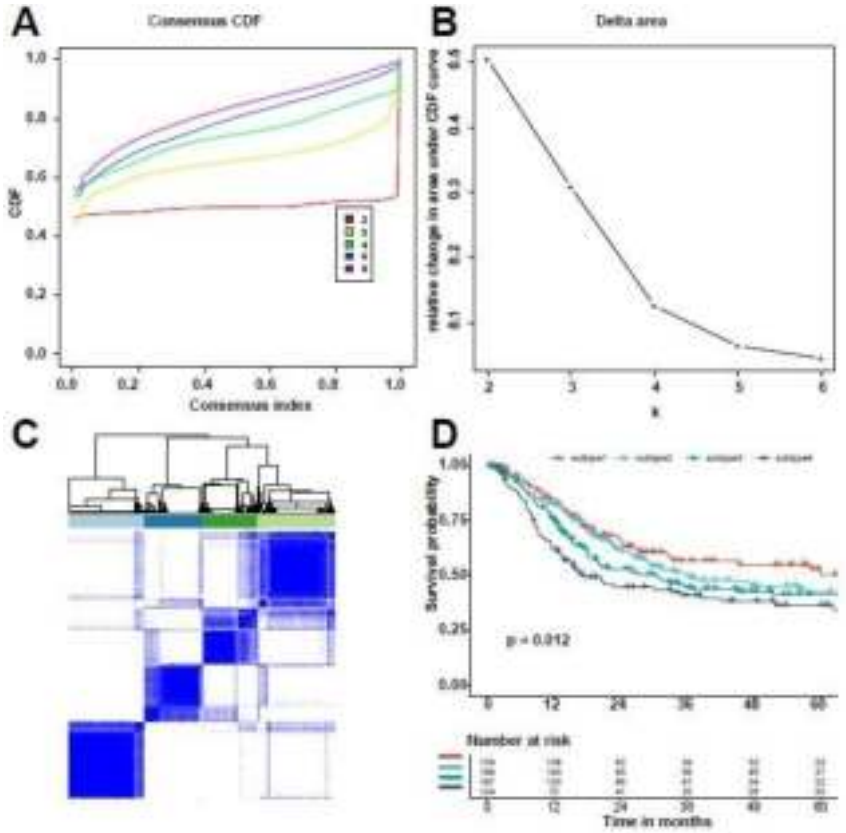


Figure 2. Identification of MIBC immune subtypes. (**a**) The cumulative distribution function (CDF) curves in consensus cluster analysis. (**b**) delta area plots in in consensus cluster analysis. Consensus scores for different subtype numbers (k = 2 to 6) are presented. (**c**) The heatmap illustrating the consensus matrix at k = 4. (**d**) Survival analysis of MIBC immune subtypes. The log-rank test was conducted to determine the significance of the differences.

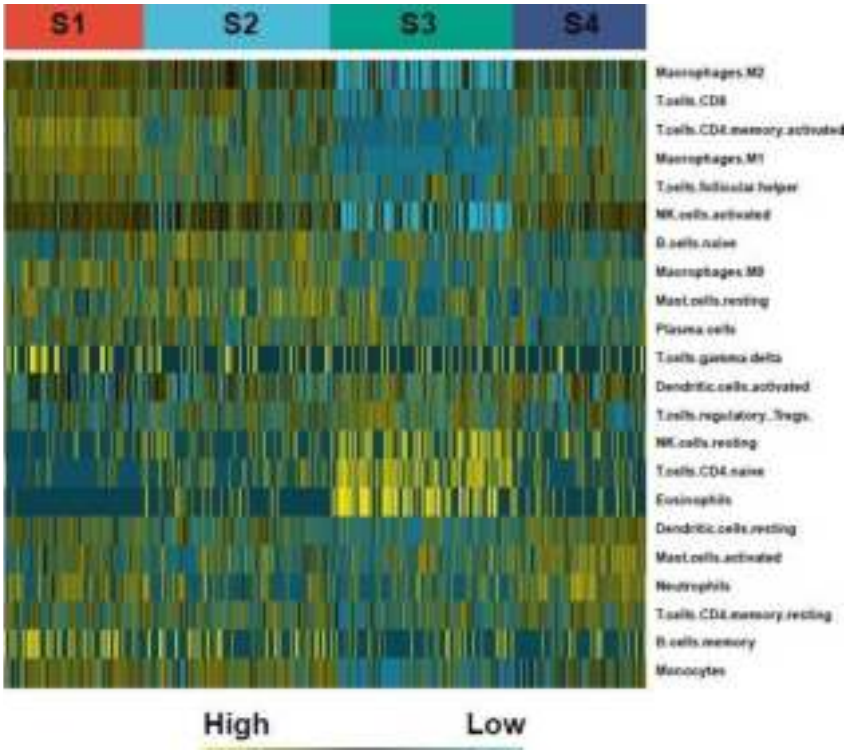


Figure 3. Immune characteristics of four MIBC immune subtypes. The heatmap showing the abundance of immune-cell populations calculated by CEBERSORT.

highest in S1, and lowest in S4 (immune scores: S1 > S4 > S2 > S3; stromal scores: S1 > S2 > S4 > S3). However, these immunological subtypes' tumor purity was in reverse order: (S3 > S4 > S2 > S1) (**Figure 4A-C**). S1 (Basal, N:80, P:82 percent ; Luminal, N:17, P:18 percent) and S4 (Basal, N:70, P:83 percent ; Luminal, N:14, P:17 percent) had different distributions of Basal and Luminal subtypes than S2 (Basal, N:43, P:36 percent ; Luminal, N:75, P:64 percent) and S3 (Basal (**Figure 4D**). It's worth noting that the Basal and Luminal subtype information was only accessible in the MINC samples from the TCGA-BLCA dataset.

Subtype-Specific Signaling Pathways among Immune Subtypes

GSEA analysis were used to uncover signaling pathways unique to the immunological subtypes observed (**Figures 5A, B**). Immune-related pathways including Cytokine-cytokine Receptor Interaction and Antigen Processing and Presentation were found to be overrepresented in subtype S1. Subtype S2 was shown to be particularly rich in extracellular matrix-related pathways such as Cell Adhesion Molecules (CAMs) and Vascular Smooth Muscle Contraction. Subtypes S3 and S4 were found to be associated with metabolism-related pathways (Metabolism of Xenobiotics by Cytochrome P450, Linoleic Acid Metabolism, and Fatty Acid Metabolism) and cancer-related pathways (Pathways in Cancer and Cell Cycle). Overall, we were effective in identifying immunological subtype characteristic signaling pathways.

DISCUSSION

There are two major molecular subgroups among MIBC patients, namely the Basal and Luminal subtypes, according to studies.[37,38] Because it is associated with a more aggressive

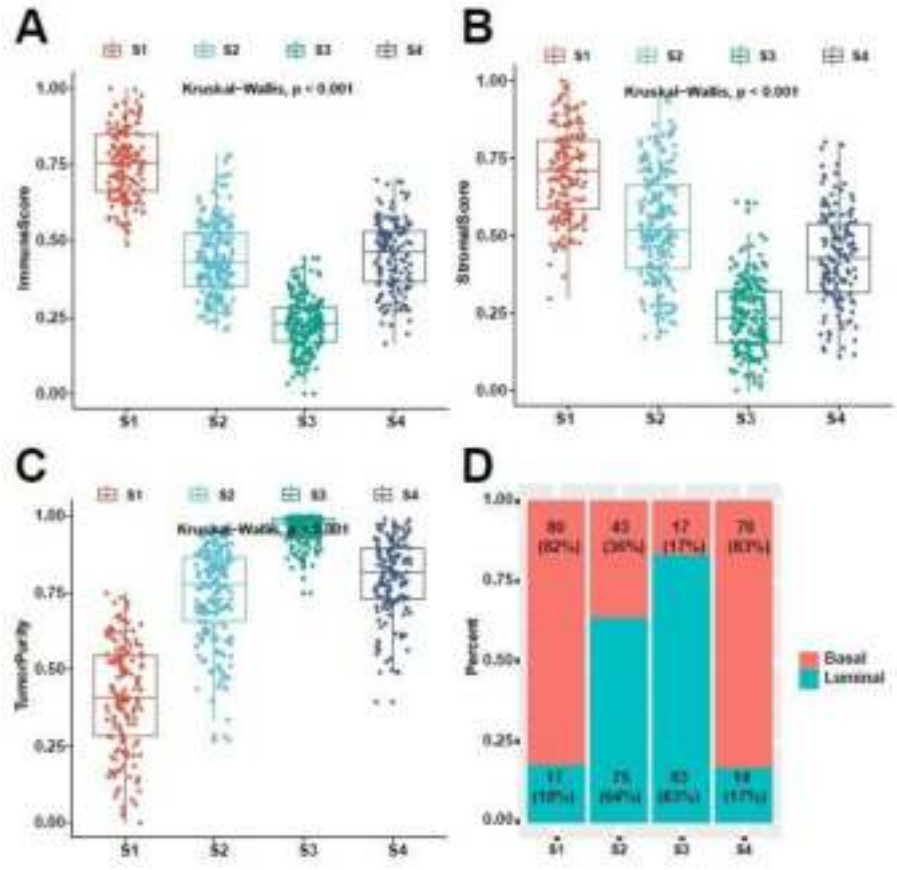
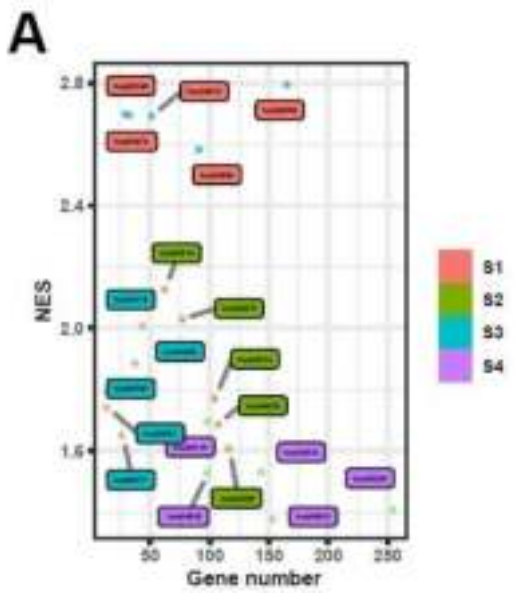


Figure 4. The correlation of stromal scores, immune scores, tumor purity, and molecular subtypes with the identified immune subtype. (**a-c**) Evaluation of stromal scores, immune scores, and tumor purity for the four immune subtypes by Kruskal-wallis test. (**d**) The distribution of molecular subtypes (Basal and Luminal subtype) in the four immune subtypes.



B

ID	Pathway
hsa04060	CYTOKINE-CYTOKINE RECEPTOR INTERACTION
hsa05340	PRIMARY IMMUNODEFICIENCY
hsa04672	INTESTINAL IMMUNE NETWORK FOR IGA PRODUCTION
hsa04612	ANTIGEN PROCESSING AND PRESENTATION
hsa04650	NATURAL KILLER CELL MEDIATED CYTOTOXICITY
hsa04006	NEUROACTIVE LIGAND RECEPTOR INTERACTION
hsa04020	CALCIUM SIGNALING PATHWAY
hsa04514	CELL ADHESION MOLECULES CAMS
hsa04270	VASCULAR SMOOTH MUSCLE CONTRACTION
hsa05414	DILATED CARDIOMYOPATHY
hsa03010	RNA DEGRADATION
hsa00980	METABOLISM OF XENOBIOTICS BY CYTOCHROME P450
hsa00040	PENTOSE AND GLUCURONATE INTERCONVERSIONS
hsa00591	LINOLEIC ACID METABOLISM
hsa00071	FATTY ACID METABOLISM
hsa05200	PATHWAYS IN CANCER
hsa04810	REGULATION OF ACTIN CYTOSKELETON
hsa04510	FOCAL ADHESION
hsa04110	CELL CYCLE
hsa04630	JAK STAT SIGNALING PATHWAY

Figure 5. Bubble plots for 5 enriched KEGG pathways with the lowest p.value in each immune subtype. (**a**) The plot of KEGG pathways. (**b**) The annotation of KEGG pathways.

phenotype and a higher risk of distant metastasis than the Luminal subtype, the Basal subtype has gotten a lot of attention.[38] Although significant progress has been made in the MIBC molecular subtype, more study into the MIBC immunological subtype is required. The identification of immunological subtypes is becoming increasingly important since it may aid in the selection of suitable candidates for immunotherapies.

TMB, which is independent of PD-L1 expression, is a powerful predictor of tumor behavior and immunotherapy response in patients with small-cell lung cancer.[39] On the other hand, TMB criteria for predicting response in a variety of different malignancies aren't well established.[40] Apart from limited correlation research, the mechanism by which TMB predicts immunotherapy sensitivity is mainly unknown.[34] Furthermore, molecular subtypes may provide additional information for predicting immunotherapy response. The basal and luminal subtypes are derived from separate progenitor cells, according to various studies, and the basal subtype has a higher ORR in immunotherapy treatment.[09,41,42] Immunotype A patients exhibited the best ORR and had the most immunological checkpoints, TMB, and CD8+ T cells, indicating that immunotherapy was highly recommended for them. It's because immunotype A corresponds to previously identified "hot tumors".[43] Patients with Immunotype B exhibited a lower ORR, a lower level of immunological checkpoints and CD8+ T cells, and a moderate number of TMB. More research is needed to establish if this tendency is analogous to "cold tumors," which are characterized by insufficient T cell priming (low tumor mutational load, poor antigen presentation, and intrinsic T cell death insensitivity).[43–45] To increase T cell responses and turn cold tumors into "hot tumors," treatment techniques include cancer stem cell (CSC) vaccination or adoptive T cell transfer.[43, 46] Immunotype C patients, on the other hand, had the lowest ORR. They had strong immunological checkpoints, intermediate CD8+ T cells, and low TMB, implying that immunotherapy may not be suited for this patient population. TTN, TP53, KMT2D, MUC16, ARID1A, KDM6A, and SYNE1) were identified as cancer risk genes after they were found to be changed often among three immunotypes. Seven more genes are as important: PIK3CA, RB1, FGFR3, KMT2C, MACF1, RYR2, and EP300. Three immunotypes have varied mutation rates for these genes, allowing for a more thorough and comprehensive understanding of MIBC immunotype mutation rates. Individual genes

in a co-expression network are less stable than modules because the overall function of a module can be maintained when individual gene expression can be replaced by other genes with similar redundant functions [02]. Network analysis revealed eight hub genes for the MIBC immunotype-related module (ACTA2, ACTA1, COL1A1, COL1A2, COL5A1, DCN, SPARC, VIM). The disease stage-related hub gene involvement of COL1A1, COL1A2, and COL5A1 was previously discovered by another group,[47] which is compatible with our findings. Multiple datasets should be used to find the robust immune subtype among MIBC patients. When merging disparate datasets, the batch effect will be a key stumbling block for researchers. Fortunately, 'sva' package[33] has been shown the ability to remove the batch effect in studies.[48,49] According to the PCA results, the batch effect was successfully removed. Because we analyzed 683 samples from six separate cohorts, the four immunological subgroups we discovered may be more robust than a single dataset. Among the four immune subtypes, S1 received the highest immunological and stromal evaluations, whereas S3 had the lowest. The ESTIMATE approach did not produce the same findings as the CIBERSORT approach. S1 has a lot of CD8 T cells, M1 macrophages, M2 macrophages, Monocytes, and Memory CD4 T cells. As a result, S1 patients should receive immunotherapy, but S3 patients should not. Based on the distribution of immunological scores and molecular subtypes in these four immune subtypes, we could determine that 1) S1 was the Basal subtype with more immune cells and S4 was the Basal subtype with fewer immune cells. 2) Tumor cells were lower and higher in the Luminal subtypes S2 and S3, respectively.

CONCLUSION

Finally, the findings of this study improved immunological subtype research in MIBC samples by identifying four immune subtypes with varying immunological scores. Immune subsets revealed may aid doctors in deciding on treatment for MIBC patients. These findings will pave the way for new immunotherapy approaches in the future.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

ACKNOWLEDGMENTS

The work was carried out within the framework of research at the Institute of Immunology and Physiology, Ural Branch of the Russian Academy of Sciences, project number AAAA-A21-121012090091-6.

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The Association of Kidney Function Monitoring Adherence and Estimated Glomerular Filtration Rate Changes Among Patients At-Risk for Chronic Kidney Disease

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ABSTRACT

Background: *Kidney Disease: Improving Global Outcome in 2012 has provided recommendations to prevent CKD progression by monitoring kidney function periodically according to the CKD stage and the clinician's adherence to these guidelines is important. This is the first study on the relationship between adherence to monitoring renal function and changes in estimated glomerular filtration rate (eGFR) in patients at risk for CKD in Indonesia.* ***Methods:*** *This study was a comparative observational study with a cross-sectional approach. Research subjects were electronic medical record data from the Hasan Sadikin General Hospital information system (SIRS) data collected with the SQL Server Report Builder and "HCLAB" applications on patients at risk for CKD at the Hasan Sadikin General Hospital's Outpatient Clinic from January 2018 to March 2020. The patients' data were taken by the total sampling technique and then processed with the Chi-Square test.* ***Results:*** *From 376 subjects, the results showed that poor adherence in renal function monitoring would increase the risk of decreasing eGFR by 1.51 times compared to good monitoring adherence (PR 1.51 95% CI (1.172 - 1.935); p-value 0.007). The eGFR changes were significant (p-value 0.002) with mean 10.84 ml/min/1.73m^2 (95% CI: 4.17 – 17.50).* ***Conclusion:*** *The study demonstrated that poor renal function monitoring adherence had an association with a decrease in eGFR in a group of patients at risk for CKD.*

Keywords: *Frequent monitoring, CKD Progression, eGFR.*

INTRODUCTION

Chronic kidney disease (CKD) presents a global public health problem with an increasing prevalence and incidence, poor prognosis, and high cost. Based on the Global Burden of Disease report, CKD is the 27th leading cause of death globally and increased to the 12th in 2017. CKD treatment ranks as the fourth most expensive cost of the National Health Insurance after heart disease in Indonesia.[1-3]

Early detection of CKD and frequent monitoring of kidney function in a patient with diseases at risk of CKD complications are vital. Early detection means assessing renal function based on laboratory tests when the underlying disease is diagnosed for the first time, usually asymptomatic. At the same time, frequent monitoring is scheduled to assess the progress

of the complications which is CKD. Those are essential because the first onset of CKD is difficult to assess. Therefore, concurrent management of both underlying disease and complications (CKD) is an important step to reduce the risk of cardiovascular disease, progression of kidney disease, and death.[4]

In 2012, *Kidney Disease: Improving Global Outcome* recommends frequent monitoring of renal function for patients at risk according to CKD stage.[2] This is following The National Institute for Health and Care Excellence (NICE) guideline that stated early detection of CKD should be implemented for patients with diabetes, hypertension, history of acute renal impairment, cardiovascular disease, renal structural abnormalities, multisystem diseases (such as systemic lupus erythematosus), and patients with nephrotoxic drugs (such as lithium, cyclosporin, and NSAIDs).[4-6]

On the contrary, some guidelines based on expert opinions such as the United States Preventive Service Task Force (USPSTF) and the American College of Physicians (ACP) do not recommend assessing renal function in asymptomatic patients. To date, there are no randomized clinical trial (RCT) study that examines the role of early detection of kidney injury with patient's clinical outcome. Both USPSTF and ACP stated that early detection of kidney damage has potential adverse effects, including discomfort during blood collection, psychological effects related to CKD stigma, drug side effects from treatment with an uncertain diagnosis, and financial impacts. [7, 8] There is no data on the clinician's adherence to monitor the population at risk of CKD in Indonesia. Since there is a discrepancy in the recommendation to monitor the population of risk of CKD, this study aims to determine the association between monitoring adherence and changes in estimated glomerular filtration (eGFR) in the population at risk.

METHODS

This study was a comparative observational study with a cross-sectional approach. We retrospectively analyzed outcomes of at-risk CKD patients who underwent early detection and monitoring between March 2018 and March 2020, at one tertiary-care outpatient clinic government hospital.

Ethics

The institutional ethics committee of Hasan Sadikin Hospital approved the ethical clearance for this study (LB.02.01/X.6.5/001/2021). Patients' data from medical records were de-identified and analyzed anonymously.

Inclusion and Exclusion Criteria

We retrospectively extracted and examined patient data from the Hasan Sadikin General Hospital information system (SIRS). Data were collected with the SQL Server Report Builder and "HCLAB" applications on patients at risk for CKD at the Hasan Sadikin General Hospital's Outpatient Clinic from January 2018 to March 2020.

We chose patients at risk for CKD covering congestive heart failure patients in the cardiology clinic, hypertension patients in the nephrology clinic, spondyloarthropathy patients who routinely used non-steroidal anti-inflammatory drugs, systemic lupus erythematosus (SLE) patients in the rheumatology clinic, diabetes patients in the endocrinology clinic, and cancer patients who underwent platinum-based chemotherapy in the oncology clinic. Information on age, sex, CKD risk factors, baseline eGFR, and proteinuria were recorded. Demographic data were collected at the time of study enrollment. We then recorded eGFR at the first encounter with a doctor in our clinic (early detection) and 1 year later (monitoring) to see the changes and counted the number of creatinine examination that was performed within one year to assess the adherence to KDIGO 2012 monitoring recommendation. We used CKD classification based on GFR category and albuminuria category according to KDIGO 2012 (**Table 1**).

The inclusion criteria required at-risk patient aged >18 years who had creatinine results at the first encounter with the doctor in our clinic and one year later. We excluded patients who had previously undergone hemodialysis and patients with the possibility of acute kidney injuries such as infection and acute heart failure.

Table 1. Recommended eGFR Monitoring Frequency for At-Risk Patients Based on KDIGO 2012

		Albuminuria Category		
		A1 (<30 mg/g)	**A2 (30–300 mg/g)**	**A3 (>300 mg/g)**
eGFR category (ml/min/1.73m2)	G1 ³90	1 time/year	1 time/year	2 times/year
	G2 60-89	1 time/year	1 time/year	2 times/year
	G3a 45-59	1 time/year	2 times/year	3 times/year
	G3b 30-44	2 times/year	3 times/year	3 times/year
	G4 15-29	3 times/year	3 times/year	³4 times/year
	G5 <15	4 times/year	³4 times/year	4 times/year

The outcome of the study was CKD progression, shown by changes in estimated glomerular filtration rate (eGFR).

Statistical Analysis

The doctor's adherence to monitoring eGFR was categorized into adherent and non-adherent groups. Baseline characteristics were described across these groups. Estimated GFR changes were categorized into normal and decreased. The comparison of eGFR between patients in the adherent and the non-adherent group was performed using dependent t-test or Wilcoxon Signed Rank test, alternatively. Bivariate analysis between monitoring adherence and eGFR changes was performed using the Chi-square test and reported as prevalence risk (PR) with its 95% confidence interval. Statistical significance was set at ≤0.05 with a two-tailed hypothesis. Statistical analyses were performed with Statistical Product and Service Solution (SPSS) version 22.0 for Windows.

RESULTS

There were 522 subjects with underlying diseases having a risk for CKD. A total of 376 subjects met the inclusion criteria. The remaining were excluded from the study due to incomplete data. (**Figure 1**)

Baseline Characteristics

Patients in the adherent group had a risk factor of SLE (21.9%), hypertension (20.7%), and DM type 2 (20.1%). In the non-adherent group, most patients had a risk factor of congestive heart failure (34.9%) and nasopharyngeal carcinoma (23.3%). The subjects' characteristics based on adherence monitoring are shown in **Table 2**.

Overall, 64.1% of subjects were not tested

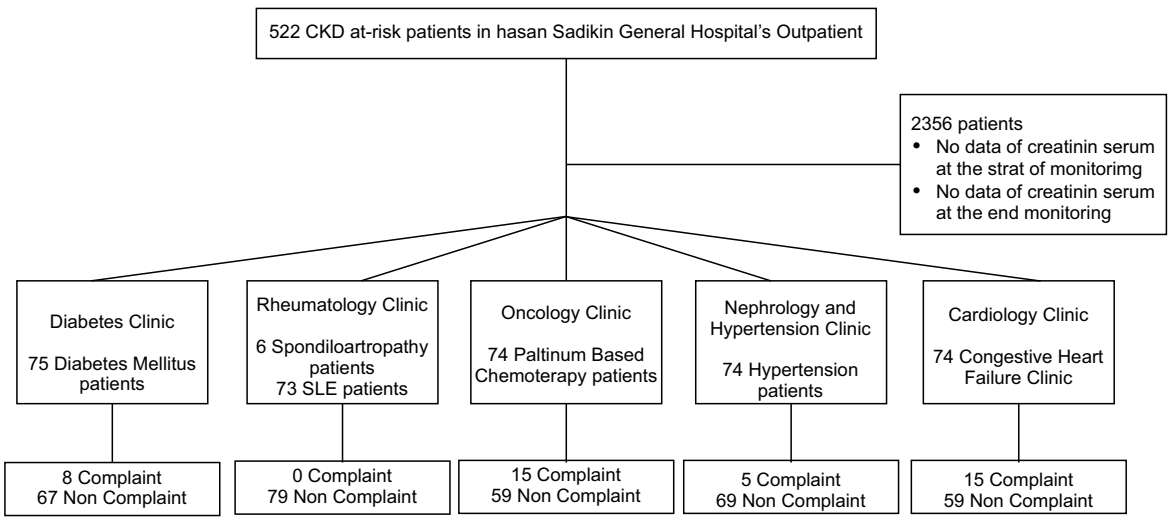

Figure 1. Study Sample Selection

Tabel 2. Baseline Characteristics

Baseline Characteristics	Total n=376	Monitoring Adherence		p value
		Adherent n=333	Non-adherent n=43	
Creatinine Monitoring Frequency per year g	5 (1 – 17)	5 (1 – 17)	1 (1 – 2)	<0.001[a]*
Proteinuria Monitoring Frequency per year [g]	1 (0 – 16)	1 (0 – 16)	0 (0 – 3)	<0.001[a]*
Age (years)[g]	61 (18 – 86)	62 (18 – 86)	58 (18 – 84)	0.184[a]
Sex, n (%)				
Male	146 (38.8)	127 (38.1)	19 (44.2)	0.444[b]
Female	230 (61.2)	206 (61.9)	24 (55.8)	
Risk Factor, n (%)				
Diabetes Mellitus	75 (19.9)	67 (20.1)	8 (18.6)	**<0.001[b]***
Congestive Heart Failure	74 (19.7)	59 (17.7)	15 (34.9)	
Cervical Cancer	13 (3.5)	11 (3.3)	2 (4.7)	
Bladder Cancer	15 (4)	13 (3.9)	2 (4.7)	
Lung Cancer	13 (3.5)	12 (3.6)	1 (2.3)	
Nasopharyngeal Cancer	33 (8.8)	23 (6.9)	10 (23.3)	
Hypertension	74 (19.7)	69 (20.7)	5 (11.6)	
Spondyloarthropaties	6 (1.6)	6 (1.8)	0 (0)	
Systemic Lupus Erythematous	73 (19.4)	73 (21.9)	0 (0)	
Baseline Proteinuria, n (%)				
Negative	101 (26.9)	101 (30.3)	0 (0)	**<0.001[b]***
1+	12 (3.2)	12 (3.6)	0 (0)	
2+	11 (2.9)	11 (3.3)	0 (0)	
3+	5 (1.3)	5 (1.5)	0 (0)	
4+	6 (1.6)	5 (1.5)	1 (2.3)	
Not Examined	241 (64.1)	199 (59.8)	42 (97.7)	
Albuminuria Stages, n (%)				
A1	101 (26.9)	101 (30.3)	0 (0)	**<0.001[b]***
A2	12 (3.2)	12 (3.6)	0 (0)	
A3	263 (69.9)	220 (66.1)	43 (100)	
Stadium				
G1	118 (31.4)	94 (28.2)	24 (55.8)	
G2	136 (36.2)	125 (37.5)	11 (25.6)	
G3a	69 (18.4)	63 (18.9)	6 (14.0)	
G3b	38 (10.1)	37 (11.1)	1 (2.3)	
G4	12 (3.1)	11 (3.3)	1 (2.3)	
G5	3 (0.8)	3 (0.9)	0 (0.0)	

[g]Median (Min-Max), [a]Mann Whitney, [b]Chi Square, *significant p<0,05

for proteinuria at their first admission. At the start of monitoring, most of the patients had stage G2 (36.2%), followed by stage G1 (31.4%), G3a (18.4%), G3b (10.1%), G4 (3.1%), and G5 (0.8%). In the non-adherent group, most patients had stage G1 (55.8%) followed by G2 (25.6%), G3a (14.0%), G3b (2.3%), G4 (2.3%) and G5 (0%) while in the adherent group, most patients were at stage G2 (37.5%), G1 (28.2%), G3a (18.9%), G3b (11.1%), G4 (3.3%) and G5 (0.9%). The median (range) of follow-up in the adherent group was 5 (1-17) times per year, while in the non-adherent group was 1 (1-2) times per year.

The adherent group had a mean ± SD eGFR of 72.02±27.04 ml/min/1.73m^2 at the start and 72.84±29.32 ml/min/1.73m^2 at the end of monitoring. The eGFR changes was not significant (p>0.05) with mean 0.08 ml/min/1.73m^2 (95% CI 1.54 to 1.70ml/min/1.73m^2 (**Figure 2**). The association between monitoring adherence with renal function and eGFR changes is shown in **Table 3**. Non-adherent monitoring had a higher decreased eGFR (65.1%) than the adherent group (43.2%). Non-adherent monitoring significantly decreased the eGFR than the adherent group (p<0.05).

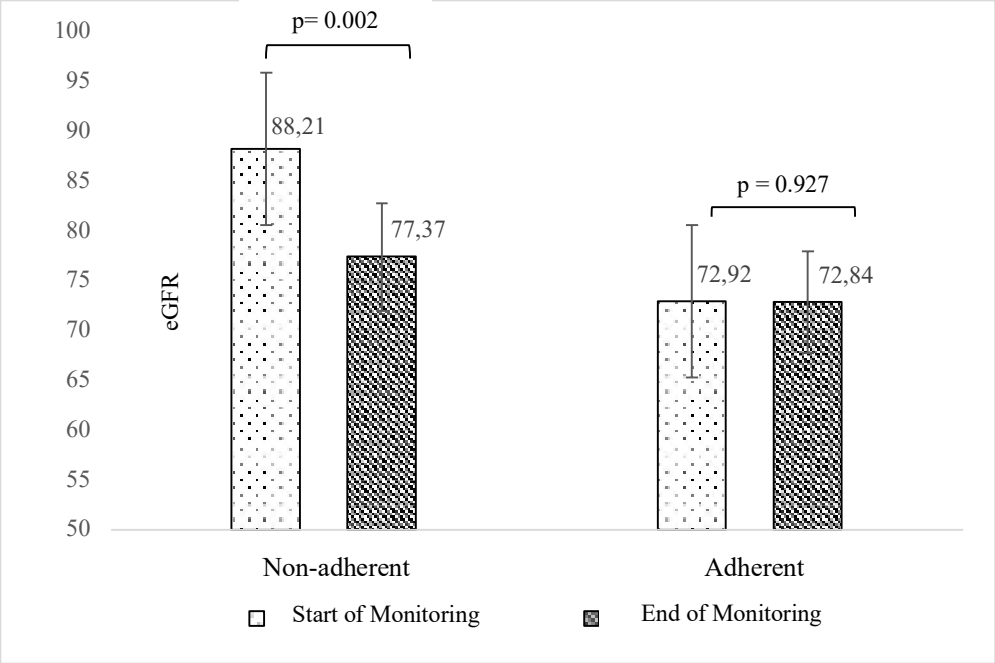

Figure 2. Comparison of renal function in one year monitoring

Table 3. Association of monitoring adherence and estimated glomerular filtration rate changes

Monitoring Adherence	n	eGFR changes		p-Value*)	PR (CI 95%)
		Decrease	Constant/Increase		
Non-Adherent	43	28 (65.1)	15 (34.9)	0.007	1.51 (1.17 – 1.93)
Adherent	333	144 (43.2)	189 (56.8)		

*) Chi-square; PR (prevalence ratio) (CI 95%)

DISCUSSION

This study was a comparative observational study with a cross-sectional method that identifies the association between monitoring adherence with renal function and eGFR changes. To the authors' best knowledge, this is the first investigation conducted in Indonesia. The International Society of Nephrology and The International Federations of Kidney Foundations, on World Kidney Day 2020, has the theme "Kidney health for everyone everywhere from prevention to detection and equitable access to care". The theme emphasizes that CKD and progression to end-stage renal disease (ESRD) can be prevented with proper access to early detection (primary prevention), frequent monitoring (secondary prevention), and simultaneous management (tertiary prevention). [9]

Kidney disease has an enormous economic burden. High-income countries allocate more than 2-3% of the annual health care budget to treat kidney failure. Based on the United States Renal Data System Report in 2019, all CKD patients require an increase in the need for care as the disease progresses, especially if the patient has reached end-stage renal disease requiring renal replacement therapy. [10,11] Our study showed that at the start of monitoring, most patients were at stage G2 (36.2%), followed by stage G1 (31.4%), G3a (18.4%), G3b (10.1%), G4 (3.1%), and G5 (0.8%). This is in accordance with USRDS 2019 data and the meta-analysis conducted by Hill et al. in 2016 that found CKD stages 1-3 are more common than stages 4-5. Therefore, the management of CKD should focus on preventing progression, not on kidney replacement.[11,12]

Many countries have implemented national policies and strategies for non-communicable diseases. However, specific policies directed at education and awareness of kidney disease with early detection, frequent monitoring,

management, and treatment of CKD are still inadequate. Until now, the management of patients with kidney disease is still not optimal. Many patients were presented with kidney failure when first referred to a nephrologist.[13] Lack of knowledge about CKD prevention is reflected in the number of proteinuria assessments as the first screening test. Our study showed that only 35.9% of patients had proteinuria examination at first admission at the outpatient clinic. The NKF-KDOQI, NICE 2008, KDIGO 2012, and CARI 2013 guidelines recommend proteinuria as one of the basics for early detection and monitoring of CKD progression. Proteinuria serves as the most common etiologic marker of CKD (DM, hypertension, and glomerular disease) and in kidney transplant recipients.[4, 14]

In our study, the non-adherent monitoring increased the risk of decreased eGFR compared to adherent monitoring with a prevalence ratio of 1.51 (95% CI 1.172 to 1.935, p=0.007). These results have very significant clinical implications. A study conducted by Matsushita et al. in 2009 found that the group that had a higher decrease in eGFR per year, had an increased incidence of acute coronary syndrome events and mortality. Our study also strengthens the KDIGO 2012 guideline recommendations stating that more frequent monitoring of renal function is needed as renal injury progress. The KDIGO 2012 recommendations were based on one of the main studies, namely the Prevention of Renal and Vascular End-stage Disease (PREVEND). The PREVEND study found that the rate of decrease in eGFR in the proteinuria and hypertension group was faster than in the other groups, indicating the importance of eGFR and proteinuria assessment to determine the number of monitoring frequencies. In addition, our study shows an association between monitoring adherence with renal function and CKD progression. Therefore, our results strengthen the confidence of KDIGO 2012 recommendations serving the basis for determining the minimum amount of renal function monitoring in at-risk patients.

Our study provides epidemiological evidence to reduce the level of trust in other guidelines such as USPSTF 2012 and ACP 2013. These guidelines do not recommend frequent monitoring of the CKD population, especially in asymptomatic patients stages 1 – 3. These recommendations are made only based on expert opinion because no studies have assessed the accuracy, precision, specificity, or sensitivity of frequent monitoring to detect eGFR changes. Both USPSTF and ACP hesitate that the benefit of early detection and frequent monitoring is greater than the harm of adverse event. [7, 8] Our study has shown that frequent monitoring is essential to reduce CKD progression.

However, some limitations should be noted. Most of the patients (64.1%) had no baseline proteinuria data. Baseline proteinuria data in the adherent group reached 59.8%, while in the non-adherent groups, almost all patients were not assessed (97.7%). This follows a study conducted by Plantinga et al. in 2010 revealing awareness of damage detection and frequent monitoring of kidney function both at the patient and doctor level is very low.[15,16] To overcome this limitation, we determined the A3 grade if proteinuria was not checked with the worst assumption so that it could describe the milder A1 or A2 condition.

This study did not include the variables of management changes made, whether appropriate or not since the data were retrospectively taken. There is the potential for selection bias. This bias mainly lies in temporal ambiguity. We cannot conclude that exposure is a risk factor for a particular disease. This may be because one patient may have more than one risk factor. Therefore, we assessed risk factors based on data from the main polyclinic where their underlying disease was controlled. Further research is needed with more accurate information regarding the timing and occurrence of the underlying disease and sensitivity analysis is required.

CONCLUSION

Renal function monitoring adherence is associated with changes in eGFR in a group of patients at risk for CKD. Patients with poor adherence monitoring were likely to develop decreased eGFR by 1.51 times compared to the adherent monitoring group.

ACKNOWLEDGMENTS

We thank Evan Susandi, S. Si, M. Stat for the useful suggestions on statistic matters.

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Identification and Validation of Entrustable Professional Activities in Indonesian Internal Medicine Residency Programs

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ABSTRACT

***Background*:** *Entrustable professional activities (EPAs) are tasks entrusted to students who assist supervisors in determining their competencies. However, the competencies required and the end-educational stage in which each EPA item is assigned have yet to be determined by the stakeholders of internal medicine residency programs in Indonesia. This study aimed to identify and determine the activities in internal medicine residency programs which could be defined as EPAs in the competency-based curriculum of Indonesian internal medicine residency programs.* ***Methods*:** *A literature review was conducted to identify activities which could be examined as EPA items in Indonesian internal medicine residency programs, which were then validated by 10 educational experts. Two rounds of the Delphi method were conducted with participants consisting of the Indonesian Board of Internal Medicine professionals, residency program directors, internal medicine specialists, and internal medicine residents to evaluate the importance of the identified EPA items. The EPA items were rated on a Likert scale ranging from 1 to 5, and their variances were analyzed. The participants also rated the end-educational stage appropriate for each EPA item. The effect size was calculated between groups as (1) small, <0.3; (2) moderate, approximately 0.5; and (3) large, >0.8.* ***Results*:** *The literature review identified 29 modified items from the Royal College of Physicians and Surgeons (RCPS) and three items from other academically developed EPA designs. The expert discussion resulted in the validation of 28 EPA items (out of the 32 items in the initial EPA draft). All 28 items were accepted after two rounds of the Delphi method, and a decrease in their variances was found.* ***Conclusion*:** *This study formulated 28 EPA items for Indonesian internal medicine residency programs. Further collaboration between the Board of Internal Medicine and residency program directors will be needed for the application of these EPA items at each residency year.*

***Keywords*:** *entrustable professional activities; internal medicine; residency program.*

INTRODUCTION

An entrustable professional activity (EPA) is defined as a clinical practice task which can be entrusted to medical residents in incremental stages.[1–3] These stages include the observational stage, the direct supervision stage, the indirect supervision stage, and the minimal supervision stage. Some medical residents will go further, achieving the capability of supervising other students when they have shown the ability to maintain the required competence.[1–3] Since competency assessments do not always predict performance, the assessment of students should focus on their abilities to perform professional tasks in the workplace. The assessment of medical residents using EPAs supports the goal of high-quality internal medicine medical education without supervision.

An ideal EPA, according to Cate et al., includes several attributes[1]: (1) It is required in professional clinical activities; (2) requires sufficient knowledge, abilities, and attitudes obtained through learning as a prerequisite of completion; (3) results in professional actions which are useful in daily practice; (4) indicates the quality of the professional when achieved; (5) can be implemented independently; (6) can be completed within a certain timeframe; (7) can be observed and rated; and (8) represents one or more competencies.[3–6] EPAs are essential in competency-based medical education curricula as they help supervisors determine student competencies and ensure that high-quality patient-centered care would be provided by the students.

The development of EPAs in internal medicine residency programs has already been carried out in several studies conducted by the Alliance for Academic Internal Medicine (AAIM), the European Board of Internal Medicine (EBIM), the Royal College of Physicians and Surgeons (RCPS), and the University of California, San Francisco (UCSF). However, there were differences in the total number and descriptions of the EPAs in each study.[3–6] Therefore, the identification and adoption of EPAs relevant to the Indonesian internal medicine curriculum and the importance of the established EPAs should be determined by stakeholders in internal medicine residency programs in Indonesia. Hence, this study aimed to identify several activities as EPAs in the competency-based educational curriculum in Indonesian internal medicine residency programs.

METHODS

We conducted a literature review to identify relevant clinical activities to be considered as EPAs for Indonesian internal medicine residency programs. The authors of this paper originally used the Indonesian language to identify and validate EPAs based on a questionnaire by Taylor and Hauer.[3,5] The translation of the chosen EPA texts into the Indonesian language was conducted by a certified and sworn translator, and the texts were then translated back into the English language by a postgraduate student in medical education with English language experience (**Supplementary Table S1**). The results of the translation into Indonesian and then back into English were revalidated by a panel of experts in medical education.

Additionally, a round of expert discussion was conducted to validate the EPAs according to the 10 attributes described by Cate et al. using the questionnaire developed by Taylor et al.[1,3] Finally, two rounds of an online Delphi method were conducted to evaluate the importance of each activity as an EPA and the end-educational stage at which each EPA could be issued.

Literature Review

A comprehensive literature review was carried out by the authors on the EPA items designed by AAIM, EBIM, RCPS, and UCSF.[3–6] The EPA design from RCPS was established as the primary reference, as it included junior student supervision in various clinical units. Then, a search for duplications in the EPA items designed by AAIM, EBIM, and UCSF was carried out for each EPA item designed by RCPS. EPAs designed by AAIM, EBIM and UCSF which had not yet been included in EPA items designed by RCPS were added as new EPA items.

Expert Discussion

Ten educational experts were recruited for this study to discuss the resultant EPA draft written by the authors. The selection of experts

was based on their professional backgrounds, which were internal medicine residency program coordinators, former internal medicine residency program coordinators, former managers of education, and former medical science Bachelor's program coordinators. The expert discussion was conducted online using 14 questions adapted from Taylor et al. to assess the relevance of selected EPA points with EPA attributes outlined by Cate et al.[1,3] The 14 questions were divided into three sections: EPAs as work units, EPAs as professional tasks, and EPAs as curriculum items. The experts were then asked to provide a score on a Likert scale ranging from 1 to 5, and they were also asked to provide comments on each EPA item.

The mean score for each EPA item was calculated by the sum of scores from each question divided by 14. EPA items that were accepted in the EPAs design were those with a mean score of 4.07 or more.[3] EPA items with mean scores less than 4.07 were excluded or revised. The consensus on EPA items was determined if 80% or more of the experts had accepted the EPA items and if less than 20% of the experts had suggested revisions. EPAs were excluded if 50% or more of the experts had rejected the EPA.

Delphi Method Round 1

In this round, four groups of participants (i.e., Indonesian Board of Internal Medicine professionals, residency program directors, internal medicine specialists, and internal medicine residents) provided their evaluations on the degree of importance of each activity using a Likert scale ranging from 1 to 5 (1 = not at all important, 2 = very insignificant, 3 = rather important, 4 = important, and 5 = very important) and chose the educational stage at which an EPA item could be assigned. The results of Delphi round 1 were analyzed by calculating the content validity indices (CVIs), namely, the number of participants who gave a score of 4 or 5 on the questionnaire divided by the number of total participants.

Delphi Method Round 2

In Delphi round 2, a reevaluation of the Likert scale ratings, the educational stages for each EPA item, and CVI were carried out. Participants provided evaluations using the same questionnaire and received feedback and individual answers from the previous Delphi round. Activities designated as EPAs for an internal medicine residency program were activities with a CVI values of ≥80%. The results were then analyzed by calculating the CVI.

Statistical Analysis

The difference in the mean ranks/scores of the two rounds was analyzed using a *t*-test, while the mean differences of more than two groups were analyzed using a one-way ANOVA test. The effect size (size/size of effect) between the two groups was calculated by dividing the difference in the mean rank/score of the two groups (i.e., pooled standard deviation divided by two). The effect size criteria included: (a) small, <0.3; (b) moderate, approximately 0.5; and (c) large, approximal and >0.8. The effect size between the three groups was evaluated by the partial eta-squared generated in the one-way ANOVA analysis. The difference in variance between Delphi rounds 1 and 2 was analyzed by a *t*-test to evaluate the statistical significance of the difference in the means of the two variances in one paired group.

Ethics

The Faculty of Medicine, Universitas Indonesia Institutional Review Board approved the study with approval number: KET.203/UN2.F1/ETIK/PPM.00.02/2020.

RESULTS

Literature Review

The literature review identified 29 EPA items from RCPS, 30 EPA items from UCSF, 16 EPA items from AAIM, and 40 EPA items from EBIM.[3–6] The EPA items from RCPS were selected for the aforementioned reasons. Next, modification of the EPAs designed by RCPS was carried out based on the similarity in content and meaning of the EPA items from UCSF, AAIM, and EBIM. For example, EPA 1 from RCPS was similar to EPA 5 from UCSF, as they were both concerned with taking patient histories and physical examinations.

The EPA modification process resulted in 29 EPA items modified from RCPS which had been reworded according to the relevant literature, and we added three EPA items from other EPA designs which had not yet been included in the EPA design from RCPS. The total number of EPA items identified at this stage was 32 (**Figure 1**). A list of the EPA items can be seen in the Supplementary Materials.

Expert Discussion

After the literature review had been completed, the resultant EPA draft was further verified through an expert discussion process. Seven EPA items from the EPA draft were 100% accepted by experts. Meanwhile, five EPA items were accepted by 90% of the experts, and eight EPA items were accepted by 80% of the experts **(Supplementary Table S2),** resulting in 20 EPA items accepted by 80% or more of the experts and 12 EPA items accepted by less than 80% of the experts. Finally, we decided to include EPA items which were accepted by 70% and 60% of the experts in the EPA design, as some of the experts had commented that the EPA was eligible to be accepted **(Supplementary Tables 3 and 4**). Meanwhile, the EPA items accepted by 50% of the experts were excluded, and none of the experts' comments for these EPAs indicated them eligible to be accepted. Thus, the expert discussion process was able to validate 28 EPAs **(Figure 1)**.

Delphi Method Round 1

The Delphi round 1 participants included 11 out of the 13 invited Indonesian Board of Internal Medicine professionals, 16 out of the 29 residency program directors, 14 out of the 26 internal medicine specialists, and 13 out of the 14 residents in internal medicine residency education programs.

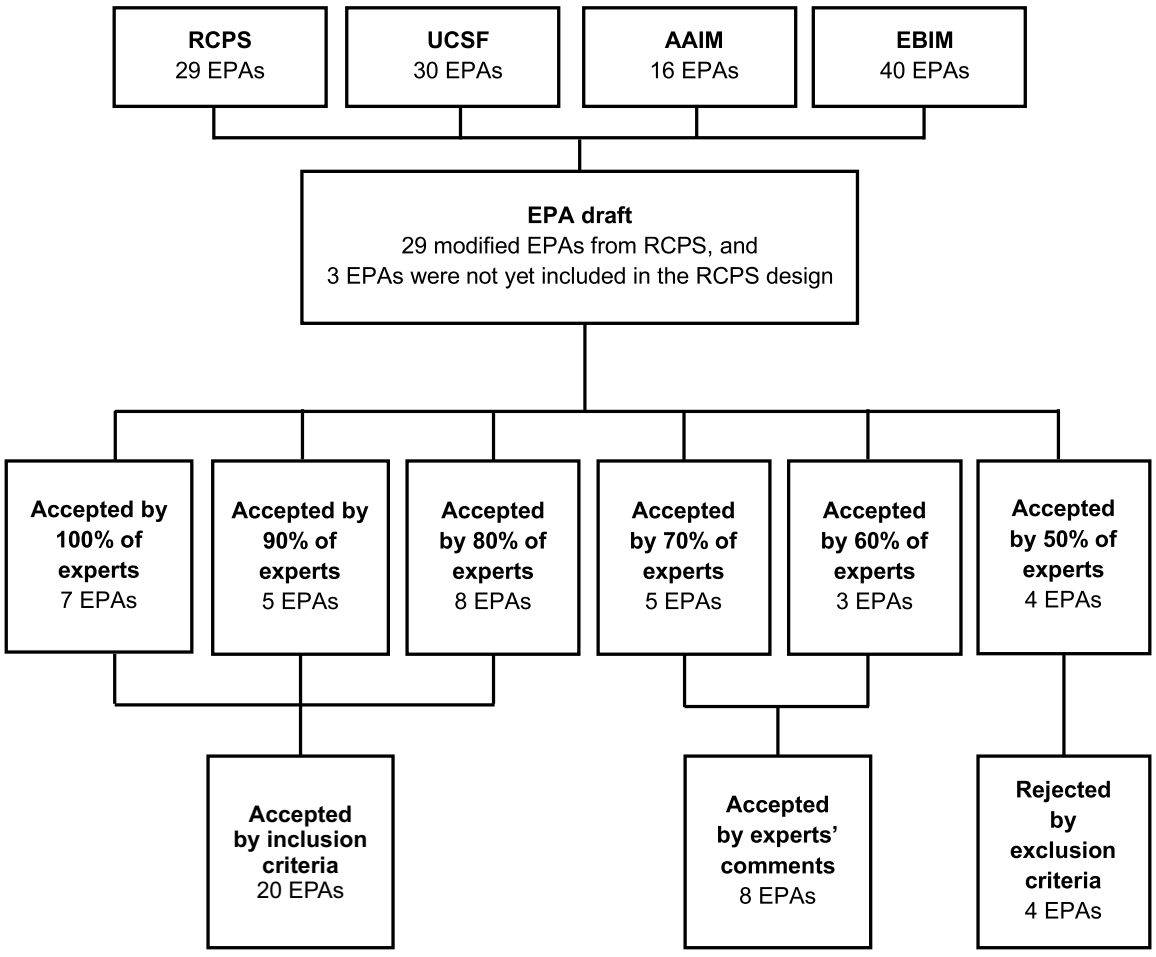

Figure 1. Results obtained from the literature review and expert discussion method.

Twenty-six EPA items were acceptable to the experts (CVI ≥80%). Only two EPA items received received CVIs of <80% from all of the participants, namely, EPA 26 (CVI 72.4%) and EPA 27 (70.4%). None of the EPA items received a 100% CVI. EPA items which received the highest CVI (98.2%) were EPA items 1, 3, 4, 5, 12, 13,14, and 24.

Delphi Method Round 2

The Delphi round 2 participants included eight out of the 11 invited Indonesian Board of Internal Medicine professionals, eight out of the 16 residency program directors, nine out of the 14 internal medicine specialists, and nine out of the 13 residents in internal medicine residency education programs. In this round, 28 EPA items were accepted by all participants (CVI ≥80%). There was an increase in the CVI of EPA item 26 (72.2% to 97.1%) and 27 (70.4% to 100%). Twelve EPA items were accepted with CVIs of 100%, namely, EPA items 1, 2, 3, 4, 8, 11, 13, 14, 22, 23, 27, and 28. The two EPA items with the lowest CVIs (94.1%) were EPA items 9 and 24 (**Table 1**).

Table 1. Validity results and effect sizes for each EPA item in Delphi round 2.

Entrustable Professional Activities (EPA) Item	Content Validity Indices %	Effect Size					
		(A) vs. (B)	(A) vs. (C)	(A) vs. (D)	(B) vs. (C)	(B) vs. (D)	(C) vs. (D)
EPA 1: Performing histories and physical examinations and documenting and presenting findings across clinical settings for initial and subsequent care	100%	0.71	1.33	1.01	0.49	0.24	0.24
EPA 2: Identifying and assessing unstable patients, providing initial management, and obtaining assistance	100%	0.71	1.33	0.67	0.49	0.04	0.53
EPA 3: Performing the basic procedures of internal medicine	100%	0.25	0.17	0.35	0.08	0.62	0.53
EPA 4: Assessing the degree of severity, diagnosing, and providing initial management for patients with common acute medical presentations in acute care settings	100%	0.31	0.06	0.35	0.22	0.04	0.29
EPA 5: Managing patients admitted to acute care settings with common medical problems and advancing their care plans	97.1%	0.25	0.13	0.07	0.39	0.14	0.18
EPA 6: Consulting with specialists and other health professionals, synthesizing recommendations, integrating these into the care plan, and referring when appropriate to other specialty care	97.1%	0.48	0.35	0.29	0.13	0.11	0.00
EPA 7: Formulating, communicating, and implementing discharge plans for patients with common medical conditions from acute care settings	97.1%	0.76	0.13	0.29	0.62	0.33	0.18
EPA 8: Assessing unstable patients and providing targeted treatments and consulting, as needed; providing emergency multidisciplinary care to medical inpatients	100%	0.00	0.13	0.62	0.13	0.62	0.77
EPA 9: Discussing and establishing patient goals of care with family and other health providers	94.1%	0.48	0.07	0.48	0.48	0.07	0.47
EPA 10: Identifying personal learning needs while caring for patient needs and accessing medical information to provide evidence-based care to address those needs in developing the practice of life-long learning	97.1%	0.25	0.62	0.14	0.35	0.07	0.36

EPA 11: Assessing, diagnosing, and managing patients with complex or atypical acute medical presentations, with complex medical conditions and/or with comorbidities	100%	0.00	0.08	0.57	0.08	0.57	0.67
EPA 12: Assessing and managing patients with complex chronic conditions that require other specialists or subspecialty care	97.1%	0.48	0.57	0.29	0.08	0.11	0.18
EPA 13: Providing internal medicine consultations to other clinical and perioperative services	100%	1.08	0.39	0.62	0.62	0.39	0.21
EPA 14: Assessing emergency and participating or leading in resuscitating and managing unstable and critically ill patients	100%	0.50	0.57	0.57	0.06	0.06	0.00
EPA 15: Performing the procedures of internal medicine	97.1%	0.61	0.08	0.32	0.69	0.92	0.24
EPA 16: Identifying and addressing any need for quality improvement to increase capacity in decision making in any clinical setting	97.1%	0.24	0.35	0.29	0.10	0.09	0.00
EPA 17: Discussing serious and/or complex aspects of care with patients, families, and caregivers, as well as with members of the interdisciplinary team	97.1%	0.76	0.62	0.71	0.13	0.07	0.18
EPA 18: Providing palliative care when needed and caring for patients at the end of their life	97.1%	0.76	0.39	0.51	0.35	0.11	0.18
EPA 19: Implementing health promotion strategies in patients with or at risk for disease and performing behavioral counseling with patients	97.1%	0.76	0.06	0.51	0.84	0.11	0.57
EPA 20: Supervising junior learners in the clinical setting	97.1%	0.50	0.46	0.32	0.93	0.17	0.76
EPA 21: Managing an inpatient medical service as a team member or multidisciplinary team leader	97.1%	0.48	0.84	0.48	0.32	0.07	0.19
EPA 22: Providing continuity of care under any clinical condition	100%	0.24	0.57	0.35	0.32	0.10	0.22
EPA 23: Assessing and managing patients with uncertain diagnoses and/or treatments	100%	0.48	0.35	0.57	0.13	0.08	0.22
EPA 24: Providing consultations to off-site healthcare providers	94.1%	0.71	0.54	0.84	0.07	0.13	0.18
EPA 25: Initiating and facilitating transfers of care according to healthcare system protocols	97.1%	0.64	0.37	0.74	0.32	0.10	0.43
EPA 26: Working with other physicians and healthcare professionals to develop collaborative patient care plans	97.1%	0.61	1.23	0.92	0.62	0.32	0.29
EPA 27: Identifying learning needs in clinical practice and addressing them with a personal learning plan	100%	0.76	0.62	0.62	0.13	0.13	0.00
EPA 28: Developing and implementing a management plan based on a review of outcome data for ambulatory patient population	100%	0.76	0.39	0.62	0.35	0.13	0.21

* A = Indonesian Board of Internal Medicine professionals
B = Residency program directors
C = Internal medicine specialists
D = Internal medicine residents

Table 2. Average variance and trend for each group of participants.

Participant Groups	Variance	
	Delphi 1	Delphi 2
Indonesian Board of Internal Medicine professionals	0.334	0.247
Residency program directors	0.355	0.253
Internal medicine specialists	0.264	0.274
Internal medicine residents	0.469	0.332

The effect sizes were analyzed to identify differences in opinion between two groups **(Table 1)**. Between the Indonesian Board of Internal Medicine professionals and residency program directors, one EPA item had a large effect size (i.e., EPA item 13). The residency program directors and internal medicine specialists also only had one EPA item with a large effect size (i.e., EPA item 19) among them, while four EPA items (i.e., 1, 2, 21, and 26) had a large effect size between the Indonesian Board of Internal Medicine professionals and the internal medicine specialists. The residency program directors and internal medicine residents had one EPA item with a large effect size (i.e., EPA item 15), while the internal medicine specialists and residents had no large effect sizes among them. Some EPA items had small effect sizes across participant groups, which mainly involved acute or emergency settings and basic internal medicine procedures (i.e., EPA items 3, 4, 5, 8, and 11). The residency program directors and residents had similar opinions, as was indicated by the small effect sizes in 21 out of the 28 EPA items between these groups. The residency program directors, internal medicine specialists, and internal medicine residents agreed on items regarding basic clinical skills (i.e., EPA items 1–5), acute care settings and learning needs and improvement (i.e., EPA items 16 and 27), and, interestingly, complex case management, multidisciplinary services, and patient referral management.

The analysis of variance between Delphi rounds 1 and 2 showed a decreased variance in almost all of the participant groups except for the internal medicine specialists **(Table 2)**. The internal medicine residents group showed the largest decrease in variance compared to the other groups. The end-educational stage competencies for individual EPA items are listed below **(Table 3)**, with some EPA items showing

Table 3. End-educational stage for each EPA item.

Entrustable Professional Activities (EPA) Item	End-Educational Stage			
	Indonesian Board of Internal Medicine professionals	Residency program directors	Internal medicine specialists	Internal medicine residents
EPA 1: Performing histories and physical examinations and documenting and presenting findings across clinical settings for initial and subsequent care	1	1	1	1
EPA 2: Identifying and assessing unstable patients, providing initial management, and obtaining assistance	1	1	1, 2	1
EPA 3: Performing the basic procedures of internal medicine	1, 2	1, 2	2	1
EPA 4: Assessing the degree of severity, diagnosing, and providing initial management for patients with common acute medical presentations in acute care settings	1	1	1	2
EPA 5: Managing patients admitted to acute care settings with common medical problems and advancing their care plans	1	1, 2	1, 2	2
EPA 6: Consulting with specialists and other health professionals, synthesizing recommendations, integrating these into the care plan, and referring, when appropriate, to other specialty care	2	2, 3	2	2, 3

EPA 7: Formulating, communicating, and implementing discharge plans for patients with common medical conditions from acute care settings	1	2	1, 2	2, 3
EPA 8: Assessing unstable patients and providing targeted treatments and consulting as needed; providing emergency multidisciplinary care to medical inpatients	2	3	2,3	2
EPA 9: Discussing and establishing patient goals of care with family and other health providers	1	2, 3	2, 3	1, 3
EPA 10: Identifying personal learning needs while caring for patient needs and accessing medical information to provide evidence-based care to address those needs in developing the practice of life-long learning	1	1, 2	1, 3	1
EPA 11: Assessing, diagnosing, and managing patients with complex or atypical acute medical presentations, with complex medical conditions and/or comorbidities	2	3	2	2, 3
EPA 12: Assessing and managing patients with complex chronic conditions that require other specialists or subspecialty care	2	3	3	3
EPA 13: Providing internal medicine consultations to other clinical and perioperative services	2	2, 3	3	2, 3
EPA 14: Assessing emergencies and participating or leading in resuscitating and managing unstable and critically ill patients	3	3	3	2, 3
EPA 15: Performing the procedures of internal medicine	2	2	2	2
EPA 16: Identifying and addressing any need for quality improvement to increase capacity in decision making in any clinical setting	2, 3	2, 3	2, 3	2, 3
EPA 17: Discussing serious and/or complex aspects of care with patients, families, and caregivers, as well as with members of the interdisciplinary team	2	2, 3	3	2, 3
EPA 18: Providing palliative care when needed and caring for patients at the end of their life	3	2	3	2, 3
EPA 19: Implementing health promotion strategies in patients with or at risk for disease and performing behavioral counseling with patients	2, 3	1, 2	2	2
EPA 20: Supervising junior learners in clinical settings	3	3	3	3
EPA 21: Managing an inpatient medical service as a team member or multidisciplinary team leader	3	3	3	3
EPA 22: Providing continuity of care under any clinical condition	2	3	3	2, 3
EPA 23: Assessing and managing patients with uncertain diagnoses and/or treatments	1	1	1, 2	1,2
EPA 24: Providing consultations to off-site healthcare providers	3	3	3	2, 3
EPA 25: Initiating and facilitating transfers of care according to healthcare system protocols	2	3	2, 3	2, 3
EPA 26: Working with other physicians and healthcare professionals to develop collaborative patient care plans	3	3	3	3
EPA 27: Identifying learning needs in clinical practice and addressing them with a personal learning plan	1, 2	1	1, 3	1
EPA 28: Developing and implementing a management plan based on review of outcome data for ambulatory patient population	2	2, 3	2, 3	2

different results between participant groups. EPA items 4 and 5 were rated for end-stage 1 by all groups except for the internal medicine residents who rated it as stage 2. The residency program directors rated EPA items 8 and 18 as appropriate for end-stage 3, while the others rated it for end-stage 2. EPA item 9 was rated for end-stage 1 by the Indonesian Board of Internal Medicine professionals, while the others rated it for end-stage 2 or 3. The other EPA items showed similar end-educational stage results across groups.

DISCUSSION

This is the first study in Indonesia to identify and analyze internal medicine resident activities as EPAs. The stakeholders of internal medicine residency programs from various universities and provinces in Indonesia identified activities as EPAs which had been adjusted to the existing curriculum in Indonesia. A literature review was conducted to identify the clinical activities of residents in internal medicine programs which could be included as EPAs. Several medical organizations have also conducted literature reviews to identify activities which could be implemented into the EPAs. For example, RCPS collected EPA items through a literature review and group review in the early stages of their research.[3] UCSF also conducted a literature review to obtain 30 EPA items which were then further processed using the Delphi method.[5] Finally, AAIM performed a similar method for identifying EPAs in the early stages by conducting a literature review.[4]

Based on these examples, a thorough literature review appears to be a typical practice when developing criteria for EPAs, as well as obtaining expert opinions from educational professionals and clinical practitioners regarding essential activities as an internist.[5,7] A literature review is typically conducted by more than one researcher. RCPS conducted a peer-reviewed literature review, while UCSF appointed three clinical education and educational researchers to conduct a literature review.[3,5] The literature review in this study was carried out by the authors, who were either non-internal medicine education experts or internal medicine practitioners. Therefore, the literature review conducted in this study was similar to previous studies which had successfully established criterion for their EPAs. However, their EPAs had been previously tested via valid research methods, where ours had not yet been validated.

In addition to the elimination of duplicates, the literature review also excluded EPA items which were considered incompatible with EPA attributes specified by Cate et al.[1] EPA items mentioning "professional behavior" and "participation in an academic project" (e.g., research, quality improvement, and education) were not included in our EPA design due to them not being specific enough on the actual professional activities involved. In addition, "demonstrating professional behavior" did not meet the ideal EPA 5 attribute (i.e., can be implemented independently) and also in contrast to the study by Taylor et al. (i.e., describes the task and avoids adjectives or adverbs that refer to proficiency).[3] "Participating in an academic project" did not meet the ideal EPA 7 attribute (i.e., can be observed and rated).

After the literature review, we facilitated an expert discussion, considered as a group consensus method. Another name for this expert discussion is the nominal group technique (NGT).[7] Traditionally, NGT has stages such as silent generation, "round-robin," clarification, ranking, discussion, and re-ranking. In practice, herein, silent generation and "round-robin" were replaced by the literature review to generate ideas. The results of the expert discussion in this study were not re-ranked, but were directly presented to the educational experts to obtain their responses, similar to the RCPS study.[3] We recruited 10 experts, which met the recommended number of participants outlined by Humphrey et al. of 5–12 experts.[7,8] The experts who participated in this discussion had prior knowledge of EPAs and internal medicine residency programs. Therefore, the consensus of these experts confirmed the content validity of the proposed EPAs.

All EPA items which were accepted by 100% of the experts, including EPAs 1, 2, 3, 4, 13, 14, and 15 (**Supplementary Table S2**), were obtained from previous studies conducted by RCPS. These EPA items were considered important by the experts because most of

them are basic skills which each resident must master, such as patient history taking, physical examination, and managing emergencies. The EPA items which were accepted by 90% and 80% of the experts, including EPAs 5, 6, 7, 8, 9, 10, 11, 12, 19, 20, 22, 23, and 27 (**Supplementary Table S2**), are indeed found in daily clinical practice, such as consulting specialists and other health professionals, synthesizing recommendations, integrating these into a care plan, and referring, when appropriate, to other specialty care (i.e., EPA 6).

EPAs 16, 17, 24, 25, and 28 (**Supplementary Table S2**) were accepted by only 70% of the experts but were recommended to be accepted in the expert qualitative comments. These EPA items are rarely undertaken by residents in day-to-day clinical practice. However, these activities were accepted as EPA items as they could be carried out by residents of internal medicine specialist education programs based on the experts' positive comments; in addition, they were considered eligible according to the ideal EPA attributes of Cate et al. EPAs 21, 26, and 32 (**Supplementary Table S2**) were accepted by only 60% of the experts, although these EPAs were accepted in the final EPA design. "Supervising junior students in the clinical unit" (i.e., EPA 21) was accepted by only 60% of the expert discussion members due to a shift in supervision from being carried out by senior students to doctors in charge or supervisor since the era of Joint Committee International (JCI) accreditation. However, in the comments, some of the experts stated that these items were eligible to be accepted as EPAs. EPAs 26 and 32 were only accepted by 60% of the experts (**Supplementary Table S2**). In contrast to EPA 21, these activities were considered non-eligible as EPAs by qualitative comments, because "initiating and facilitating transfers of care according to healthcare system protocols" and "developing and implementing management plans based on the review of data for the outpatient population" are not typically undertaken by residents of internal medicine programs in day-to-day clinical practice, but rather by the nurses and hospital management. However, as EPA 21 had been accepted through qualitative comments, all clinical activities agreed upon by 60% of the experts were accepted.

EPAs 18, 29, 30, and 31 were accepted by only 50% of the experts. EPA 18 was suggested for exclusion, as it had already been addressed by another EPA item. EPAs 29, 30, and 31 were related to the role of a doctor (versus a resident), according to the Danish Health and Medicines Authority 2013, namely, as a medical expert, communicator, collaborator, manager, health advocate, scholar, and professional.[14] EPA 29 was included in the manager role, and EPA 30 reflected the roles of communicator, collaborator, and manager, whereas EPA 31 expressed the role of communicator. Furthermore, the three activities are not routinely performed as part of daily clinical practice. For example, it is rare for a doctor in Indonesia to provide telephone services to outpatients on an emergency basis, to serve non-native-speaking patients, or to manage resources at the system level.

Delphi Method

Rowe et al. provided the criteria for participants in the Delphi method, in which they must have appropriate knowledge, be heterogeneous, and within 5 to 20 people, which was implemented when choosing the participants in this study.[9] Our study extracted the activities from established EPAs in other countries, validated by expert discussion and determined by the stakeholders in Indonesia. In the second round, 26 EPA items evaluated by Delphi round 1 participants were adjusted to 28 EPA items. Two EPA items which previously received a CVI of <80% increased to 97.1% for EPA 26 and 100% for EPA 27. This change may have occurred after participants considered the results of Delphi round 1 which had already been released, as similar shown in the research by Taylor et al. showed similar changes.[3]

Although all of the EPA items were finally approved, this research provided some interesting insights. In this study, a high CVI (100%) was predominantly found in EPA items containing elements of acute, serious, and unstable medical conditions, as well as perioperative consultation. This was in contrast to the results of the study by Hauer et al., which identified a low CVI for perioperative consultations and resuscitation

(64.3% and 60.7%, respectively).[5] This suggests that the contextual differences between countries may produce differences in the EPAs approved due to culturally different expectations in clinical practices and competency standards. Therefore, it is best for the residency programs in each country to determine their own EPAs. Most of the participants considered clinical activities as more important in acute, chronic, and complex medical conditions, emergency departments, and internal medicine procedures (CVI 98.2%) than those related to outpatient care (85.2%). This may be due to the first group of activities requiring more complex skills, full concentration, and a rapid response; they are often quite challenging in daily case management.

Several EPA items had a small effect size across groups (some were 0), which were EPA items 3, 4, 5, 8, and 11 **(Table 1)**. These items were related to acute setting management, suggesting agreement in the importance of acute care setting competency as components of EPAs, as this competency would be important under any circumstances, from an acting medical doctor to an internal medicine resident. Interestingly, the residency program directors and the residents agreed on most of the EPA items, where 21 out of the 28 EPA items had a small effect size. This shows agreement on which competencies were important for daily clinical practice from those directly involved in the residency programs. The residency program directors, the internal medicine specialists, and the residents had similar evaluations regarding basic clinical skills (i.e., EPA items 1–5) and learning improvements, as these components are at the core of the foundations of study. These population groups had similar results on items regarding complex case management, multidisciplinary management, and patient referral. These similarities may be related to the program location, as well as the referral procedure in our country, as the main academic hospitals for residencies in Indonesia are generally tertiary hospitals, and even a national referral hospital.

Between the Board of Internal Medicine professionals and the residency program directors, only one EPA item showed a large effect size (i.e., EPA item 13), regarding consultation with other clinical services and perioperative consultations, on which the residency program directors placed more importance. The differing opinions may be related to the different settings in which these two groups practice. For example, hospitals that received higher referrals may require more clinical service collaboration and consultation, so those familiar with this environment would rate this activity as more important. However, the Indonesian Board of Internal Medicine professionals and the internal medicine specialists had four EPA items which showed large effect size, categorized as basic clinical skills (i.e., EPA items 1 and 2) and multidisciplinary approaches (i.e., EPA items 21 and 26). The difference in opinion regarding the multidisciplinary approach may be related to the different backgrounds of a subspecialist and a specialist, in which clinical practitioners who are mostly internal medicine specialists may require more knowledge and skills in managing patients with complex chronic conditions and conditions requiring combined care, compared to the Board of Internal Medicine professionals who typically have subspecialty backgrounds. This might also explain the large effect size for EPA item 19. The differences in opinion regarding basic clinical skills may be related to the participants' current professional work. An internal medicine specialist, being a full-time clinician, handles a wide variety of cases and gains clinical knowledge daily; therefore, he may rate these EPA items as important, but not as important as an Indonesian Board of Internal Medicine professional, who addresses the academic curriculum directly as part of their current professional responsibilities.

The residency program directors, the internal medicine specialists, and the residents typically had similar ratings for almost all EPA items, with the exception of EPA item 15 on internal medicine procedures, which could be related to the different experiences among the groups, where an internal medicine resident, who has far less experience and knowledge regarding procedures in internal medicine, may rate this aspect higher, while a residency program director with far more experience may be more familiar

with prioritizing important procedures in daily clinical practice. Surprisingly, the supervision of junior students in a clinical unit had a large size effect between the internal medicine specialists and the Board of Internal Medicine professionals (0.95; mean score, 4.93 vs. 4.55). This may have been due to changes in the education system after the Joint Commission International (JCI) accreditation period in most teaching hospitals in Indonesia, which supports the appointment of an academic medical staff member as supervisor in a clinical unit so that the supervision of each student is the responsibility of those staff members, not the senior students.[9]

This study also showed a decrease in variance in the second round of the Delphi procedure. This may be because in round 1, the informants were not familiar with the EPA items. In round 2, and after seeing the results of round 1, the participants may have been more convinced of the score they had initially given, so they may have increased it further, if possible. This was evidenced by the CVIs reaching 100% in EPA items 27 and 28. The significant decrease in mean variance between the study program directors and the study program participants indicated that these two groups agreed on the importance of the 28 EPA items. These two groups comprised those most likely to encounter daily EPA practices, as compared to the Board of Internal Medicine professionals and the internal medicine specialists. Similar findings were also found in the study of Hauer et al, who found a decrease in the variance from Delphi round 1 to round 2.[5] This decrease in variance indicates an increase in agreement due to the smaller variations in the rank scores given.

The end educational stage attributed to each EPA item could significantly determine the developmental progress of students. As Taylor et al. showed at RCPS, each EPA item was assigned to end-stages 1–4 so that the achievement of a student would be observed, estimated, evaluated, and monitored.[3] In this study, most of the EPA items were assigned to the end of educational stages 2 or 3. Differences in opinion among the groups were found in several EPA items. The internal medicine residents rated EPA items 4 and 5 as being in end-stage 2, while the others conferred these activities in end-stage 1. EPA items 4 and 5 were related to diagnosis and initial management in acute care settings. The difference in experience levels may be attributed to this disagreement, as residents may think they need more time to prepare for these competencies, while others with specialty and subspecialty backgrounds may believe that acute care settings should be learned and entrusted as early as in end-stage 1.

The residency program directors had different opinions on EPA items 8 and 18. In terms of EPA item 8 on targeted therapy and the multidisciplinary approach in acute care settings, the residency program directors, as well as the internal medicine specialists, indicated they should be assigned in end-stage 3, while others suggested them to be put on end-stage 2. The residency program directors, who developed the curriculum for these students, may feel that end-stage 3, at which point residents have passed through most of their rotations, may be more suitable for a multidisciplinary approach, as residents' clinical skills and judgements would be more refined, especially regarding acute and unstable cases. The Indonesian Board of Internal Medicine professionals assigned EPA item 9 to end-stage 1, while the others assigned it to end-stage 2 or 3. The Board of Internal Medicine professionals may have believed that comprehensive multidisciplinary care with other disciplines and the patient's family should be initiated from the first end-stage. The Board of Internal Medicine professionals may view this competency as a basic competency for a medical doctor, while the others may feel that sufficient knowledge should first be met for appropriate comprehensive care.

Overall, the participants suggested that end-stage 1 residents should be entrusted and evaluated based on their basic clinical skills (e.g., history taking and physical examination) and basic internal medicine procedures, management in acute care settings, and identifying their learning needs for future improvements. During end-stage 2, they should be allowed to consult with specialists and synthesize recommendations, formulate discharge plans based on obtained data, manage the transfer of care between healthcare

systems, and complete health promotion and behavioral consultations with patients. During the final end-stage, residents should be entrusted to manage complex and atypical cases, both in acute and unstable settings and under chronic conditions, manage palliative care, entrusted in multidisciplinary care and becoming a leader in inpatient care, planning the continuity of care for patients, supervising junior residents, and identifying quality improvements in overall service and learning. These results are similar with those of RPCS, and in implementing these EPAs, it appeared that Indonesia could adopt the RCPS EPA design with some modifications.[3]

However, compared to the suggestions of Taylor et al., some items in our study were entrusted in earlier stages. Items entrusted in end-stage 2, such as specialist and other professional consultations, formulation of a discharge plan, and establishing patient goals of care, were entrusted during the foundation of the discipline stage. Items entrusted in stage 3, such as the management of complex chronic conditions, palliative care, and junior resident supervision, were entrusted during the core of the discipline stage. These differences reflect that the study participants and curriculum providers in Indonesia were more cautious in several competencies and may view these strong foundations as required competencies in end-stage 3 prior to being entrusted with such responsibilities as multidisciplinary care, junior resident supervision, and others.

Study Limitations

The main limitation in this study is the Indonesian language and terminology used in the EPA items were sometimes difficult to understand by the experts and required further explanation from the authors. This may explain the reasons why some experts gave a low rating/ score on an EPA item but made comments indicating acceptability for the same EPA item.

CONCLUSION

Twenty-eight clinical activities were validated through a literature review, expert discussion, and two Delphi rounds as EPAs for internal medicine residency programs in Indonesia. Implementation of these EPA items will require further discussion with the relevant stakeholders to determine appropriate year of training and expected competencies required for each EPA item.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to Professor Pradana Soewondo, Professor Siti Setiati, Professor Imam Subekti, Professor Murdani Abdullah, Aida Lydia, Aulia Rizka, Kuntjoro Harimurti, Sally Aman Nasution, and Diantha Soemantri for their contributions in the validation of the items in this study.

In addition, the authors offer their gratitude to internal medicine attending physicians: M. Darwin Prenggono, Djallalluddin, Wachid Putranto, Ratih Tri Kusuma Dewi, Yenny Kandarini Ni Made Renny Anggreni Rena, Putu Moda Arsana, C. Singgih Wahono, Abdullah, Vera Abdullah, Ummi Maimunah, Novira Widajanti, Fathur Nurkholis, Setyo Gundi Pramudo, Deddy Nur Wachid Achadiono, Heni Retnowulan, Irza Wahid, Fauzar, Nova Kurniati, Yuniza, Dairion Gatot, Wika Hanida Lubis, Hasyim Kasim, Haerani Rasyid, Linda Rotty, Eko Surachmanto, Rudi Supriyadi, Indra Wijaya, Samuel Maripadang Baso, I Made Gede Darmaja, I Komang Adi Sujendra, Arfan Sanusi, M. Yusuf Hamra, Jane Estherina Fransiska, Asep Purnama, Irwin Prijatna Kusumah, Diana Novitasari, Nyoman Ati Subiantarti, Herman Kusbiantoro, Johana Prihatini, Mochamad Arief Setiawan, Poerniati Koes Andrijani, Elizabeth Merry Wintery, Endang Mardiningsih, Jon Ganefi, I Wayan Darya, Ni Gusti Putu Cilik Wiryani, Rastri, Tri Wahyu, Skandinoviar, Yunita, Adi Wijaya, Afin Tagor Harahap, and Usman Markum.

The authors also offer their gratitude to internal medicine residents: Syarif Hidayatulloh, Putu Yuliani Widiasari, Nyoman Satvika Dharma Yuda, Andrie Gunawan, Amluatul Karimah, Gama Yuniharizky, Prenali Dwisthi Sattwika, Bevi Dewi Citra, Teguh Setiadi, Dewi Fuji Lestari, Arman Mikael Singara, Revlie Towoliu, and Bayu Laksono.

Finally, the authors also offer gratitude to the staff of the Indonesian Society of Internal Medicine: Kanu Eltrantri and Agus Suhono; research assistants: Leroy David, Kevin Winston, Jessica Leoni, and Yusuf Aji Samudera.

CONFLICT OF INTEREST

The authors declare no conflicts of interest in this study.

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SUPPLEMENTARY TABLES

Supplementary Table 1. The Translation of EPA by Hauer et al into Indonesian Language

Original EPA by Hauer et al	Translation into Indonesian Language	Re-translation into English Language
Evaluate and manage a new problem in a continuity ambulatory patient requiring coordination of care between providers and across settings	Mengevaluasi dan mengelola masalah baru secara berkesinambungan pada pasien rawat jalan yang membutuhkan koordinasi perawatan antara penyedia layanan perawatan dan berbagai unit keperawatan.	Evaluate and manage new medical condition in ambulatory patient and conduct care coordination with other health care provider across various unit care.
Admit and manage a medical inpatient with a new acute problem on a medical floor	Menerima dan mengelola pasien rawat inap medis dengan masalah akut baru di unit layanan perawatan medis	Admit and manage new acutely ill patient in service care unit.
Provide medical consultation for patients receiving nonmedical services	Menyediakan konsultasi medis bagi pasien-pasien yang menerima perawatan non-medis.	Provide medical consultation for patient receiving non-medical care
Admit and manage a medical inpatient with an acute exacerbation of a chronic problem on a medical floor	Menerima dan mengelola pasien rawat inap medis dengan eksaserbasi akut dari masalah kronik di unit layanan perawatan medis / lantai medis	Admit and manage inpatient with acute exacerbation from chronic medical condition at medical service care unit/ medical floor
Discuss serious news with a patient and/or family (bad news, end-of-life care planning)	Memimpin pertemuan keluarga untuk membahas berita serius atau sensitif dengan pasien dan/atau keluarga dan penyedia layanan kesehatan lainnya	Lead family meeting to discuss serious or sensitive news with patient and/or patients' family and other health care provider
Lead a family meeting to discuss serious or sensitive news with patient and/or family and other health providers	Membina pengertian dengan para pasien, keluarganya dan anggota-anggota tim multidisiplin ilmu.	Develop understanding with patients, family, and multidiscipline team members
Perform initial H&P, develop problem list, and plan for new ambulatory patient in continuity practice	Melakukan anamnesis dan pemeriksaan fisik awal, mengembangkan daftar masalah dan rencana untuk pasien rawat jalan baru dalam praktek yang berkesinambungan.	Conduct history taking and early physical examination, develop problem lists and plan for new ambulatory patient in continuity practice
Provide continuity care, conducting interval visits, for primary care patients with multiple chronic conditions	Menyediakan perawatan berkesinambungan, melakukan kunjungan berkala bagi pasien-pasien layanan kesehatan primer.	Provide continuity care and conduct periodic visit for primary health care patient
Develop and implement a safe discharge plan for a patient from the acute care setting	Merencanakan dan menerapkan rencana pemulangan pasien yang aman di unit perawatan akut	Plan and implement safety patient discharge plan in acute care unit
Triage medically ill patients to an appropriate level of care	Melakukan triase bagi pasien-pasien yang secara medis sakit dan merujuk mereka ke tingkat layanan perawatan yang sesuai.	Conduct patient triage for medically ill patients and refer them to appropriate level of care
Provide initial management and contribute to postoperative care for patients presenting with surgical problems	Menyediakan penanganan awal dan berkontribusi dalam perawatan pascaoperasi untuk pasien-pasien yang menunjukkan masalah bedah.	Provide early management and contribute in postoperative care for patients with surgical problem
Access medical information to provide evidence-based care for adult patients	Mengakses informasi medis untuk menyediakan perawatan berbasis bukti	Access medical information for evidence based practice
Identify and manage acute, emergent problems	Mengidentifikasi dan menangani masalah-masalah gawat	Identify and manage emergency problems
Provide urgent and emergent cross-coverage care to medicine inpatients	Menyediakan perawatan kegawatan multidisiplin ilmu bagi pasien rawat inap medis	Provide multidiscipline emergency care for medical inpatient
Lead a team in managing multiple inpatients	Lead a team in managing multiple inpatients	Lead team in managing multiple inpatients
Recognize and diagnose common non–internal medicine (surgical, neurological, dermatologic, etc) problems and appropriately refer to subspecialty care	Mengenali dan mendiagnosis masalah-masalah umum non-penyakit dalam (bedah, neurologis, dermatologis, dll) dan merujuk ke perawatan subspesialis secara tepat.	Recognize and diagnose general non-internal medicine problems (surgical, neurological, dermatological, etc) and refer to appropriate subspecialists)

Supplementary Table 1. The Translation of EPA by Hauer et al into Indonesian Language

Original EPA by Hauer et al	Translation into Indonesian Language	Re-translation into English Language
Diagnose conditions for and co-manage patients with complex problems needing subspecialty care (inpatient or outpatient)	Mendiagnosis dan menangani bersama pasien-pasien dengan kondisi kompleks yang memerlukan perawatan spesialis lainnya (rawat inap atau rawat jalan).	Diagnose and co-manage patients with complex condition requiring other specialty care (inpatient or outpatient)
Manage information and knowledge for personal learning to improve care delivery and to educate others (journal club, etc)	Mengelola informasi dan pengetahuan untuk pembelajaran pribadi guna meningkatkan pemberian layanan perawatan dan melakukan edukasi bagi pihak lain (klub jurnal, dll).	Organize and maintain information and knowledge through medical practice to increase personal self-development when providing treatments and conducting education for others (club journal, etc)
Institute palliative care appropriately in collaboration with palliative care specialists	Mendirikan layanan perawatan paliatif secara tepat bekerja sama dengan para spesialis perawatan paliatif.	Establish palliative care appropriately and work together with palliative care specialist
Perform behavioral counseling with a patient	Melakukan konseling perilaku dengan pasien.	Conduct behavior counselling with patient
Admit and manage a medical ICU patient	Menerima dan mengelola pasien medis ICU.	Admit and manage medical ICU patient
Identify and address a quality improvement need in a clinical setting	Mengidentifikasi dan mengatasi masalah perbaikan kualitas yang diperlukan pada suatu situasi klinis.	Identify and manage quality improvement problems required in a clinical situation.
Provide telephone management of an acute problem for an ambulatory patient	Menyediakan pengelolaan layanan telepon untuk masalah akut bagi pasien rawat jalan.	Provide telephone management care for ambulatory patient with acute problem
Provide care to an inpatient or outpatient non–English-speaking patient, using appropriate translator services	Menyediakan perawatan bagi pasien rawat inap atau rawat jalan yang tidak berbahasa Inggris, menggunakan layanan penerjemah yang tepat.	Provide treatment for non-English speakers in inpatient or outpatient setting with appropriate translation services
Develop and implement an action plan based on review of performance data for one's ambulatory patient panel	Mengembangkan dan menerapkan rencana kerja berdasarkan kajian atas data kinerja untuk panel pasien rawat jalan.	Develop and implement working plan according to performance data study for panel ambulatory patients
Provide inpatient and outpatient care for patients with challenges in access to care that appropriately address those challenges	Menyediakan layanan perawatan rawat inap dan rawat jalan bagi pasien-pasien yang mempunyai tantangan dalam hal akses perawatan serta mengatasi tantangan-tantangan tersebut dengan tepat.	Provide inpatient and ambulatory service for patients with access difficulty to obtain appropriate health care and solve the challenge appropriately
Participate in and lead an inpatient cardiopulmonary resuscitation	Berpartisipasi dan memimpin resusitasi jantung paru bagi pasien rawat inap.	Participate and lead cardiopulmonary resuscitation for inpatient
Perform common procedures in internal medicine (LP, thoracentesis, central line, arthrocentesis)	Melakukan prosedur umum dalam bidang ilmu penyakit dalam (pungsi lumbal (LP / lumbal puncture), torakosentesis, pemasangan kateter vena sentral, aspirasi sendi / artrosentesis.	Conduct general procedures in internal medicine (lumbal punction, thoracocentesis, central vein catheterization, joint aspiration).
Conduct or participate in a scholarly project (research, QI, education, other)	Melakukan atau berpartisipasi dalam proyek akademik (riset, perbaikan kualitas (QI / quality improvement), edukasi, lainnya	Conduct or participate in academic project (i.e: degree or diploma, quality improvement, health promotion, etc)

Supplementary Table 2. List of Accepted EPA from Experts' Discussion

Entrustable Professional Activities (EPA) Item	% Mean score ≥4,07	Commentary from Experts
Accepted EPAs		
EPA 1: Performing histories and physical examinations and documenting and presenting findings across clinical settings for initial and subsequent care	100%	Eligible
EPA 2: Identifying and assessing unstable patients, providing initial management, and obtaining assistance	100%	Eligible
EPA 3: Performing the basic procedures of internal medicine	100%	Eligible
EPA 4: Assessing the degree of severity, diagnosing, and providing initial management for patients with common acute medical presentations in acute care settings	100%	Eligible
EPA 5: Managing patients admitted to acute care settings with common medical problems and advancing their care plans	90%	Eligible
EPA 6: Consulting with specialists and other health professionals, synthesizing recommendations, integrating these into the care plan, and referring when appropriate to other specialty care	**80%**	Eligible
EPA 7: Formulating, communicating, and implementing discharge plans for patients with common medical conditions from acute care settings	**80%**	Eligible
EPA 8: Assessing unstable patients and providing targeted treatments and consulting, as needed; providing emergency multidisciplinary care to medical inpatients	**80%**	Eligible
EPA 9: Discussing and establishing patient goals of care with family and other health providers	**80%**	Eligible
EPA 10: Identifying personal learning needs while caring for patient needs and accessing medical information to provide evidence-based care to address those needs in developing the practice of life□long learning	90%	Eligible
EPA 11: Assessing, diagnosing, and managing patients with complex or atypical acute medical presentations, with complex medical conditions and/or with comorbidities	90%	Eligible
EPA 12: Assessing and managing patients with complex chronic conditions that require other specialists or subspecialty care	90%	Eligible
EPA 13: Providing internal medicine consultations to other clinical and perioperative services	100%	Eligible
EPA 14: Assessing emergency and participating or leading in resuscitating and managing unstable and critically ill patients	100%	Eligible
EPA 15: Performing the procedures of internal medicine	100%	Eligible
EPA 16: Identifying and addressing any need for quality improvement to increase capacity in decision making in any clinical setting	70%	Ineligible
EPA 17: Discussing serious and/or complex aspects of care with patients, families, and caregivers, as well as with members of the interdisciplinary team	70%	Eligible
EPA 19: Providing palliative care when needed and caring for patients at the end of their life	**80%**	Eligible
EPA 20: Implementing health promotion strategies in patients with or at risk for disease and performing behavioral counseling with patients	**80%**	Eligible
EPA 21: Supervising junior learners in the clinical setting	60%	Eligible
EPA 22: Managing an inpatient medical service as a team member or multidisciplinary team leader	90%	Eligible
EPA 23: Providing continuity of care under any clinical condition	**80%**	Eligible
EPA 24: Assessing and managing patients with uncertain diagnoses and/or treatments	70%	Ineligible
EPA 25: Providing consultations to off-site health care providers	70%	Eligible
EPA 26: Initiating and facilitating transfers of care according to healthcare system protocols	60%	Ineligible
EPA 27: Working with other physicians and healthcare professionals to develop collaborative patient care plans	**80%**	Eligible
EPA 28: Identifying learning needs in clinical practice and addressing them with a personal learning plan	70%	Eligible
EPA 32: Developing and implementing a management plan based on a review of outcome data for ambulatory patient population	60%	Ineligible
Rejected EPA		
EPA 18: Caring for patients who have experienced a patient safety incident (adverse event)	50%	Ineligible

EPA 29: Identifying and analyzing system-level safety, quality, or resource stewardship concerns in health care delivery	50%	Ineligible
EPA 30: Provide telephone management for an ambulatory patient in an emergency.	50%	Ineligible
EPA 31: Providing care services for non-native speaker patients in inpatient or outpatient rooms using appropriate translation services.	50%	Ineligible

The Use of Complementary Alternative Medicine in HIV-infected Patients during COVID-19 Pandemic: Its Related Factors and Drug Interactions with Antiretroviral Therapy

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ABSTRACT

Background: *The use of complementary and alternative medicine (CAM) is widespread among patients with chronic disease despite lack of supporting evidence for most CAM types. Concerned regarding higher risk of COVID-19 for HIV-infected patients, probably increase the use of CAM during COVID-19 pandemic in this population. This study aimed to assess the prevalence and factors related to CAM use among HIV-infected patients during COVID-19 pandemic, then identify drug- to-drug interaction (DDI) of antiretroviral (ARV) drugs with CAM that they used.* ***Methods:*** *The study was conducted in HIV Clinic Cipto Mangunkusumo Hospital in September-October 2021, specifically targeting adults HIV-infected patients routinely using ARV. Demographic and clinical data, including COVID-19 and vaccine history, were taken from clinic survey and hospital medical records data.* ***Results:*** *554 of 1275 patients (43.5%) reported using any type of ingested CAM during COVID-19 pandemic, mostly vitamins and/or minerals. Factors related to CAM use were history of COVID-19 infection (aOR 2.28; 95% CI 1.65-3.14) and 2-5 years ARV duration compared to more than 10 years (aOR 1.4; 95% CI 1.02–1.91). Five known potential interactions involving 20 patients and two potential weak interactions involving 8 patients were found, but many of other interactions categorized as unknown. Only limited number of patients (3.8%) were aware about the drug interaction between ARV and CAM that they used.* ***Conclusion:*** *CAM was commonly used by HIV-infected patients on ARV during the COVID-19 pandemics, but patient awareness related to CAM-ARV drug interactions was exteremely low.*

Keywords**:** *Complementary therapies, HIV, COVID-19, herbal, drug interactions.*

INTRODUCTION

Complementary and alternative medicine (CAM) is a term for medical products and practices that are not part of standard medical care. CAM consists of various types, such as reflexology, herbal medicine, nutritional supplements, yoga, and acupuncture.[1] CAM is often used by patients with chronic diseases, including HIV. Previous studies have shown that many HIV-infected patients did not disclose their use of CAM to the doctors. The use of CAM is often based on the belief that it is more natural, and it can improve general health and ensure long term survival.[2]

Indonesia is a country with rich biodiversity, especially medicinal plants. More than 2500 species in Indonesia are recognized as medicinal plants.[3] Based on data released by WHO on Traditional and Complementary Medicine (T&CM) in 2019, 40-59% of the Indonesian population used traditional and herbal medicine. However, percentage data on the type used was not available.[4] Many studies reported that CAM was routinely used for HIV-infected patients since they were first diagnosed with HIV.[2,5–13] Research on the use of CAM among HIV-infected patients in Indonesia is still minimal. A small study involving 88 HIV-infected patients in Jambi City, showed that all of them had used CAM. The types of CAM used were prayer, aromatherapy, cupping, herbal medicine, vitamin, and massage.[14]

Since the presence of COVID-19, research is still being carried out to find a definitive therapy or prevention. Patient immunity plays an essential role in COVID-19 infection. Herbal medicine and supplement were proposed to have immunomodulatory effects that can be preventive and even therapeutic agents for patients with COVID-19 disease.[15] Public interest in CAM has increased drastically after the first confirmed case of COVID-19 was reported in Indonesia.[16] Concern over the increased risk of developing severe COVID-19 for HIV-infected patients is based on the fact that they are more likely to be immunosuppressed.[17] In a recent meta-analysis, HIV-positive individuals have a significantly higher risk of SARS-CoV-2 infection and risk of death, compared to people who are not infected with HIV.[18] On this basis, HIV-infected patients might use CAM to increase their immunity during the COVID-19 pandemic, in addition to the vaccine and their antiretroviral (ARV) therapy. However, clinicians have to be aware that almost all ARV classes have a potential drug interaction with other drugs, as substrate, inhibitor, and/or inducer of various enzymes and drug transporters in metabolic processes. Interaction of ARV drug with CAM can cause various clinical problems, particularly reducing the concentration of ARV drug, leading to treatment failure.[6,19]

Therefore, the aim of this study was to describe CAM use among HIV-infected patients with ARV during the pandemic and its related factors. In addition, we would like to evaluate the drug- to-drug interaction (DDI) of ARV with CAM and whether the patients were aware of their drug interactions.

METHODS

This was a cross-sectional study of HIV-infected patients in HIV Integrated Clinic Cipto Mangunkusumo Hospital, Jakarta. Some of the data were taken from a routine survey among patients visiting the clinic in September and October 2021. The survey was planned to complete the medical history of the patients, such as partner testing, alcohol use, cigarette use, other drug use, and recent medical event, including COVID-19. The use of CAM was assessed by asking the patients non-judgementally whether they use ingested CAM at least once, including vitamins, herbal medicines, and supplements. Inclusion criteria were HIV-infected patients, aged above 18 years, using ARV, and have completely filled the clinic survey for COVID-19. Other clinical and demographic characteristics of the participants were extracted from hospital medical records A standardized form was used when abstracting data from the survey and hospital electronic medical records, consisting of demographic and clinical information.

Ethics approvals were obtained from the Ethics Committee of the Faculty of Medicine Universitas Indonesia for the use of survey and routinely collected anonymous data with a waiver for informed consent.

Factors Associated with CAM Use

Demographic and HIV clinical data, as well as COVID-19 history, were evaluated as potential factors associated with CAM use. These include gender, age, HIV risk transmission, duration of antiretroviral therapy, using once-daily fixed-dose combination (FDC) ARV, history of COVID-19, and COVID-19 vaccine status.

Drug to Drug Interaction

For those who took CAM, drug interaction between each CAM use with ARV drug was assessed. References for classification of drug interaction were taken from the Indonesian National Guidelines of Clinical Management of HIV and Antiretroviral Therapy and Liverpool HIV interaction (Liverpool iChart).[20,21] Moreover, we asked the patients' awareness of drug interaction between their antiretroviral drugs with the CAM that they used.

Statistical Analysis

Frequencies and percentages were used to describe categorical variables, and continuous variables were expressed as mean with standard deviation (SD) or median with interquartile range (IQR). Bivariate analysis was performed to compare variables related to the use of CAM during the COVID-19 pandemic. A logistic regression model was fitted using the backward stepwise selection process. Factors with $p<0.25$ in the bivariate analysis were chosen for inclusion in the multivariate analysis. Factors with $p<0.05$ were considered statistically significant in the final multivariate analysis. Data management and statistical analyses were performed using Statistical Package for Social Science program version 20.0 (SPSS Inc, Chicago IL, USA).

RESULTS

A total of 1278 HIV-infected patients joined the survey. However, three patients did not give the correct medical record numbers that can be linked to hospital medical record data, leaving 1275 data for the analysis. Most of them were male (68.1%), with a median age of 41 years (IQR 9). All participants have used various ARV drugs, with a median duration of nine years (IQR 7). Tenofovir (TDF)-based was the most common backbone NRTI used and EFV was the most common anchor ARV used.

There were 183 participants reported to have COVID-19 infection from March 2020 to October 2021 (14,4%). Less than half of them (41.5%) have completed the first and second COVID-19 vaccination, but 33.6% have not been vaccinated at all. A total of 554 (43.5%) participants used any type of ingested CAM during the COVID-19 pandemic: 504 (39.5%) used vitamin and/or mineral, 88 (6.9%) used nutritional supplements, and 72 (5.6%) used herbal medicine, as shown in **Table 1**.

Factors Related to CAM Use

Gender, risk of HIV transmission, using once-daily FDC ARV did not relate to the use of CAM during the first and second wave COVID-19 pandemic in our participants, nor their

Table 1. Demographic and clinical description of participants

Characteristics		N = 1275
Male gender, n (%)	Male	868 (68.1)
	Female	407 (31.9)
Age, years, median (IQR)		41 (9)
HIV risk factor, n (%)	Heterosexual	641 (50.3)
	Homo/ bisexual	168 (13.2)
	IVDU	355 (27.8)
	Others	47 (3.7)
	Unknown	64 (5.0)
Backbone ARV, n (%)	AZT	594 (46.6)
	TDF	681 (53.4)
Anchor ARV, n (%)	NVP	433 (34.0)
	EFV	628 (49.3)
	LPV/r	144 (11.3)
	DTG	69 (5.4)
ARV duration, year, median (IQR)		9 (7)
History of COVID-19 infection, n (%)	Ever infected	183 (14.4)
	Never/ unknown	580 (45.5)
	Unknown	512 (40.2)
COVID-19 vaccine, n (%)	Never	428 (33.6)
	1st dose	273 (21.4)
	2nd dose	529 (41.5)
	3rd dose	8 (0.6)
	Unknown	37 (2.9)
Use ingested CAM, n (%)	Yes*	554 (43.5)
	No	(56.5)

*Vitamin or mineral: 504 participants, nutritional supplement: 88 participants, and herbal medicine: 72 participants.
IVDU = intravenous drug user; ARV = antiretrovirus; AZT = azidotimidine/zidovudine; TDF = tenofovir disoproxil fumarate; NVP = nevirapine; EFV = efavirenz; LPV/r = lopinavir/ritonavir; DTG = dolutegravir

COVID-19 vaccine history, as shown in **Table 2**. HIV-infected patients who had experienced COVID-19 infection used CAM 2.28 times more often than those who never got infected with COVID-19 (95% CI 1.65-3.14). CAM use was also more common in patients who had been treated with ARV drugs for 2-5 years compared to more than 10 years (aOR 1.41; 95% CI 1.02-1.91).

Table 2. Factors related to CAM use among HIV-infected patients on ARV during COVID-19 pandemic (n=1275).

Variable	CAM User n (%)	Non-CAM User n (%)	Bivariate		Multivariate	
			OR (95%CI)	p	aOR (95% CI)	p
Gender						
Female	183 (45)	224 (55)	1.11 (0.87-1.40)	0.445		
Male	369 (42.5)	499 (57.5)				
Age						
50 years and more	58 (36.3)	102 (63.8)	1.48 (0.89-2.44)	0.130	1.73 (0.99-3.03)	0.056
40-49 years	251 (44.2)	317 (55.8)	1.06 (0.70-1.62)	0.787	1.24 (0.77-2.02)	0.376
30-39 years	196 (44.1)	248 (55.9)	1.06 (0.69-1.63)	0.784	1.13 (0.71-1.81)	0.609
Less than 30 years	47 (45.6)	56 (54.4)	1		1	
Risk of HIV transmission						
Heterosexual	287 (44.8)	354 (55.2)	1.00 (0.77-1.30)	1.00	0.95 (0.71-1.25)	0.697
Homosexual	66 (39.3)	102 (60.7)	1.25 (0.86-1.82)	0.236	1.24 (0.81-1.91)	0.321
Others/Unknown	40 (36)	71 (64)	1.44 (0.93-2.24)	0.105	1.42 (0.87-2.31)	0.156
IVDU	159 (44.8)	196 (55.2)	1		1	
Using once-daily FDC ARV						
Yes	178 (44.2)	254 (58.8)	0.88 (0.70-111)	0.308		
No	374 (44.4)	469 (55.6)				
ARV duration						
0-1 years	52 (47.7)	57 (52.3)	0.94 (0.62-1.43)	0.788	0.94 (0.62-1.44)	0.777
2-5 years	107 (38.1)	174 (61.9)	1.40 (1.04-1.89)	0.028	1.41 (1.02-1.91)	0.027
6-10 years	169 (42.1)	232 (57.9)	1.18 (0.91-1.54)	0.218	1.17 (0.89-1.52)	0.265
More than 10 years	224 (46.3)	260 (53.7)	1		1	
COVID-19 infection history						
Ever	111 (60.7)	72 (39.3)	2.28 (1.65-3.14)	< 0.001	2.28 (1.65-3.14)	< 0.001
Never/Unknown	441 (40.4)	651 (59.6)				
Primary COVID-19 vaccine history						
Never/Unknown	195 (41.9)	270 (58.1)	1.17 (0.91-1.50)	0.218	1.24 (0.96-1.60)	0.098
First dose only	111 (40.7)	162 (59.3)	1.23 (0.92-1.66)	0.163	1.30 (0.96-1.75)	0.089
Complete (1st and 2nd dose)	246 (45.8)	291 (54.2)	1		1	

ARV = antiretrovirus; FDC = fixed-drug combination.

Description of CAM Use

Of 554 participants who reported using ingested CAM, the most common vitamin and mineral used was vitamin C (48.6%), followed by multivitamin (31.8%) and vitamin D (20.9%). Honey, omega 3, and propolis were the most common nutritional supplements used by the participants (7.4%, 5.2%, and 1.8%, respectively). Echinacea, curcumin, and black cumin extract were the three most commonly used herbal medicines during the COVID-19 pandemic (2.9%, 2.5%, and 2.5%, respectively), as seen in **Table 3**.

Table 3. Description of CAM use and possible drug interaction with ARV drugs (n = 554)

	Frequency (%)	Drug interaction with ARV drugs	no of patients
Vitamin and Mineral			
Vitamin C	269 (48.6)	No interaction	
Multivitamin	176 (31.8)	potential interaction with DTG	10
Vitamin D	116 (20.9)	No interaction	
Vitamin B	69 (12.5)	No interaction	
Vitamin E	35 (6.3)	No interaction	
Iron	23 (4.2)	potential interaction with DTG	5
Zinc	20 (3.6)	No interaction	
Folic acid	12 (2.2)	potential interaction with DTG	3
Vitamin A	6 (1.1)	No interaction	
Nutritional supplement			
honey	41 (7.4)	Unkown interaction with ARV	41
Omega 3	29 (5.2)	Unkown interaction with ARV	29
Propolis	10 (1.8)	Unkown interaction with ARV	10
Virgin olive oil	8 (1.4)	Unkown interaction with ARV	8
Protein drink	3 (0.5)	Unkown interaction with ARV	3
Palm date	2 (0.4)	Unkown interaction with ARV	2
Albumin	1 (0.2)	Unkown interaction with ARV	1
Garlic	1 (0.2)	Potential interaction with NVP	1
Virgin coconut oil	1 (0.2)	Unkown interaction with ARV	1
Herbal			
Echinacea	16 (2.9)	No interaction	
Curcumin	14 (2.5)	Potential weak interaction with TDF	7
Black cumin extract (*habbatussauda*)	14 (2.5)	Unkown interaction with ARV	14
Traditional indonesian medicine (TIM)	10 (1.8)	Unkown interaction with ARV	10
Cordyceps spp (fungi) extract	8 (1.4)	Unkown interaction with ARV	8
Garcinia mangostana (*kulit manggis*) extract	8 (1.4)	Unkown interaction with ARV	8
Phylantus niruri (*meniran*) extract	6 (1.1)	Unkown interaction with ARV	6
Green tea extract	2 (0.4)	Potential weak interaction with TDF	1
Elderberry extract	2 (0.4)	Unkown interaction with ARV	2
Morindae spp (*mengkudu*) extract	2 (0.4)	Unkown interaction with ARV	2
Schisandra spp	2 (0.4)	Unkown interaction with ARV	2
Zingiber officinale (*jahe merah*) extract	2 (0.4)	No interaction	
Annona muricata (*daun sirsak*) extract	1 (0.2)	Unkown interaction with ARV	1
Cantella asiatica (*daun pegagan*) extract	1 (0.2)	Unkown interaction with ARV	1
Ginkgo biloba	1 (0.2)	Potential interaction with EFV	1
Moringa spp (*daun kelor*) extract	1 (0.2)	Unkown interaction with ARV	1
Panax ginseng	1 (0.2)	No interaction	-
Pandanus conoideus (*buah merah*) extract	1 (0.2)	Unkown interaction with ARV	1
Sausera costus extract (*Qustul Al Hindi*)	1 (0.2)	Unkown interaction with ARV	1

ARV = antiretrovirus; TDF = tenofovir disoproxil fumarate; NVP = nevirapine; EFV = efavirenz; DTG = dolutegravir.

Drug interaction of CAM with ARV drugs

As shown in **Table 3**, we found 5 potential (moderate) interactions of CAM with ARV drugs: 1). multivitamin with DTG, involving 10 patients; 2). iron with DTG (5 patients); 3). folic acid with DTG (3 patients); 4). garlic with NVP (1 patient); 5). ginkgo biloba with EFV (1 patient). Potential weak interactions were found between curcumin and TDF (7 patients) and green tea extract with TDF (1 patient). No known major drug interaction was found. However, we could not define the interaction between 8 of 9 types of nutritional supplements and 13 of 19 types of herbal medicines with ARV drugs.

Of 554 patients who used CAM with ARV drugs, only 21 patients (3.8%) were aware of drug interactions, some of them had discussed with the physicians before. Many others just thought that CAM would improve their health and well-being during the pandemic.

DISCUSSION

The prevalence of CAM users found in this study was 43.5%. This result was comparable to other studies among HIV study population before the COVID-19 pandemic, ranging from 1.8% to 96.8%. This wide range of variability was likely to occur due to the differences in CAM definition used between studies. The CAM reported in this study was ingested CAM, consisting of vitamins and/or minerals, nutritional supplements, and herbal medicines, while other studies included spiritual therapy, energy therapy, and mind-body therapy as part of CAM modalities.[2,5–13] The other possible cause in this varied result was the different methods to assess the CAM use among the study populations, sample size, and period of study data collection. In this study, the data was reported from a routine survey among patients visiting the HIV Integrated Clinic Cipto Mangunkusumo Hospital by directly asking the patient whether they used ingested CAM during the COVID-19 pandemic. Face-to-face interview to 343 patients using a questionnaire in Trinidad reported 32.8% patients using CAM.[7] Anonymous survey conducted to 1211 adult patients in clinics across Australia reported 53% patients using CAM.[9] This difference of method might partly be a factor that affected patients' disclosure of CAM use, but the rate of disclosure was not further evaluated in this study.

Among CAM modalities used in our patients, vitamins and/or minerals were the most common (39.5%), followed by nutritional supplements (6.9%), and herbal medicines (5.6%). Studies conducted in high-income countries like Australia and USA reported vitamins and minerals supplementation as the most common CAM,[9,11] while in low-middle income countries, such as Trinidad, Ethiopia, and Lebanon, herbal medicines emerged as the most common type of CAM used.[2,7,10,13] Nevertheless, we could not directly compare our findings with these studies since our study described only the situation during the COVID-19 pandemic. Unfortunately, we did not have the data on the use of CAM before 2020.

This study was conducted after the second wave of the COVID-19 pandemic in Indonesia when a total of 14.4% participants had ever been infected with COVID-19. The prevalence might be higher because we only included HIV-infected patients who had recovered from COVID-19 infection and joined the survey in the clinic. Referring to two recent meta-analysis, HIV-positive individuals had a higher risk of getting SARS-CoV-2 infection and risk of death.[18,22]

When the survey was conducted, the free COVID-19 vaccine had become an Indonesian government program for the general public for about 4 months. A total of 63.5% of participants had been vaccinated: 21.4% had first dose vaccination only, 41.5% had second dose vaccination, and 0.6% had the third dose. The Indonesian Food and Drug Administration has granted emergency use permits for ten types of COVID-19 vaccines: Sinovac, AstraZeneca, Sinopharm, Moderna, Pfizer, Novavax, Sputnik-V, Janssen, Convidencia, and Zifivax.[22] However, 33.6% of subjects have not been vaccinated against COVID-19 for several possible reasons. One of the reasons was fear of being rejected when registering for vaccinations because of their HIV status. In addition, some vaccination sites require a certificate of eligibility for vaccination from a doctor. A study that predicts COVID-19 vaccine acceptance and practices among HIV-infected patients in

Indonesia is warranted.

This study investigated the use of complementary alternative medicine in HIV-infected patients during the COVID-19 pandemic along with the related factors. We found that history of COVID-19 infection was a significant factor of CAM use among people with HIV (aOR 2.8, 95%CI 1.65–3.14). In Iran, a study in the general population also showed that participants with COVID-19 infection history used CAM to treat and improve the symptoms of the disease during the COVID-19 outbreak.[23] During the COVID-19 pandemic, there has been an increase in demand for alternative medicines to increase immunity, prevent the body from being exposed to the virus, and provide additional treatment for COVID-19. Clinical pharmacists have being increasingly requested an information about dietary supplementation, vitamins, and any options on the shelves that could offer symptom relief and boost the immune system since the start of the COVID-19 pandemic.[24] A study in the general population also showed there was a significant increase in the use of CAM in those who have experienced symptoms of COVID-19 such as fever, cough and dyspnoea.[25]

We also found that in people with HIV, ARV treatment duration of 2-5 years was a significant factor of CAM use with ARV duration of more than 10 years as reference (aOR 1.4; 95% CI 1.02–1.91). This result was different from a review that showed longer disease duration/ time on ARV being one of the most common predictors of CAM use before the COVID-19 pandemic. Furthermore, CAM is often used to address the limitations of or problems with ARV during the period of ARV use.[26]

Starting in 2020, the new class of ARV drug integrase inhibitors (INI) was introduced in Indonesia. Dolutegravir (DTG) as the only INI available was recommended in the first line and second-line ARV regimen due to its simplicity and high barrier of resistance. Polyvalent minerals such as calcium, magnesium, and iron can reduce the absorption rate of INI by chelation. Plasma DTG AUC, Cmax, C24 were reduced up to 39%, 37%, 39%, respectively when co-administered with 480 mg elemental calcium under fasting condition, and up to 54%, 57%, 56%, respectively when co-administered with 107 mg elemental iron under fasting condition.[27,28] One study reported that virological failure was observed in 15% patients with DTG-based regimens with concomitantly used multivitamin/supplements containing polyvalent cations. Thus, calcium, magnesium, iron, zinc, or multivitamin supplement should be co-administered with DTG during meals or at least 6 hours before/2 hours after taking DTG if they are not co-administered with a meal.[29,30] As vitamins and/or minerals were the most common CAM used in our setting, drug interaction with DTG was also common. With the increasing number of patients using a DTG-based regimen, clinicians should consider discussing CAM with HIV-infected patients as part of routine care, to ensure the success of ARV treatment.

In the community, supplements containing garlic were claimed to have several benefits for the health such as anti-cholesterol, antioxidant, antimicrobial, and immune-modulating effects. Despite these positive health effects, garlic has been shown to have drug interactions with some antiretrovirals. Garlic can inhibit or induce CYP450 and P-gp enzymes which can reduce the concentration of protease inhibitor antiretroviral drugs, especially saquinavir and ritonavir-boosted atazanavir. Garlic can reduce saquinavir AUC by up to 50%.[19] In addition, treatment failure has also been reported in a one case report in a patient who took ritonavir-boosted atazanavir with 6 cloves of garlic 3 times a week, decreasing the drug concentration by up to 70%.[31] The same effect is expected in HIV-infected patients who used nevirapine (NVP)-based regimen because they can induce CYP3A4 and/or P-gp, leading to reduce NVP concentration. Patients should be advised against the use of garlic supplements.[21]

Ginkgo biloba extract is an herbal supplement that contains flavonoids and terpenoids which have been implicated in the inhibition and induction of CYP3A4, UGT, and P-gp. This supplement has several positive effects on cognitive function, including improvement in concentration. memory, dementia, and depression.[19,27] HIV-infected patients need to be cautioned on the use of this product because

of drug interaction with ARV, especially EFV. Ginkgo biloba extract would accelerate the hepatic clearance of EFV that affected the EFV serum concentrations to become lower by 38-62% following the initiation of supplements that contain GBE that can lead to a viral breakthrough. Naccarato, et al. [32] reported one case that experienced virological breakthrough after 10 years of being on the same antiretroviral regimen due to drug interaction caused by a negative interaction of Ginkgo biloba on EFV. Until now, the interaction of TDF-based regimen with herbal supplements containing curcumin and green tea extract is still unknown. However, in vivo study showed that both curcumin and green tea extract inhibit P-gp enzyme that potentially makes unfavorable outcomes to patients taking TDF.[21] Moreover, for most herbal medicines and nutritional supplements, no drug interaction information had been available of studied before, including for black cumin (habbatussauda) and traditional Indonesian medicine. Pharmacokinetic studies that evaluate the effect of various herbal medicines and nutritional supplements co-administered with current ARV are needed.

In this study, of 554 patients taking CAM, only 3.8% had knowledge of the interaction between ARV and CAM. This number was much lower than a study conducted in Lebanon that revealed nearly half of the CAM users were unaware of the potential interaction between the drug and the CAM they used.[2] The lack of knowledge about the potential interactions between ARV and CAM is due to the lack of education related to the potential interactions of drugs and CAM by the physicians and nurses who treat them and the lack of awareness of the patient to notify the doctor about the use of CAM. Based on our findings, special education for ARV drug interactions need to be designed for health care workers and patients.

This is the first report for the use of CAM in HIV-infected patients on ARV therapy during the COVID-19 pandemic. Although, the study involved a large number of participants, the selection of the patients was from a single HIV clinic in Jakarta. The attendees of the clinic tended to be lower to middle socioeconomic status, which is not fully representative of HIV-infected patients in Indonesia. Secondly, we used a cross-sectional study design that has limited ability to define the causal association between the use of CAM and other related factors. Moreover, we were unable to follow the consequences of drug-to-drug interaction on the HIV treatment outcome. Finally, we utilized self-reported question which might not describe the actual condition and may cause recall bias.

CONCLUSION

This study revealed a prevalent CAM use among HIV-infected patients during the COVID-19 pandemic, with vitamins and/or minerals being the most reported modality. CAM usage was more common among COVID-19 survivors and patients who used ARV for 2-5 years. The lack of awareness related to CAM-ARV drug interactions shows the need to enhance the education for clinicians and patients on the proper use of CAM as complementary to ARV medication.

CONFLICT OF INTERESTS

All authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The authors received no financial support for the research, authorship, and/or publication of this article.

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Possible Cases of SARS-CoV-2 Reinfection In Pekanbaru, Indonesia

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ABSTRACT

Confirmed and possible reinfection cases of SARS-CoV-2 have been reported from various countries. Here we present two cases of possible SARS-CoV-2 reinfection in Pekanbaru, Indonesia. A 26 years old female and a 27 years old male healthcare workers were first confirmed by PCR with high Ct-value (>35) while presenting no or mild symptoms, respectively. In more than one month since the last negative test results, both patients developed typical COVID-19 symptoms; fever and anosmia. RT-PCR results for SARS-CoV-2 were positive with Ct-value less than 30. The timeframe between 1st and 2nd episode, negative test result between episodes, and epidemiological risk factor strengthened the possibility of reinfection. However, we did not have whole genome sequence (WGS) or viral viability data to further confirm reinfection with different viable virus. The requirement of viral WGS data to confirm true reinfection cases calls for investment in whole genome sequencing platform in public health laboratories. We encourage standardized definition of SARS-CoV-2 reinfection case in order to be able to investigate and observe such cases.

Keywords*: COVID-19, Indonesia, reinfection, SARS-CoV-2*

INTRODUCTION

Severe-acute-respiratory-syndrome coronavirus 2 (SARS-CoV-2) has caused the worldwide Coronavirus disease 2019 (COVID-19) pandemic since early 2020. While the medical and scientific community have speedily studied the virus, there are still many things to learn, including the possibility of reinfection. Investigation and observation of reinfection cases could provide better understanding on immunity to SARS-CoV-2.[1] Currently, at least there have been 4 cases supported by whole genome sequencing (WGS) data to confirm reinfection with genetically-distinct virus.[2–5] There are also several other possible reinfection cases without WGS data.[6–9]

To the best of our knowledge, there are no published reinfection cases from Indonesia.

We present two cases of immunocompetent, young persons that indicated the possibility of SARS-CoV-2 reinfection from Pekanbaru city, Riau Province, Indonesia.

CASE ILLUSTRATION

Case 1

The timeline of Case #1 is summarized on **Figure 1**. A 26-years old female healthcare worker was tested on August 4th, 2020 as part of contact tracing. One of her flat-mates was confirmed positive for SARS-CoV-2. The initial case was also a healthcare worker in the same hospital. Last shift of Case #1 in the hospital was a night shift on August 3rd, 2020. Naso-

oropharyngeal sample was taken on August 4th with positive result for SARS-CoV-2 (Ct-value of 35.50 RdRP) using AllPlex™ 2019-nCoV Assay (Seegene, South Korea). The patient did not have any complaints, and her lab results such as routine hematology (**Table 1**), blood gas analysis, and chest x-ray were within normal range. The patient was admitted to the hospital for isolation purpose on August 6th – 11th and remained asymptomatic. The patient was prescribed azythromicin, oseltamivir, paracetamol, omeprazole, acetylcystein, and vitamin D. Case #1 was tested negative twice on August 9th and 11th, thus declared to be recovered.

The Case 1 was tested on August 23rd, 2020 as another part of contact tracing while still presented no symptoms. The result of naso-oropharyngeal swab came back negative using Standard M nCoV Real-Time Detection Kit (SD BIOSENSOR, South Korea).

On November 3rd, Case 1 developed fever, cough, sneezing, and anosmia. The patient was on duty as nurse in COVID-19 ICU. Therefore, the patient's naso-oropharyngeal swab sample was taken in Influenza-Like Illness unit on November 4th. The RT-PCR (SD BIOSENSOR, South Korea) result was positive for SARS-CoV-2 with Ct-value of 22.19 (ORF1ab) and 21.78 (E). The patient was further hospitalized. Laboratory test showed neutropenia and lymphocytosis (**Table 1**) while chest x-ray was within normal limits. The patient had no comorbidities. The patient was prescribed levofloxacin, dexamethasone, enoxaparine, favipiravir, acetylcysteine, vitamin D, vitamin B, and curcuma.

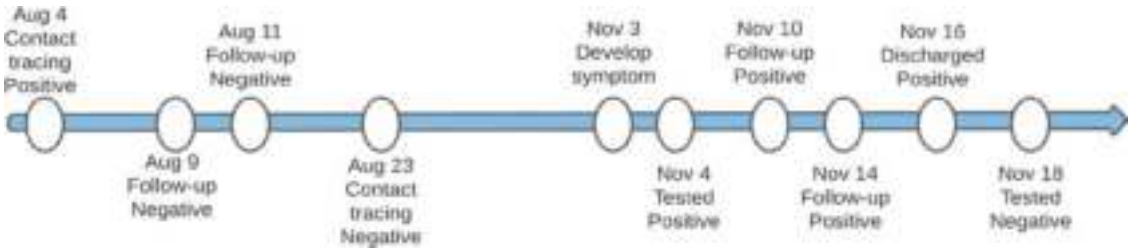

Figure 1. Timeline of Case #1

Table 1. Routine hematology result from first and second COVID-19 episode of Case #1.

Parameter	First COVID-19 Episode	Second COVID-19 Episode	Reference
Hemoglobin (g/dL)	13.1	13.8	11.7 – 15.5
Hematocryte (%)	38.9	39.9	35 – 47
Erythrocyte (mio/µL)	4.66	4.88	3.8 – 5.2
MCV (fL)	83.5	81.8	79 – 99
MCH (pg)	28.1	28.3	27 – 31
MCHC (g/dL)	33.7	34.6	33 – 37
Leucocyte (mio/µL)	9.3	5.8	4.8 – 10.8
Basophil (%)	0	0	0 – 1
Eusinophil (%)	1	1	2 – 4
Neutrophil (%)	58	39	50 – 70
Lymphocyte (%)	36	53	25 – 40
Monocyte (%)	5	7	2 – 8
Thrombocyte (mio/µL)	269	240	150 – 440
Absolute lymphocyte (mio/µL)	3.32	3.08	1 – 4
Neutrophil-Lymphocte Ratio	1.61	0.74	< 3.13
C-reactive protein	1.12	N/A	<5
D-Dimer	N/A	159.53	<500

The patient was followed up on November 10th and 14th. Both came back positive, with Ct-value of 33.78 (ORF1ab) & 31.66 (E gene) for the first follow-up and 35.29 (ORF1ab) and 33.82 (E gene) for the second follow-up. After symptom resolution, patient was discharged on November 16th with positive SARS-CoV-2 RT-PCR result of Ct-value 34.85 (ORF1ab) and 35.19 (E). All three follow-ups used the same RT-PCR kit (SD BIOSENSOR, South Korea). On November 18th, her naso-oropharyngeal swab was tested negative for SARS-CoV-2 RNA (GB SARS-CoV-2 Real-Time RT-PCR, GBC, Taiwan).

Case 2

The timeline of Case 2 is summarized on **Figure 2**. A 27-years old male self-reported malaise on September 15th, 2020. The patient's naso-oropharyngeal swab was tested for SARS-CoV-2 on September 18th using DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit (SolGent, South Korea) resulting in positive result with Ct-value of 38.62 (ORF1a) and 38.08 (N). The same sample was re-extracted and retested using the same kit, resulting in same positive result (36.90 ORF1a, 36.74 N). His blood parameter and chest x-ray were within normal range, thus the patient conducted self-isolation at home. The patient had paracetamol, omeprazole, vitamin C, vitamin D, zinc, azythromicin, oseltamivir.

The patient reported going out for lunch with 3 people on September 10th. His contacts were additionally tested on September 19th. Two of them were negative, but one contact was tested positive (37.12 ORF1a, 37.70 N, DiaPlexQ™ kit). The contact reported sorethroat and cough starting on September 12th. Additional contact tracing from the abovementioned contact found another positive contact. The patient Case #2 was followed up on September 21st using the same test kit and his naso-oropharyngeal swab was tested negative. The patient continued self-isolation for additional 1 week. However, when tested for antibody using STANDARD Q COVID-19 IgM/IgG Combo Test Kit (SD Biosensor, South Korea) on October 26th, the patient was non-reactive for both IgM and IgG. The patient had no history of immunocompromised and was not taking any immunosuppressive drugs.

The Case #2 travelled inter-province on October 31st evening by car with a driver and

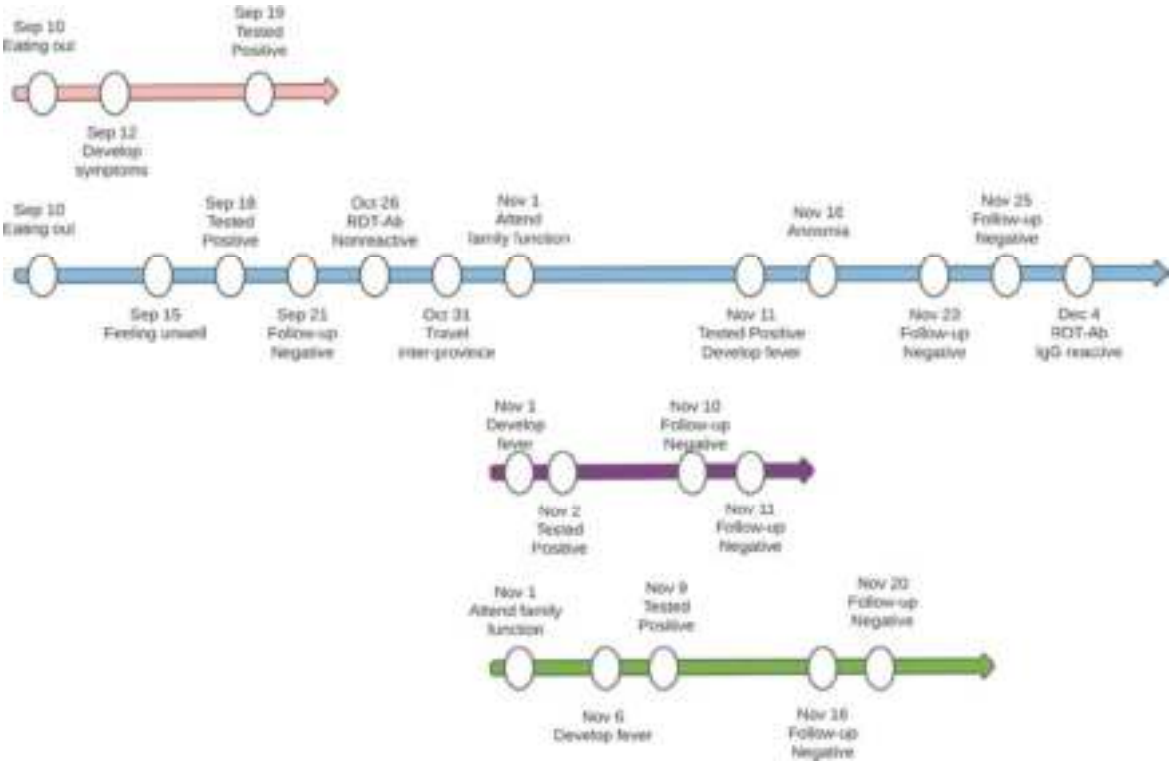

Figure 2. Timeline of Case #2 (blue) and of his contacts, friend (red), father (purple), and mother (green). RDT-Ab: rapid antibody test

arrived on November 1st morning to attend a family function. In the same morning, the patient's father developed fever and did not attend the family function. The father's nasopharyngeal swab was tested for SARS-CoV-2 (mBioCoV-19, BioFarma, Indonesia) on November 2nd with positive result (32.01 ORF1b, 35.21 RdRp). The patient's mother, who attended the family function on November 1st, developed fever on November 6th. Her nasopharyngeal swab was tested for SARS-CoV-2 (STANDARD M, SD Biosensor, South Korea) on November 9th with positive result (27.54 ORF1ab, 28.38 E).

Nasopharyngeal swab was taken from Case #2 for SARS-CoV-2 testing on November 11th morning as part of contact tracing while reporting no symptoms. However later in the evening, the patient developed fever (37.8°C) which on the following day reached 38.8°C. On November 13th, the SARS-CoV-2 test result came back positive (28.39 ORF1ab, 26.39 E, STANDARD M kit). Chest x-ray and blood analysis were not performed. The patient conducted self-isolation at home. The patient had paracetamol, omeprazole, vitamin C, vitamin D, zinc, azythromicin, oseltamivir. On November 16th the patient started to develop anosmia.

The Case #2's father was tested negative on November 10th and 11th, the mother was tested negative on November 16th and 20th, and the Case #2 himself was tested negative on November 23rd and 25th. All follow-up tests were conducted on nasopharyngeal swab sample using STANDARD M kit (SD BIOSENSOR, South Korea). The

Table 2. Possibility of reinfection based on CDC (2020) and Yahav (2020) criteria

Definition	Criteria	Case #1	Case #2
US CDC (2020)			
Suspected reinfection			
Characteristic clinical symptoms on 2nd episode	+	Fever, cough, sneezing, anosmia	Fever, anosmia
RT-PCR of 2nd episode	Ct < 33	22.19 (ORF1ab), 21.78 (E)	28.39 (ORF), 26.39 (E)
Timeframe from 1st episode	≥45 days	92 days	56 days
Close-contact	+	n/a	+
Viral RNA sequence	Different strain	n/a	n/a
Yahav et al. (2020)			
Confirmed reinfection			
True 1st episode	Ct value < 35	35.50 (RdRP)	38.62 (ORF1a) 38.08 (N) Same specimen retested: 36.90 (ORF1a) 36.74 (N)
Characteristic clinical symptoms on 2nd episode	+	+	+
RT-PCR of 2nd episode	Ct < 35	+	+
Negative test between 1st and 2nd episode	At least 1, ideally 2	3	1
Viral culture / subgenomic RNA*	+	n/a	n/a
Timeframe from 1st episode	>90 days†	92 days	56 days‡
Viral RNA sequence	Different strain	n/a	n/a
Clinical reinfection			
Characteristic clinical symptoms on 2nd episode	+	+	+
RT-PCR (Ct < 35)	+	+	+
Viral culture / subgenomic RNA[a]	+	n/a	n/a
Epidemiological risk factor	+	+	+

* Optional to provide evidence of replicating virus.
† Could be <90 days if recovery proven by negative PCR tests and current known COVID-19 exposure.
‡ Had one negative RT-PCR after 1st episode and close-contact with two laboratory-confirmed COVID-19 cases before 2nd episode.

Case #2 was tested on December 4th for using COVID-19 IgM/IgG Combo Test Kit (SD Biosensor, South Korea), resulting in IgG strong reactivity and IgM weak reactivity.

In most reported reinfection cases (**Table 3**), the first positive results were mainly asymptomatic or mildly symptomatic with high Ct-value and followed by nonreactive antibody test. Our Case #2 was nonreactive for both IgM and IgG within 6 weeks after the first, mildly symptomatic infection. To the extent of our knowledge, Case #2 had neither immunocompromised nor immunosuppressed condition which was shown by the presence of IgG after the second, symptomatic infection. Sensitivity and specificity of the rapid antibody test kit could play a factor. However, it could also be due to lower antibody response in mild cases compared to more severe cases.[12–13] Studies regarding antibody against SARS-CoV-2 and its persistence have also been contradictory. Several studies showed waning response while others showed lasting immunity. Non-hospitalised patients have been shown to have more rapid decline of antibody titer.[12] Ibarrondo et al. showed declining antibody with half-life of 36 days.[14] Jeewandara et al. also showed that 4 out of 13 mild COVID-19 patients had no detectable neutralizing antibody (NAb) at 40 days since illness onset.[13] On the other hand, Choe et al. showed that antibody against SARS-CoV-2 was still present at 8 months after asymptomatic or mild COVID-19.[15] Rodda et al. showed that not only antibody but also both memory B and memory T cell persisted at least 3 months after mild SARS-CoV-2 infection.[16] It is important to note that in both Choe et al. and Rodda et al. not all mild patients had seropositivity, with only 85% and 69.0-91.4%, respectively.[15–16] Another possible factor for reinfection is the low viral load

Table 3. Summary of several SARS-CoV-2 reinfection reports

Cases	Sex	Age (years)	1st Episode (RT-PCR)	2nd Episode (RT-PCR)	Timeframe (days)	Negative test between episodes	Epidemiological risk faktor	Viral RNA sequence
Pekanbaru Case 1	F	26	Asymptomatic 35.50 (RdRP)	Symptomatic 22.19 (ORF1ab) 21.78 (E)	92	3	Healthcare worker	N/A
Pekanbaru Case 2	M	27	Mild 38.62 (ORF1a) 38.08 (N)	Worse 28.39 (ORF) 26.39 (E)	56	1	Close-contact	N/A
Hong Kong (*2*)	M	33	Mild (Positive)	Asymptomatic 26.69	142	2	Travel abroad	Different clade
USA (*3*)	M	25	Mild 35.24	Hospitalized 35.31	48	2	N/A	Same clade, Genetically distinct
Belgium (*4*)	F	51	Mild 25.6 (N1) 27.2 (N2)	Milder 32.6 (N1) 33.2 (N2)	93	N/A	N/A	Different clade
Ecuador (*5*)	M	46	Mild 36.85 (ORF3)	Worse 30.82 (N)	63	1	N/A	Different clade
UK (*6*)	M	25	Mild (Negative, reactive antibody)	Milder (Positive)	>90	-	Close-contact	N/A
USA (*7*)	M	82	Hospitalized (Positive)	Hospitalized (Positive, high Ct- value)	55	2	N/A	N/A
Bangladesh (*8*)	M	40	Mild (Positive)	Mild (Positive)	53	1	Healthcare worker / Contact	N/A
Israel (*9*)	F	20	Mild (Positive)	Asymptomatic (Positive)	~90	2	Close-contact	N/A

during the first episode. Kim et al. showed in ferret model, lower viral load in the first episode resulted in lower NAb titer which correlated to reinfection when challenged with heterologous virus three weeks after primary infection.[17] In both of our cases, low viral load (indicated by high Ct-value) during the first episode and low antibody titer (showed by non- reactive rapid antibody test of Case #2) might had left them susceptible to reinfection after more than 45 days or 7 weeks from primary infection.

Our case report also highlights important public health messages. Our cases had considerably easier testing access therefore could be tested while presenting no or mild symptoms. It is possible that we are missing many reinfection cases with asymptomatic or mild SARS-CoV-2 infections due to limited access to testing. Widespread testing and data management might enable us to observe more possible SARS-CoV-2 reinfection cases. The current national report system, New All Record, has continuous data per personal ID number therefore it could be utilized to observe possible reinfection cases.

Additionally, investment in WGS platform, especially automated platform will surely be beneficial to confirm reinfection cases during current COVID-19 pandemic. Adaptation of routine whole genome sequencing in public health laboratory will support epidemiological analysis to detect, monitor, and control circulating or emerging pathogens in Indonesia.[18–19] Lastly, as shown by our case report, natural infection might result in varied immune response due to the varied viral load. We will require safe and effective vaccines as well as robust vaccination program to achieve herd immunity against SARS-CoV-2.

CONCLUSION

We presented two possible SARS-COV-2 reinfection cases from Pekanbaru, Indonesia. Both cases mostly fulfilled US CDC criteria for suspected reinfection, namely presence of typical clinical COVID-19 on 2nd episode, RT-PCR with Ct-value of less than 33 on 2nd episode, as well as timeframe of more than 45 days between 1st and 2nd episode. One of the cases, Case #2, had close-contact while Case #1 had epidemiological risk factor as healthcare worker.

However, in both cases, no viral RNA sequences or viral viability data were obtained. Both data are relatively laborious to obtain and not routinely conducted in public health laboratories, hindering many to observe and report (possible) reinfection cases. As reinfection cases could provide better understanding on immunity to SARS-CoV-2, definition and criteria of SARS- CoV-2 reinfection case are needed to capture and observe such cases. Combination of reinfection criteria from CDC[10] and Yahav et al.[11] could be adapted. Additionally, in order to fulfill the requirement of WGS data, investment in routine use of automated whole genome sequencing platform in public health laboratories is needed.

ACKNOWLEDGMENTS

Figures were designed by Fajri Marindra Siregar. We thank members of Biomolecular Laboratory for insightful discussion regarding this case report.

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Liver Cirrhosis in Woman with Ciliopathy Syndrome

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ABSTRACT

Ciliopathy syndrome is a congenital abnormality of structure and/or function of cilia, which causes pleiotropic disorder, including liver cirrhosis. This study aimed to describe a unique case of liver cirrhosis with possible aetiology of ciliopathy syndrome. A 44 year-old woman with chief complain of hematemesis had diabetes mellitus, obesity, dyslipidaemia, amenorrhoea and often became unconscious. We found short stature, brachydactyly, hyperpigmented maculae in trunk and four limbs, and hepatosplenomegaly. The laboratory results showed: haemoglobin 7.4 g/dl; albumin 2.42 g/dl; urea 84.8 mg/dl; creatinine 2.4 mg/dl; prolactin 138.8 ng/ml, while HBsAg was negative and anti-HCV was non-reactive. Abdominal ultrasonography showed liver cirrhosis; endoscopy showed grade 3 oesophageal varicose; FibroScan® showed 75 kPa; liver biopsy showed hydropic degeneration and cirrhosis; and head CT scan showed chronic lacunar infarction of corona radiata and mega cisterna magna occipital. We reported female with oesophageal varicose rupture, short stature, brachydactyly, obesity, diabetes mellitus, dyslipidaemia, hyperpigmented maculae, liver cirrhosis and mega cisterna magna, which was likely to suffer from ciliopathy syndrome.

***Keywords**: short stature, brachydactyly, insulin resistance, cirrhosis, ciliopathy.*

INTRODUCTION

Rare aetiologies of liver cirrhosis include disorders.[1] One of the most rare aetiologies of liver cirrhosis associated with genetic disorders is the entity of cilia abnormality. Ciliopathy involves a variety of anatomical abnormalities.[2] Unfortunately, genetic examination is still out of reach due to lack of diagnosis tools in our country. Ciliopathy is rarely discussed in daily clinical practice because of its rarity, and will be discussed in this case report.

CASE ILLUSTRATION

A 44 year old unmarried woman from Javanese ethnic, worked as a tailor, was referred from private hospital to our hospital due to main complaint black tarry stool and bloody vomiting. She passed black tarry stool and vomited blood 2 days before admission, 3-4 times a day about a half glass volume each vomit. She did not feel any pain at her stomach, only bloating sensation and dizziness. She had similar condition one year earlier, had been hospitalised and got transfusion with packed red blood cells at that time. Since one year ago, she suffered enlarged abdomen, and after treated with medication, known by her as diuretics and some other drugs, her abdomen circumference gradually reduced. The patient never experienced a yellowish skin and tea like colour urine. Patient experienced itchy skin almost the entire body, especially her arms and legs; she often scratched it and leaving black spots on it.

She had suffered from diabetes mellitus since for four years before, and took oral anti-diabetes. She also often had high levels of blood

cholesterol. She complained temporary blurred vision and sometimes experienced fainting with unknown causes, and did not remember the incident before it. Several times she woke from fainting in various rooms at home including bathroom without anyone knowing and helping. Her menstrual period was irregular, and the last four months she did not have menstruation. She was short statured since childhood, and other family members who had short stature was her older sister. She was obese, but her weight slowly decreased since the last two years. She never consumed herbs, analgesics, alcohol and smoking.

At admission, she was moderate ill, fully conscious, blood pressure was 130/70 mmHg, pulse rate 70 beats per minute regular, respiratory rate 20 times per minute, axillary temperature 36 °Celsius, weight 50 kilograms, height 145 centimetres, and body mass index 24 kilograms/centimetres^2 (overweight). She looked anaemic and had asymmetric facial expression which we concluded as paresis of right nerve VII upper motor neuron type (**Figure1**). We found her had hepatomegaly with liver span of 14 cm, splenomegaly Schuffner 2, and shifting dullness test was positive which we concluded as ascites. She had short fingers and toes (*brachydactyly*) (**Figure 2**) and multiple hyperpigmented macules in the upper and lower extremities (**Figure 3**).

Abnormal laboratory findings were haemoglobin 7.4 g/dl, albumin 2.42 g/dl, urea 84.8 mg/dl, creatinine 2.4 mg/dl, prolactin 138.8 ng/ml, while HBsAg was negative and anti-HCV was non reative. Abdominal ultrasonography showed liver cirrhosis (**Figure 4**) and FibroScan® showed 75 kPa (F4). We conducted endoscopy, and it showed grade 3 oesophageal varicose (**Figure 5**). Liver biopsy showed hydropic degeneration and cirrhosis. We also conducted head CT scan with contrast because the patient had neurological deficits and often fainted, and it showed a chronic lacunar infarction at right corona radiata and mega cisterna magna occipital (**Figure 6**). We consulted the patient to Neurology Department to evaluate neurological problem. They diagnosed her with benign peripheral positional vertigo.

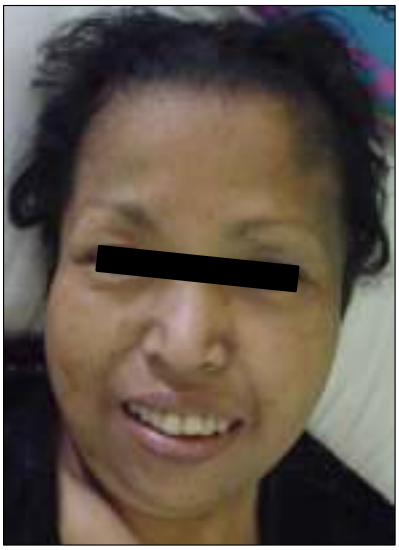

Figure 1. The patient's face looked asymmetry, concluded as paresis of right nerve VII upper motor neuron type.

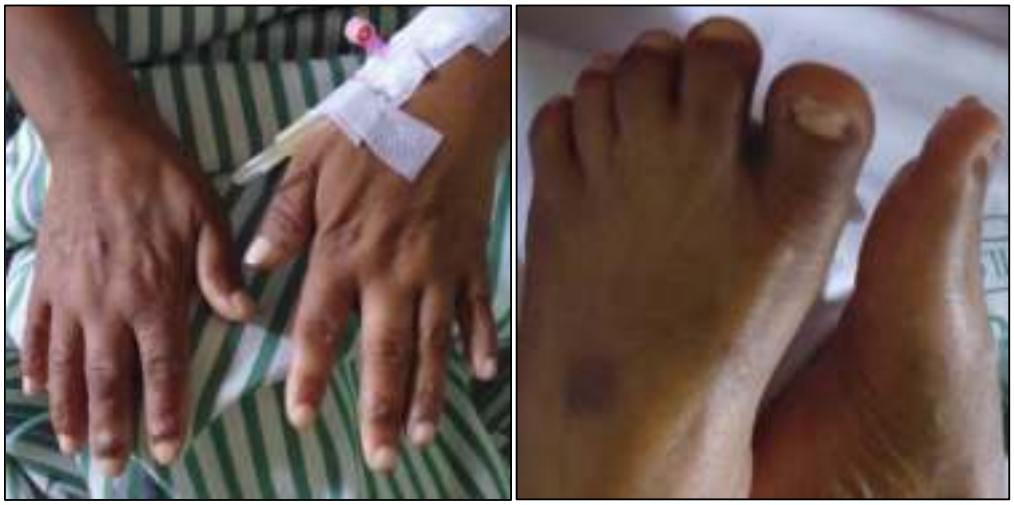

Figure 2. The fingers and toes of the patient were short (*brachydactyly*). The patient's stature is also short and obese.

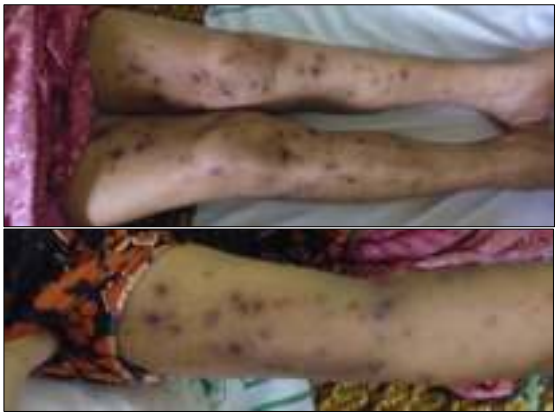

Figure 3. Upper and lower extremity of the patient had multiple hyperpigmented macules.

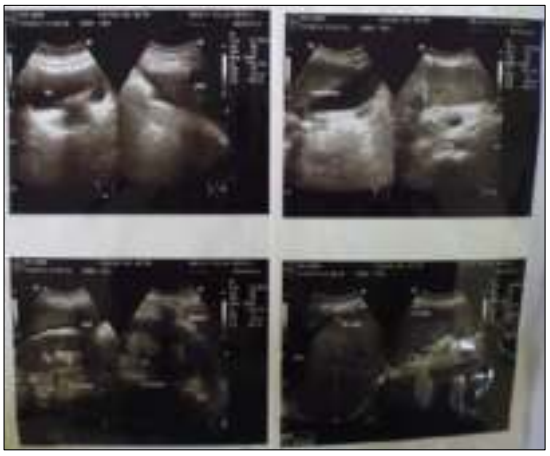

Figure 4. Abdominal ultrasonography showed liver cirrhosis with ascites.

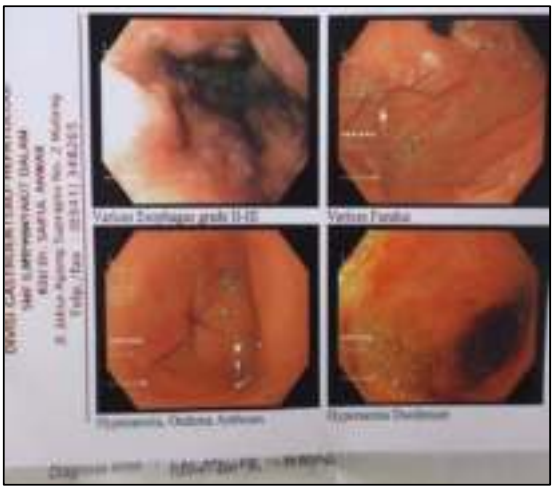

Figure 5. Endoscopy showed grade 3 oesophageal varicose.

We treated the patient with octreotide bolus 50 mcg iv, continued with drip 50 mcg/ hour, lansoprazol 30 mg iv every 12 hours, metoclopramide 10 mg iv every 8 hours, ceftriaxone 1 gram iv every 24 hours, insulin long acting 10 units subcutaneous at bed time, lactulose 1 table spoon 3 times a day, betahistine mesylate 24 mg pro re nata, PRC transfusion and albumin transfusion. After the hematemesis was resolved, we gave her propranolol 20 mg every 8 hours and spironolactone 100 mg a day. With several anatomical abnormality in this patient, we suspected her to had ciliopathy syndrome, but definitive diagnosis, gen abnormality, could not be performed due to lack of diagnostic tool in our hospital.

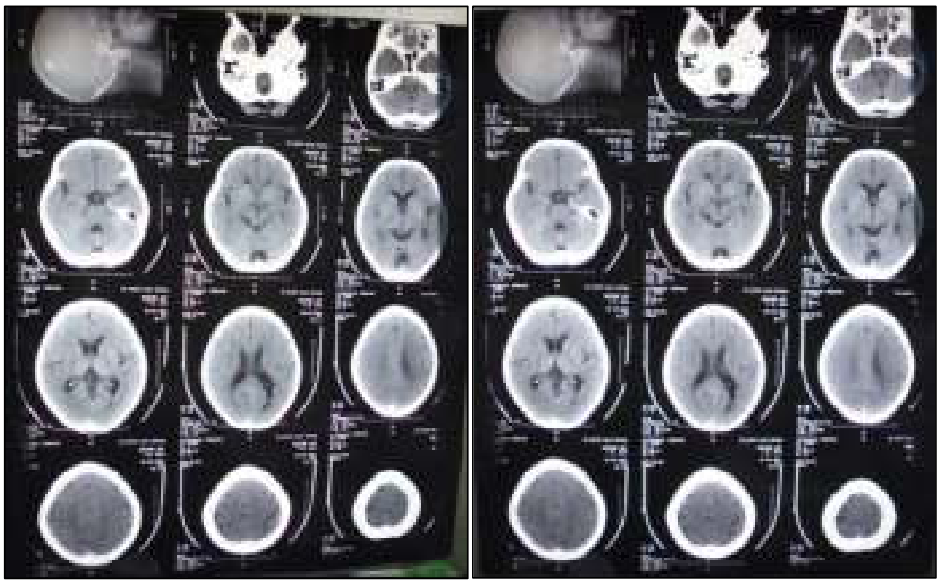

Figure 6. Head CT scan showed a chronic lacunar infarction at right corona radiata and mega cisterna magna occipital

DISCUSSION

Patient presented with hematemesis and melena. We found hepatosplenomegaly, abdominal ultrasonography described liver cirrhosis and FibroScan® showed high degree liver fibrosis. Generally, in hepatic cirrhosis, we find liver in smaller size. However, there are several conditions of liver cirrhosis with hepatomegaly, such as cardiac cirrhosis (Laennac cirrhosis) and fatty liver as seen in non-alcoholic fatty liver disease (NAFLD) that develops to liver cirrhosis.[3] Endoscopy revealed that she had grade 3 oesophageal varicose as the cause of hematemesis. This supported diagnosis of liver cirrhosis. Liver biopsy result was hydropic degeneration which was usually caused by hepatic cell injury. Chronic liver injury can cause cirrhosis and it is important to determine the specific aetiology given its implications in patient management and its long-term outcomes. Certainly, if the aetiology of a disease remains unknown, effective therapy can not be performed. We had excluded common aetiology of liver cirrhosis in the patient, such as viral hepatitis infection and autoimmune hepatitis. Epidemiologically, the most common aetiology of liver cirrhosis other than hepatitis viral infection is NAFLD, which usually revealed fatty degeneration on liver biopsy, but we found no fatty degeneration in the patient.[4,5] However, we considered that she had diabetes, dyslipidaemia and obesity. NAFLD is associated with metabolic syndrome and insulin resistance.[5,6] Obesity is common and well-documented risk factor for NAFLD.[4,5,6] There is a very high prevalence of NAFLD in individuals with type 2 diabetes mellitus. High serum triglyceride levels and low serum HDL levels are very common in NAFLD patients.[7] The prevalence of NAFLD in individuals with dyslipidaemia is estimated to be 50%.[8,9]

Although metabolic disorders such as obesity and type 2 diabetes are increasing in global pandemics, their pathophysiology and molecular basis are not fully understood.[8,9] The cause of obesity is complex, because many confusing genetic and environmental factors are not obviously affecting it.[6] In addition, metabolic disorders involve interconnected disease which can be exemplified by the association of obesity with insulin resistance, leading to the development of type 2 diabetes.[6,7] The genetic factors for obesity are poorly understood. Genomic association studies support the idea that some genes, tissues, and pathways, contribute to this disease. An interesting gene subsets associated with obesity are caused by primary ciliary dysfunction, resulting in a rare pleiotropic disorder in humans called ciliopathy syndrome.[8]

Primary cilia may act as sensory cell antennae, coordinating intercellular communications via receptor clustering and signalling.[10] Bardet-Biedl syndrome (BBS) is the archetypical example of a ciliopathy with profound appetite dysregulation. BBS children are unable to resist the drive to eat, becoming massively obese at an early age, and about half develop type 2 diabetes mellitus and metabolic syndrome. Another childhood obesity syndrome that may be ascribed to a ciliopathy is Alström syndrome (AS).[10,11] In addition to their respective specific features (skeletal, retinal, renal and hepatobiliary fibrocystic abnormalities, hearing defects and infertility), BBS and AS are both associated with hyperphagic obesity, early onset of insulin resistance, type 2 diabetes mellitus and (best described for AS) severe fatty liver disease leading to cirrhosis.[12] An additional exciting finding is that pre-adipocytes also express primary cilia, and these play a role in their capacity to differentiate and form triglyceride-storing adipocytes and secrete adiponectin.[11] The mice carrying a gene mutation for the basal body protein of cilia underwent NAFLD.[12,13] This is very appropriate with the condition of our patient who had characteristics of ciliopathy syndrome and also suffer from diabetes mellitus and history of obesity and dyslipidaemia.

It was very interesting that in this case, the patient also had body dysmorphism. She had asymmetrical facial expression, short stature and brachydactyly. Short stature and brachydactyly was also found in her older sister. She also suffered oligomenorrhea and even amenorrhoea in the last 4 months, had high level of serum prolactin and mega cisterna magna in the brain. We suggested that these abnormalities were related to one disease entity or syndrome and

associated with her liver cirrhosis. When the structure or function of the cilia is defective, it affects most of the body's organs such as kidneys, brain, limbs, eyes, ears, liver and bones.[11,13,14] The unique characteristics of ciliopathy show a broad phenotypic spectrum determined by the degree of damage to the affected cilia and tissue specificity. The symptoms of ciliopathy vary greatly depending on the affected genes and their role in ciliogenesis and ciliary function.[10,11]

Cilia falls into two broad categories: motile and immotile.[10] Primary cilia are typically immotile and consist of nine peripheral doublet microtubules; while motile cilia, in addition, contain a central pair of singlet microtubules ("9+2" arrangement) to which they are connected by the radial spike proteins. Immotile cilia are characterised by the absence of the central pair of singlet microtubules ("9+0" arrangement).[11] Motile cilia are distinguished from primary cilia by their ability to beat rhythmically, an activity that is powered by adenosine triphosphate (ATP), hydrolysed by dynein proteins, which are anchored to the inner and outer aspects of peripheral doublet microtubules.[11,12] Motile cilia are utilised in both unicellular and multicellular organisms for locomotion. Primary cilia have chemosensory, osmosensory and phototransduction functions.[10,11,12]

As cilia are a component of almost all vertebrate cells, ciliary dysfunction can manifest as a constellation of features include congenital fibrocystic diseases of the liver and pancreas, diabetes, obesity and skeletal dysplasia.[10,11] Phenotypically heterogeneous, ciliopathic features can manifest from variation at a single locus while mutations affecting a number of different loci can, at the same time, result in similar phenotypes. Within each organ, diseases can be developmental phenotypes presenting at birth or later in childhood.[13] Often this may depend on the severity of the underlying mutation in addition to the number of defective proteins encoded where more than one mutation in a ciliary gene occurs.[14] Ciliary membranes contain receptors and ion channel proteins mediating cell signalling, including roles for Sonic Hedgehog (SHH), Wnt and PDGFa signalling pathways that control diverse processes (e.g., cell differentiation, migration, axonal path finding, and planar cell polarity).[14] The SHH pathway is important for dorsal-ventral patterning of the neural tube and, later, for proliferation of cerebellar granule cells. Defects of this pathway can cause anomalies of the cerebral commissures.[15,16]

Intact cilia-based signalling is required for normal development of the biliary and portal system in the liver. The majority of diseases manifesting with hepatic fibrocystic pathology are caused by defective ciliary proteins.[12] Congenital hepatic fibrosis is a histopathological diagnosis with three main components; that is, defective remodelling of the ductal plate; abnormal portal veins; and progressive fibrosis of the portal tracks. The major morbidity associated with congenital hepatic fibrosis is portal hypertension.[12,13] Congenital fibrocystic diseases of the liver are a heterogeneous group of disorders that are characterised by a spectrum of biliary dysgenesis that includes congenital hepatic fibrosis, bile duct dilatation and cyst formation. Defects in cholangiocyte ciliary structure and/or their integrated transducing function lead to a decrease in intracellular calcium and increased cAMP, causing cholangiocyte hyperproliferation, abnormal cell matrix interactions and altered fluid secretion/absorption, which can result in hepatic cystogenesis.[13]

Emerging data indicate that hedgehog signalling, one of signalling pathway in primary cilia, mediates both adaptive and maladaptive responses to liver injury, depending upon the balance between its actions as a regulator of progenitor cell growth and its ability to promote liver inflammation and fibrogenic repair.[14] Synthesis of hedgehog ligands is stimulated by diverse factors that trigger liver regeneration, including both liver cell mitogens and liver cell stressors. These Hh ligands, in turn, are released from ligand-producing cells into the local environment where they engage receptors on Hh-responsive cells. The latter include progenitor cells, hepatic stellate cells, sinusoidal endothelial cells and certain types of resident hepatic immune cells. In general, Hh ligands function as trophic factors and promote the viability of Hh-target cells.[13,14] This enhances the outgrowth

of liver progenitor populations, triggers tissue remodelling, and promotes liver regeneration. However, Hh ligands also stimulate certain cell types (e.g., hepatic stellate cells, immature liver epithelial cells) to acquire a less epithelial and more mesenchymal state during which such cells generate inflammatory mediators and scar tissue, therefore, induces liver fibrogenesis. Hence, excessive or persistent Hh pathway activity actually aborts successful regeneration of damaged liver tissue and contributes to the pathogenesis of liver fibrosis.[14]

Findings of skin disorders in this patient led us to make the differential diagnosis of neurocutaneous disorder due to ciliopathy. Several case reports also presented skin disorders in patients with ciliopathy syndromes in the form of hyperpigmentation macules and sometimes also in the form of multiple nevus.[16,17,18] These skin disorders often coincided with pigmented abnormalities in the patient's cerebral meninges.[17,18] We tested the patient for serum prolactin level because of amenorrhoea. The condition of hyperprolactinemia in her could be caused by liver cirrhosis or stood alone. Hyperprolactinaemia could also be caused by her obesity.

Management of liver cirrhosis in patient with ciliopathy syndrome is same with that of other aetiology. The aetiology of cirrhosis in ciliopathy is related to development of NASH; so, the management of metabolic condition related to obesity and insulin resistance and dyslipidaemia should be optimized.

CONCLUSION

We reported woman with hematemesis, short stature, brachydactyly, hyperpigmented maculae, liver cirrhosis, and mega cisterna magna, which was likely to suffer from ciliopathy syndrome; however, genetic tests has not been performed on the patient yet. Management of patients with this syndrome is same as liver cirrhosis caused by other aetiology. Follow-up related to other organ abnormalities in the future, is necessary. Appropriate genetic counselling and family member screening should be performed. The definitive diagnosis necessarily requires chromosome and gene analysis, which is not available here.

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Successful Treatment Bilateral Panuveitis with Multiple Systemic Infection in HIV/AIDS Patient: A Case Report

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ABSTRACT

There is an increasing number of HIV/AIDS patients in Indonesia, starting from <0.1% in 2010 to 0.4% in 2012, which warrants awareness of ocular manifestation in HIV. This might appear in 70-100% of patients with HIV. A 47 years old man came to the infection and immunology clinic with blurry vision on both eyes. He had been treated before but there was no clinical improvement. Examination showed both eyes had vitreous haziness. Visual acuity was 1/60 in both eyes with appearance of flare and cells within +3. Uveitis workup showed positive results for HIV, HSV and syphilis. Patient was given 100 mg of doxyciclin two times daily and fixed dose tablet which contains the combination of antiretroviral. Three months later, final acuity was 6/10 on the right eye and 6/18 on the left eye. Prompt diagnosis and treatment warrant good prognosis including multidisciplinary approach by ophthalmologist, clinical allergist and immunologist, and dermato-venerologist.

Keywords**:** *HIV/AIDS, syphillis, ocular manifestation in HIV, bilateral panuveitis.*

INTRODUCTION

Increasing number of HIV/AIDS patients in Indonesia, from <0.1% in 2010 to 0.4% in 2012, warrants awareness of ocular manifestations of HIV. Ocular manifestation of HIV might appear in 70-100% patients. In some cases ocular manifestations might be the initial presenting clinical finding in patients with HIV/AIDS.[1] Various causative agents might cause uveitis in these population, such as CMV, syphilis, toxoplasma, herpes, and tuberculosis.[1-3]

In this case report we present a successful management of panuveitis in HIV patients. The aim of this report is to demonstrate the management of uveitis in HIV patients and highlighting the importance of prompt and accurate diagnosis as well as proper treatment.

CASE ILLUSTRATION

A 47 years old man came with complaint of blurry vision for 2 months. One year before the patient complaint of having blurry vision and floaters on his left eye. Two months before, the vision worsen accompanied with red eyes. The patient was initially treated with prednisolone acetate eye drop. There was history of promiscuity and tattoo.

Visual acuity was 1/60 in both eyes with cells +3 and flare. The vitreous was hazy, the optic nerve can not be examined in detail, exudate was found, other details was hard to be evaluated. The patient was initially assessed with panuveitis on both eyes caused by toxoplasma dd/ cytomegalovirus and AIDS without ARV treatment. The patient was planned to have

fundus photograph taken, uveitis workup. And was treated with by the attending ophthalmologist with trimetripim + sulfametoxazole 2 x 960 mg.

Complete uveitis workup was done to the patient to rule in or out the possibility of infectious disease as the cause of uveitis, and the patient return three days later with the result. Uveitis workup showed positive HIV; positive IgG and IgM for anti-HSV 2; reactive VDRL (1/512) and TPHA test (>1:5120); positive IgG for anti-CMV. CD4+ lymphocyte count was 9% and absolute count was 261 cells/mcL. Other uveitis screening workup such as tuberculin test for tuberculosis, IgM and IgG for toxoplasma, showed negative result excluding the diagnosis. Chest X-Ray showed no signs infiltrate in both lungs.

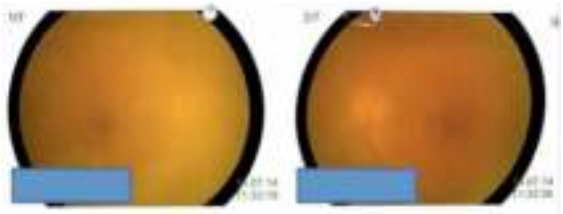

Figure 1. The first fundus photograph showing he optic nerve head was hazy and other details were hard to be evaluated

The patient was treated as syphilitic panuveitis. Because the patient was allergic to penicillin, he was treated with doxyciclin 2 x 100 mg. The HIV infection was treated with fixed dose tablet consisting zidovudine 300 mg and lamivudine 150 mg and efavirenz 600 mg once daily. Because negative result test for toxoplasma, the treatment trimetropim and sulfametoxazole was stopped.

Two weeks later the patient's acuity was 6/20 on the right eye and 6/18 on the left eye. Anterior segment was quiet. Posterior segment shows vitreous cells +1, with normal appearing optic nerve head and retina (**Figure 2**).

Three months after treatment for syphilis, the patient's visual acuity was 6/10 in the right eye and 6/18 in the left eye. Anterior segment and posterior segment were within normal limit. His final titer for VDRL and TPHA was 1:128.

1.

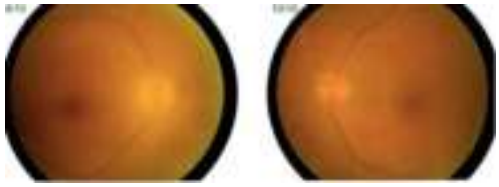

Figure 2. Second fundus and photograph taken 2 weeks later. Optic nerve head was round with clear margin, artery to venous ratio was 2/3, with cup to disc ratio 0.3-0.4, no hemorrhage or exudate can be found on both eyes.

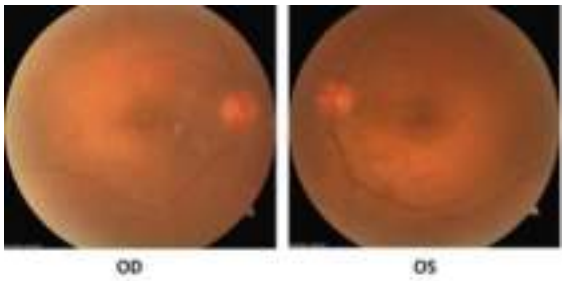

Figure 3. Final fundus photographed. Optic nerve head was round with clear margin, artery to venous ratio was 2/3, with cup to disc ratio 0.3-0.4, no hemorrhage or exudate was found.

DISCUSSION

Most common ocular manifestation in HIV/AIDS patients includes HIV retinopathy, opportunistic infections, Kaposi sarcoma, and adnexal disease, and might occur in 70-100% patients in this population.[1] Uveitis might be the first manifestations of AIDS stage of HIV infections. In patients with HIV/AIDS the course of the uveitis tend to be severe and produce more sequelae.[1-3]

Uveitis in patient with HIV/AIDS might be caused by various etiology. Study in Taiwan in patients with uveitis as initial manifestations of AIDS, showed CMV, syphilis, and toxoplasmosis is the leading pathogen.[2] In HIV patients with CD4+ lymphocyte count >200/mcL, one study showed infectious uveitis is mostly caused by syphilis and herpes virus.[3]

The diagnosis of syphilitic uveitis requires thorough history taking, clinical findings and established through serological test. The most common finding in the fundus are multifocal chorioretinitis associated with vitritis or severe vitritis alone but, necrotizing retinitis, retinal vasculitis, exudative retinal etachment, isolated papilitis and neuroretinitis can also be found.

Panuveitis is not a rare manifestation of syphilitic uveitis, comprising of 40% cases in one study.[4] Two type of serological test are available for syphilis classified as non-treponemal, (e.g. VDRL test) , and treponemal test (e.g. TPHA, FTA-ABS or MHA-TP). Current CDC recommendation is to test patient suspected of having syphilis with treponemal test, then if positive, should be tested for non-treponemal test.[5,6]

Because of the wide possibility of the cause of uveitis in patients with HIV, our patients was tested with various tests to confirm the causative agent. The most common viral infection of uveitis in patient with HIV is cytomegalovirus, thus testing for this disease is very important. Other important causative agents are varicella zoster virus and herpes simplex virus, which, unlike CMV that cause slowly progressive disease, causes rapidly developing and confluent retinitis. Ocular toxoplasmic retinochoroiditis is also one of the most common ocular manifestation in patients with HIV, thus testing for toxoplasma infection is very important in these cases. Infectious agents that can be commonly seen in HIV-negative uveitis patients such as syphilis and tuberculosis should also be tested since it these infection is commonly found in HIV positive patients.[(2-,4)]

The treatment for ocular syphilis is the same with syphilis with neural involvement. CDC only recommended aqueous penicillin G 18-24 MU/d given IV as 3-4 MU every 4 hours for 10-14 days.[5] European guidelines recommend the administration of oral doxycyclin 2 x 200 mg daily in cases of penicillin allergy, but the evidence is very weak (graded IV C).[7] There is one case series that reports the improvement of clinical findings and visual acuity in one patients with ocular syphilis and penicillin allergy.[8]

Our patient was a 47 years old HIV positive male with remarkable risk factors for sexually transmitted diseases. Patients with HIV with history of promiscuity is at risk for other sexually transmitted infection. Examination showed dense vitritis hampering proper examination of retina, and serological test showed positive test for both treponemal and non-treponemal test for syphilis, establishing the diagnosis of syphilitic uveitis. Test for other diseases should be done, because the dense vitritis hampers the evaluation of the retina, making the diagnosis for disease that might cause retinal necrosis worth being considered. Although test for HSV-2 IgM and IgG also showed positive result, clinical examination that support acute retinal necrosis or progressive outer retinal necrosis can not be found, thus excluding this diagnosis. But the serological test is still justifiable because on initial examination dense vitritis hamper the examination of the fundus. Because our patient was diagnosed with syphilitic uveitis in both eyes as well as HSV-2 infection, we advise to cooperate with other fields of expert in order to properly manage this patient.

Our patient was allergic to penicillin, and given doxycycline 100 mg orally twice daily for two weeks. In spite of not adhering to CDC recommendation, and low evidence based on European Guideline it is interesting to note that our patient had dramatic clinical improvement.

The prognosis of patient with ocular syphilis is favorable if treated promptly. One research study showed that the final BCVA for patients who had been treated with penicillin regimen ranging from 20/50 – 20-20 and no recurrences in 2 years. [9] Another report stated that treatment more that 28 days since symptoms occur is a risk factor for poor prognosis.[10]

CONCLUSION

Prompt diagnosis and treatment warrant good prognosis in these population. Our patient came with poor visual acuity and severe inflammatory reaction, but with proper antibiotic and anti-retroviral treatment improve the visual acuity of our patient improve dramatically. The treatment involving multidisciplinary approach by ophthalmologist, clinical allergist and immunologist, and dermato-venerologist is required in these cases.

ACKNOWLEDGMENTS

Address for reprints and sources of support that require acknowledgement is none.

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Sight-Threatening Condition in Severe Thyroid Eye Disease: How We Should Manage

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ABSTRACT

Thyroid eye disease (TED) is an autoimmune disorder that is associated with thyroid gland dysfunction which causes muscle and orbital fat enlargement. This case report is aimed to present a case of sight-threatening TED and how we should manage this condition. We present a case of patient with chief complaint of vision loss and prominent eyes for 5 months prior to the visit to our eye hospital. Patient had sought advice from an ophthalmologist and internist. TED was eventually diagnosed 2 months after consulted with an ophthalmologist in the rural area. According to EUGOGO guidelines, TED with sight-threatening condition should be treated with glucocorticoid IV 500-1000 mg for 3 days consecutively. Although the patient was already given steroid injection for the initial treatment, the dosage was inadequate. After the inflammation process was reduced, the patient was reluctant to have an orbital decompression that was suggested. Hence, TED progressed continuously besides sight-threatening complications arising. He indeed underwent fat decompression and tarsorrhaphy as eyelid surgery to prevent corneal exposure. In follow-up, both visual acuity and corneal improvement were finally achieved. In the management of TED, collaboration between ophthalmologist and internist, who may be specialized in endocrinology, is imperative. They should be able to manage TED promptly and correctly, hence sight-threatening and other complications can be prevented and satisfactory results are achieved. Fat decompression should be considered as a good help to improve visual acuity nevertheless orbital decompression cannot be done.

Keywords: *grave's disease, thyroid eye disease, dysthyroid optic neuropathy, orbital decompression, fat decompression.*

INTRODUCTION

Graves' disease is an autoimmune disease that leads to a generalized overactivity of the entire thyroid gland (hyperthyroidism). It is the most common cause of hyperthyroidism in the United States. Generally occurring in patients with hyperthyroidism, sometimes thyroid-associated ophthalmopathy or thyroid eye disease (TED) occurs in patients with euthyroid or hypothyroid chronic autoimmune thyroiditis. The condition has an annual adjusted incidence rate of 16 women and 3 men per 100.000 population.[1]

TED is an autoimmune disorder that is associated with thyroid gland dysfunction which causes muscle and orbital fat enlargement. TED generally accompanies Graves hyperthyroidism where the course of eye disease does not always parallel with the activity of thyroid gland. The onset of TED can precede, together, or after the onset of hyperthyroidism.[2-5]

TED pathophysiology is complex and the treatment has not fully focused on the pathogenesis of the disease. Although the majority of TED cases are mild, around 3-7% of patients develop vision-threatening complications such as corneal damage or dysthyroid optic neuropathy (DON). The strategy for handling this disease consists of various methods such as medical therapy, surgery, and radiotherapy.[4-6]

The ability of ophthalmologists and internists to diagnose and assess the activity and severity of this disease correctly is expected to help determine the right treatment for patients. In an active TED, proper handling can improve vision, appearance, and quality of life. Therefore, TED is a challenge for ophthalmologists and internists both in diagnosis and appropriate management.[4-6] This case report discussed severe TED with corneal damage and DON. Eventually, TED was diagnosed, yet the treatment given was not according to guidelines, so the patient was deteriorating and in high risk of losing his sight.

CASE ILLUSTRATION

A 64-year-old male was referred to our eye hospital presented with complaint of vision loss, protrusion and soreness on both eyes. Five months prior, he complained of redness, swelling, and blurred vision. There was no other sign of systemic infection, previous illness, and family illness. All of these conditions lead his first-seen-ophthalmologist to diagnose his red eyes with an eye infection. Two months later, he went to an internist with a complaint of trembling, sweating, weight loss, heart palpitations, and sleep deprivation. There was no data about physical examination because he was seeking the internist in another hospital in rural area. The laboratory tests supported the diagnosis of hyperthyroidism and type two diabetes mellitus because he has high level of Total T3 (5.15 ng/mL), high level of Total T4 (20,7 μg/dL) and low level of TSHs (<0,02 μIU/mL). There was no data on blood glucose level. He was a heavy-smoker although he had already been advised to quit smoking. The patient was given Thiamazole 3x10 mg, Glimepiride 1x2 mg, and Univoxy® 1x1 tablet per day from the internist.

One month later, both of his eyes became protruded. Right eye (RE) was blind and left eye (LE) was otherwise normal. Clinically suspected with pseudotumors, the doctor evaluated him with orbital Computed Tomography (CT) Scan, but neither retrobulbar nor pseudotumor was found. This patient was then referred to ophthalmologist at another hospital in urban area.

One month later, he felt his LE vision began to be blurred. Orbital CT-scan depicted an orbital muscle enlargement with a coke-bottle sign and proptosis in both eyes, hence the patient was eventually diagnosed with TED and received steroid injections twelve times for three days (divided doses of steroid) of hospitalization consecutively from the first ophthalmologist. **(Figure 1)** The patient did not remember the dosage of steroid injection that was given by the ophthalmologist. In spite of the inflammation process that was subsided, one week later, swelling of both eyes became recurrent and the visual acuity of his LE got worsened until he lost his vision. The laboratory tests revealed a hypothyroid condition with low FT4 (0.28 ng/dL), high TSHs (28.834 μIU/mL) and low Total T3 (0.46 ng/mL). Hence, the internist in the second hospital reduced the dosage of Thiamazole to 1x10 mg. This patient did not have a regular schedule of follow up for thyroid and diabetes mellitus.

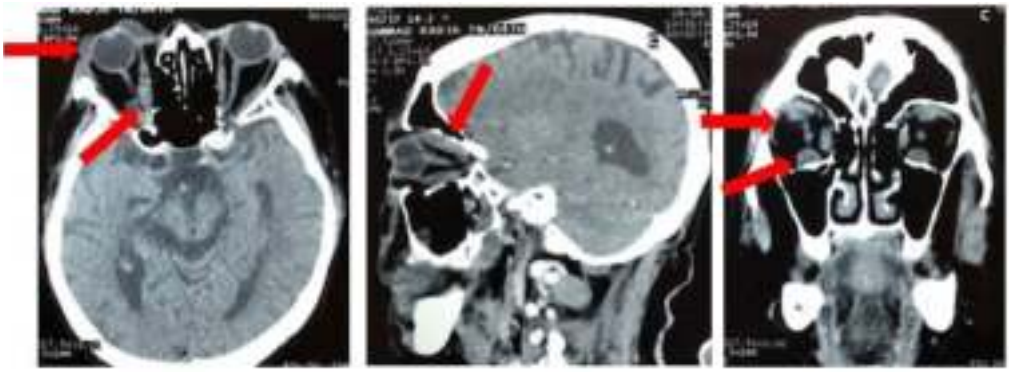

Figure 1. Axial, sagittal, and coronal CT-scan showed proptosis and extraocular muscles enlargement (coke-bottle sign).

The patient came to our eye hospital. On examination, there was no light perception (NLP) for the RE and light perception (LP) for the LE. He has 30° exotropia. Both eye movements were -4 in all directions (fixed eyeball). There were proptosis, edema on upper and lower eyelids, retraction of upper and lower eyelids, lagophthalmos, severe chemosis, and caruncular edema in both eyes. Corneal ulceration in the RE and corneal infiltrate in the LE were also recognized. Pupillary light reflexes were reduced for both eyes, so relative afferent pupillary defect (RAPD) was difficult to be assessed. Cataract could also be identified in both eyes. The result of Hertel exophthalmometers on each eye was 25 mm. Diabetes mellitus was again confirmed by laboratory tests with high level of HbA1c (NGSP 6.4% and IFCC 46 mmol/mol). At the same time, his hypothyroidism condition was supported by laboratory tests such as low FT3 (1.55 pg/mL), low FT4 (0.68 ng/dL), high TSHs (0.375 µIU/Ml), low Total T3 (0,51 ng/mL), low Total T4 (4,49 µg/dL), high TRAb (15,74 IU/L), and low Anti-TPO (<0.5 IU/mL).

Based on all manifestations and work-up studies, this patient had complex problems due to the complications of Grave's disease, such as bilateral proptosis, RE corneal ulcer, LE exposure keratitis, bilateral dysthyroid optic neuropathy (DON), and bilateral immature cataracts. He was hospitalized and treated with Methylprednisolone intravenous (IV) 500 mg for three consecutive days, Chloramphenicol eye ointment 3 times/day, Citicoline 2x500 mg, Mecobalamin 2x500 µg, Gatifloxacin eye drops every 3 hours, and Solcoseryl® eye gel every hour. After the methylprednisolone IV administration, patient's blood glucose level increased to 355 mg/dL indeed. He was given insulin (Lantus 1x14 unit and Novorapid 3x10 unit) and Thiamazole 1x10 mg from the internist.

His LE vision was improved to 2/60 on his fourth day of hospitalization. However, he still had DON and at high risk for corneal perforations on both eyes, hence he was suggested to undergo an orbital decompression. As he was being reluctant to proceed, he underwent fat decompression and blepharorrhaphy. The procedures included chemosis incision, eyelid fat removal, upper and lower blepharotomy, amniotic membrane transplant, bandage contact lenses, and blepharorrhaphy. Two consecutive days after the surgery, he was given an infusion of 250 mg MP per day. The blepharorrhaphy was then released after 3 days. Central corneas were healed but left scars at the inferior corneas of both eyes.

During hospitalization, intraocular pressures (IOP) were high (20.7 mmHg for RE and 34.3 mmHg for LE). He was given Latanoprost-Timolol Maleate eye drops 2 times/day, Acetazolamide 3 x 250 mg, Potassium L-Aspartate 3 times/day, Calcium-Vitamin D_3 2 x/day, Acetylsalicylic Acid 2 x 160 mg, Tobramycin-Dexamethasone eye drops 1 x/day, Gatifloxacin 4 x/day, Solcoseryl® 4 x/day, Selenium 1 tablet/day, Vitamin C 500 mg IV, Ciprofloxacin 2 x 500 mg and Mefenamic Acid 2 x 500 mg.

On the 11th day of hospitalization, his eye movements improved to -3 in all directions for both eyes and his dosage of Methylprednisolone was increased to 500 mg. He was finally allowed to go home on the 12th day of hospitalization. **(Figure 2, Figure 3)**

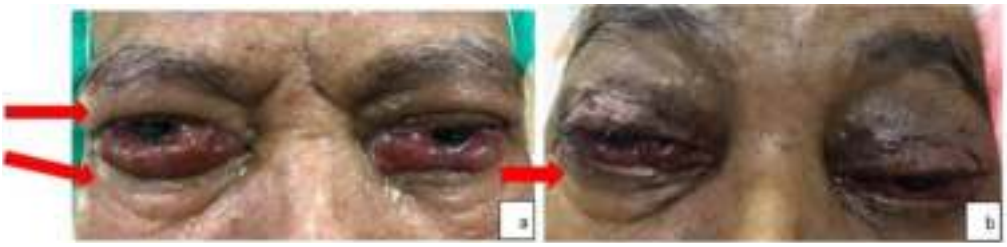

Figure 2. a) before surgery, there were eyelid retraction on the RE and chemosis on both eyes; b) eye condition after fat decompression and blepharorrhaphy was released. Chemosis was gradually diminished but caruncular oedema was still recognized. On the LE, eyelid oedema was slowly subsided.

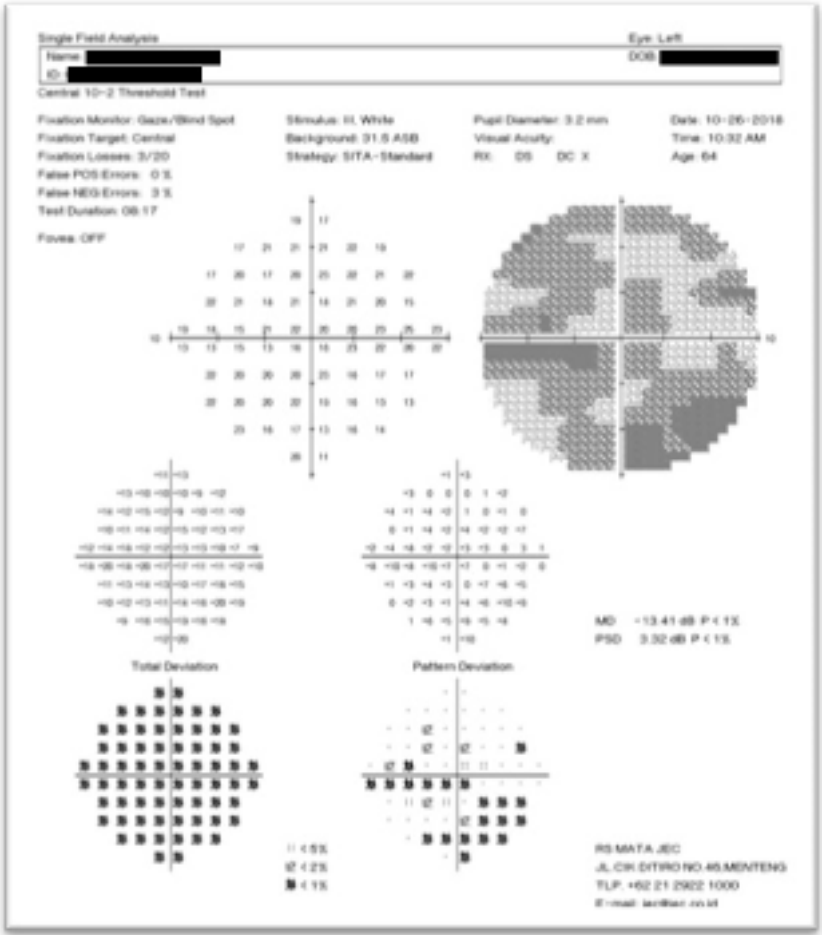

Figure 3. Perimetry of the LE demonstrated a visual field defect.

On the last visit, his eye pain was alleviated, vision of the RE remained NLP and vision of the LE improved to 0.1. Exotropia improved to 15º and both eye movements were improved in all directions as well. In spite of eyelid oedema, caruncular oedema and conjunctival chemosis that were still found, his eyes were generally improved compared to the initial condition. RAPD was positive in the RE. Ishihara test for the LE showed total colour blindness. **(Figure 4)**

DISCUSSION

The assessment recommended by The 2018 European Thyroid Association (ETA) guidelines for suspected Grave's hyperthyroidism is Thyroid-Stimulating Hormone (TSH), free T3 (fT3), free T4 (fT4), TSH Receptor Antibodies (TRAb), and thyroid gland ultrasound examination. TSHs should be used as an initial screening test because it has the highest sensitivity and specificity for evaluating suspected Grave's hyperthyroidism.

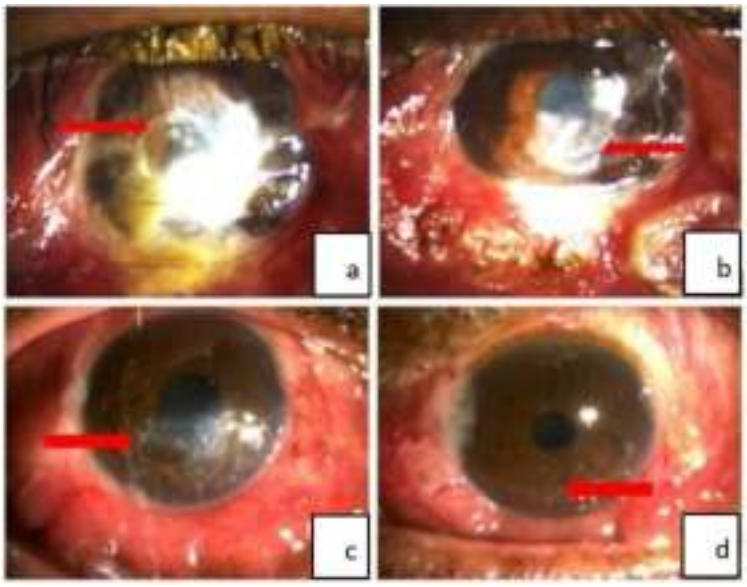

Figure 4. a) corneal ulcer on the RE; b) corneal infiltrate was seen on the LE; c) corneal leucoma (improvement on the RE); d) corneal nebula (improvement on the LE).

Serum TSH levels are more sensitive than thyroid hormone (T3, T4) tests for assessing thyroid hormone excess. Diagnostic accuracy improves when both serum TSHs and fT4 are assessed at the initial evaluation. TRAb measurements are also sensitive and specific in evaluating suspected Grave's hyperthyroidism. TRAb is a specific biomarker for the extrathyroidal manifestations of Grave's disease and correlates with TED activity and severity.[7-10] In Grave's hyperthyroidism, 30-50% of patients will have ocular involvement. In one-third of TED cases, symptoms and signs appear altogether with hyperthyroidism.

Diagnosis of TED is made when 2 of the following 3 signs are present: (1) typical orbital signs (1 or more of the following): unilateral/bilateral eyelid retraction with typical temporal flares (with/without lagophthalmos), unilateral/bilateral proptosis, restrictive strabismus, compressive optic neuropathy, eyelid oedema/erythema, or caruncular oedema/chemosis; (2) immune-related thyroid dysfunction (1 or more of the following): Grave's hyperthyroidism, Hashimoto thyroiditis, or presence of thyroid antibodies without a coexisting dysthyroid state (TSH-receptor antibodies, thyroid-binding inhibitory immunoglobulins/TBII, thyroid-stimulating immunoglobulins/TSI, anti microsomal antibody); (3) radiographic evidence of TED (enlargement of 1 or more of the following): inferior/medial/superior/lateral rectus muscle or levator palpebral muscle.[1-3]

In this patient, the diagnosis of TED was made based on ophthalmology examination, laboratory tests, and orbital CT-scan findings. Orbital CT-scan of this patient demonstrated an extraocular muscle enlargement with tendon sparing and optic nerve compression at the orbital apex.[11,12] Prior ophthalmologists were unable to recognize the disease despite all orbital signs, laboratory findings and CT-scan that supported the diagnosis of TED. As a result, the patient did not get the proper treatment immediately. Even after the patient was eventually diagnosed with TED, he was not given an appropriate treatment according to The European Group on Grave's Orbitopathy (EUGOGO) guidelines.

This patient had a poor prognosis because of several risk factors of TED including old age, smoking habits, and diabetes mellitus. Furthermore, the patient came to us when he already had DON. Therefore, these conditions deteriorated and lead his eyes to a severe sight-threatening TED consequently. In the age of 64 years old, he was considered as in the peak incidence of TED (age 45-49 years old and at 65-69 years old in men). DON generally

occurs at older ages (over age 60 years old).[2,13] Hyperthyroid patient who smokes is five times higher to suffer from TED and will suffer more severe form than non-smokers. The risk of TED in an active smoker is related to the number of cigarettes smoked per day which causes the progression of TED. Smokers have a poor response to immunosuppressant therapy, hence, the patient should be advised to taper and stop smoking.[2,5,6] Diabetes mellitus (DM) is also an important risk factor for the occurrence of DON. The incidence of DON in patient with DM is greater (15-35%) than without DM (3-4%) due to vasculopathy. Visual improvement is not significant in DON, smoker and diabetic patients.[4,5,14]

In accordance to EUGOGO guidelines and Italian consensus, the priorities in TED cases are prompt correction of thyroid dysfunction and stable maintenance of euthyroidism, smoking cessation, conservative therapy for eyes, and assessing the activity and severity of TED. Based on 7/7 Clinical Activity Score (CAS), the patient was indicated as an active TED.[2,4,6,15] It is imperative to determine TED activity because high-dose glucocorticoid is only effective in an active phase. Severity assessment is also important to decide whether it is worth running the risk of high-dose glucocorticoid or it is preferable to limit the therapeutic intervention to local and preventive measures.[3,6,7]According to EUGOGO guidelines, the severity of TED in this case is categorized as sight-threatening (very severe) TED due to corneal damage and DON in both eyes. Both of these conditions are emergencies that must be treated immediately.[4,6,7]

Until the third day of hospitalization, even though the patient already received an infusion of methylprednisolone 500 mg/day for 3 consecutive days, both eyes were still protruded, eyelids were still retracted, and lagophthalmos were still present. Thus, the risk for corneal perforation in both eyes was still inevitable. We decided to do chemosis incision and fat decompression, blepharotomy, amniotic membrane transplant for corneal ulceration, bandage contact lenses, and partial blepharorrhaphy in both eyes. This appears in line with EUGOGO recommendations that stated severe corneal exposure should be treated immediately, medically or with surgery, to avoid corneal perforation. After blepharorrhaphy was released, both corneas were healed and the vision of the LE could finally improve.

In this case, methylprednisolone IV was continued until the second week with total of 3 grams. Vision of the RE did not improve but vision of the LE improved from LP to 2/60. The pupillary light reflexes of both eyes were still decreased, so DON has not been resolved. Therefore, it was required to do orbital decompression surgery for the LE. Instead, the patient was reluctant to undergo such surgery. We decided to continue giving Methylprednisolone IV, corresponding to EUGOGO guidelines for severe TED treatment (infusion of Methylprednisolone 500 mg/week for 6 weeks, followed by infusion of Methylprednisolone 250 mg/week for 6 weeks, with the total limit should not exceed 8 grams).[6] On the last day of treatment, RE vision remained NLP and LE vision improved to 0.1.

Surgery for TED is not advised until a euthyroid state is maintained and it has been in the stable phase for at least 6-9 months. Exceptions include DON or corneal damage, in which cases urgent surgical intervention is warranted.[18,19] Studies from Kahaly *et al* and Vaphiades *et al* revealed case series of 3 patients who had DON with NLP vision and onset of 5 days to 3 months, showed a return of vison following orbital decompression surgery suggesting that axonal death may be delayed by months after total nerve function loss. Orbital decompression surgery may still be effective in reversing compressive optic neuropathy in patients with NLP vision of up to 3 months.[7,20]

The TED course is conceptualized by the Rundle's Curve. TED consists of 2 phases, the inflammatory/active phase which lasts for 6-24 months, followed by the inactive phase where fibrotic changes occur.[4-6] TED treatment during the active phase aims to reduce the immune and inflammatory reactions and to limit destructive consequences. In our case, the active phase had occurred 5 months before the patient was treated with methylprednisolone infusion. Referring to Rundle's Curve, it was more difficult to shift this

disease to be inactive. Therefore, TED is indeed a challenge for ophthalmologists and internists, both in the diagnosis and management. It is crucial to be able to diagnose TED immediately because early initiation of therapy lead to a better prognosis.[4,5,6]

CONCLUSION

The ability to diagnose TED promptly and manage this disease correctly is importantly needed to prevent sight-threatening complications and to achieve satisfying results. A collaboration between internist, who may be subspecialized in endocrinology, and ophthalmologist should be established to control and treat the associated systemic disease.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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Atrial Failure: What Do We Know About It?

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ABSTRACT

Heart failure is the end of all pathological conditions in the heart. Most accepted paradigms in heart failure are always preceded by left ventricle disfunction. Currently, there are several clinical studies that show that heart failure may occur without prior left ventricular dysfunction. Left atrial dysfunction may play a more important role in heart failure than previously expected. Failure of the left atrium can exist independently of left ventricle dysfunction and mitral valve abnormalities. Atrial failure, just like left ventricular failure, can lead to global heart failure. Etiology, pathomechanism and clinical symptoms of atrial failure are complex and not well understood. This review will explain atrial failure.

Keywords*: atrial dysfunction, failure, etiology, pathomechanism, clinical symptoms*

INTRODUCTION

To date, heart failure (HF) has been a never-ending story for cardiologists. Despite many attempts to untangle its complexity, there is no single conceptual paradigm that can rationalize this precise event. HF syndrome is defined as the inability of the heart to provide adequate blood to the body and is classified according to whether it is systolic and/or diastolic, acute/ chronic, compensated or uncompensated, and uni- or bi-ventricular.[1] Many cardiologists consider neurohormonal factors and ventricular function to be one the key player in the pathomechanism of HF and under-estimate the role of the atria.[2] HF is initiated when either the cardiac myocytes are damaged or myocardium are altered while generating force. As a consequence, the heart is not able to contract normally. Recently, through the development of cardiac imaging modalities, many clinicians have agreed that the atria play a more critical role than previously expected. Alteration of atria function arises as a result of alteration of its mechanical or hemostatic function, or electrical physiology of the ventricle.[3]

HF has an extensive variety of clinical symptoms, although in left ventricle (LV) dysfunction, asymptomatic conditions may occur and there is no rigid explanation to explain this phenomenon.[2] However, many scientists believe that neurohormonal and cytokine interactions result in heart remodeling, including the atria chambers.[2-3] The left atrium (LA) is not only involved in LV filling, but also has a bigger role via its multiple mechanisms, such as its endocrine function (atrial natriuretic peptide/ ANP) and regulator function (regulation of the autonomic nervous system and antidiuretic

hormone/ADH).[4] Recently, concerns have risen considering atrial failure to be a new separate entity, which may reduce heart function without significant valvular or ventricular abnormalities.[3] Failure of the LA may trigger neurohumoral overactivity, vasoconstriction, and volume overload.[5]

STRUCTURE AND FUNCTION OF THE LEFT ATRIUM

McAlpine classifies the muscular wall of the LA into the superior, posterior, left lateral, septal/ medial, and anterior regions, especially for interventionist purposes.[6] Nevertheless, the thickness of the muscular LA wall is varied, and the anterior part is especially thin near the vestibule of the mitral annulus and is defined as the "unprotected" area by McAlpine, in that it has a greater risk of perforation.[6-7] Posteriorly, the area around the orifices of the left and right pulmonary veins tend to be thinner and also border with the vagal nerve.[6] Muscle sleeves that spread from the left atrium to the outer aspects of the venous wall are considered to be important in electrical heart activity, especially due to their association with focal activity which initiates atrial fibrillation (AF).[8] The epicardial fat pads at the veno-atrial junction contain autonomic nerve bundles and intrinsic ganglia.[6]

Anatomically, the pulmonary vein (PV) is a varied anastomosis connected to the left atrium and found at the posterior aspect of the LA.[9] At the veno-atrial junction, there are no clear separating structures between the atrium and vein. The atrial musculature extends to the pulmonary vein and acts as a sphincter avoiding reflux during atrial systole. It has been associated as a source of ectopic beats.[8] Moreover, PV attachment also favors early diastolic LV filling and avoiding blood stasis.[10] In the LA wall, there is an infolding that protrudes to the external part of the heart called the left atrial appendage (LAA). It is small, narrow, and tubular in shape, and the left appendage mirrors the right appedage.[6] A postmortem study showed that the atrial appendages from patients with atrial fibrillation had 3 times the volume of those with a normal heart beat.[11] Several investigations also concluded that the LAA is associated with atrial fibrillation and thrombus formation.[11,12]

The LA mechanically consist of three phases; the filling phase, passive emptying phase and active emptying phase.[3,13] The LA stretches during the filling phase and blood flows from the PV into the LA chamber. The filling phase is followed by passive emptying, which is marked by the opening of the mitral valve and the blood flowing passively downstream from the atria to the ventricle. The filling phase is affected by the size, function, relaxation and stiffness of the LA.[13,14] Then, the muscle in the LA is immediately shortened (active emptying) to ensure that the entire volume of the LA is transferred into the ventricle chamber. This process is referred to as the atrial systolic or LA booster pump function. The LA's systolic function is affected by the diastolic myocardial length, afterload, and myocardial contractility.[14] This can be seen in **Figure 1**.

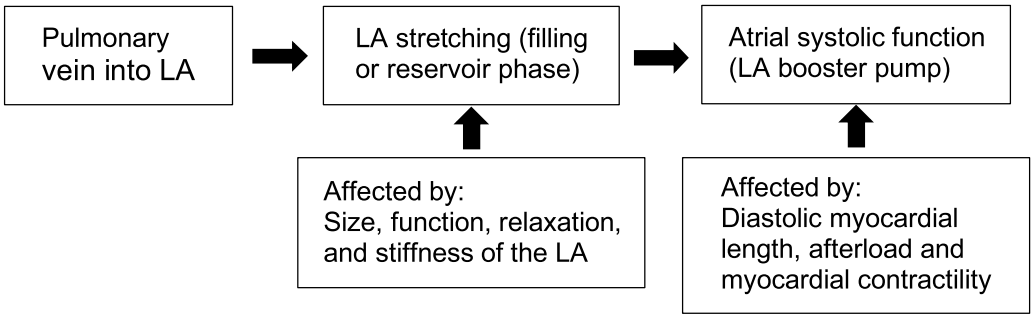

Figure 1. LA mechanism. LA: left atrium

LEFT ATRIA FAILURE

Recent studies have shown LA dysfunction without prior LV disfunction and/or mitral valve abnormalities.[3,15-17] Bisbal, et al. suggest that atria failure be defined as any dysfunction (anatomical, mechanical, electrical, and/or rheological, including blood stasis) that could alter heart performance and symptoms, and worsen quality of life or life expectancy, in the absence of significant valvular or ventricular abnormalities.[3] Additionally, another study defines LA dysfunction as an LAA peak emptying velocity of < 40cm/s, or the presence of spontaneous echo contrast, and/or thrombus in the LA/LAA detected by transesophageal echocardiography (TEE).[16]

Several anatomical spots in atria can initiate their own (ectopic) rhythm.[8,11] Electrical conduction problems in the atria, such as atrial fibrillation (AF), are a common rhythm problem encountered by cardiologists. As a result, LA pumping may be altered and the LA chamber dilatated, which could result in LA failure.[5] A cohort of studies found a correlation between LA disfunction in AF patients, despite having recovered for 3 months.[16] The alteration of LA function in sinus rhythm patients who have previously been diagnosed with AF conditions may occur as a result of mechanical and neurohormonal remodeling, leading to atrial failure.[16,18] Moreover, in subpopulations that receive different types of drugs (beta blockers, angiotensin converting enzyme inhibitors, angiotensin receptor blockers/ARB, anti-arrhythmic drugs, digitalis, diuretics, and calcium channel blockers/CCB), the incidence of LA dysfunction was not significantly different. The LA dimension chamber was significantly larger in patients with LA dysfunction compared to the control group (40±6mm vs 36±8mm, p=0.018).[16] Atrial Fibrillation Investigators concluded that there are several clinical risk factors independently associated with LV dysfunction, such as being aged >65 years old, or having a history of hypertension, diabetes mellitus, coronary artery disease (CAD), and previous TIA or stroke.[19] Other than AF, distortion of the atrioventricular (AV) conduction system and atrial dyssynchrony may also trigger atrial failure.[3]

Cardiomyopathy of the atria, caused by isolated primary or secondary atria pathology, may lead to atrial failure.[3,6] In recent consensus, cardiomyopathies have been described as any complex structural, architectural, contractile, or electrophysiological changes affecting the atria with the potential to produce clinically relevant manifestations.[20] The isolated etiologies of atria cardiomyopathies other than AF are genetic (amyloidosis), infective (myocarditis), infiltrative, inflammatory, and toxin causes.[21] Any specific causes that affect the atrial chamber lead to tachyarrhythmias and result in impairment of atrial systolic contraction and further atrial dilatation, which can persist even after the heart's rhythm returns to normal.[22] Atrial cardiomyopathies can progress into atrial fibrosis, electrical dysfunction, or a procoagulant state, which can worsen the preexisting condition (**Figure 2**).[20,23]

The third mechanism of atrial failure is atrial remodeling.[3] Left atrial remodeling consists of a spectrum of structural and electrical alterations, which lead to atrial dilatation and disrupt atrial function.[6] The problem with atrial remodeling is that it is caused by volume/pressure overload, although not exclusively. Other clinical factors predisposing remodeling are obesity, exercise, obstructive sleep apnea, and modifiable atherosclerosis.[24] Maladaptive responses of atria cells in high stress conditions (such as volume or pressure overload) are myocyte growths, hypertrophy, necrosis, apoptosis, alteration of the extracellular matrix (ECM), recalibration of energy production and expenditure, and changes in the expression of cellular ionic channel and atrial hormones.[6,25] Maladaptive responses result in atrial fibrosis and can lead to shortening of atrial refractoriness, re-entrant wavelengths, and create local conduction heterogeneities (arrhythmias).[26] The connection between electrical arrhythmias and cardiac remodeling remains poorly understood, but the complexity of pathogenesis may involve multiple agents, such as oxidative stress, calcium overload, atrial dilatation, micro-RNAs, inflammation, and myofibroblast activation.[27] These changes are the underlying mechanisms behind atrial remodeling.

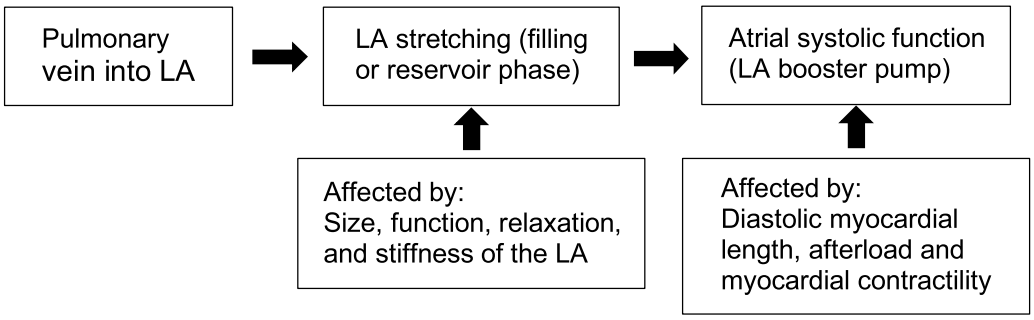

Figure 2. Concept of atrial cardiomyopathy[20-23]

DETERIORATION MECHANISM IN LA FAILURE

After several etiologies, LA function finally fails (Table 1). Fat pads around the ostium of the PV consist of ganglionated plexus (GP) and are innervated with both adrenergic and vagal nerve endings.[28] A volume receptor reflex is activated by a mechanical stretching at the pulmonary venous-atria junction. Over expansion of the blood volume leads to an inhibition of renal sympathetic nerve activity, increasing diuresis, and affecting the heart rate (Bainbridge and reverse Bainbridge reflexes).[29] On the other hand, lack of blood volume leads to thirst and vasopressin hormone release.[30] LA failure downregulates sympatho-inhibition and, unfortunately, upregulates sympatho-excitatory.[6] A recent study conducted by the New York Heart Association (NYHA) was successful in finding a correlation between sympathetic over-activation and functional capacity and prognosis in heart failure patients.[31] As a result, alteration of sympathetic regulation in LA failure can worsen the atria's condition and, in turn, overall heart performance.

The natriuretic peptide (NP) family consists of atrial -type, brain -type, and c-type peptides with their own receptors. Atrial natriuretic peptides (ANP) are stored inside the atria and appendages, and are released during disruption to the LA wall.[31] A precursor for atrial natriuretic peptides is proANP with 126 amino acids that are stored in secretory granules of atrial cardiomyocytes. The proANP secreted from the atria has 3 major forms: proANP with 126 amino acids, proANP with 98 amino acids N terminal peptide (NT-proANP), and pro ANP with 28 amino acids C terminal (ANP) which hormonally actives.[33] ANP plays an important role in cardioprotective mechanisms through several functions.[34,35] However, when LA failure occurs, ANP processing becomes defective and desensitized.[6]

Tabel 1. Causes and triggers of atrial failure

A	Electrical dyssynchrony:
	Atrioventricular dyssynchrony
	Atrial dyssnchrony
B	Booster-Pump and Reservoir Dysfunction
	Fast/disorganized atrial activation
	Extensive atrial fibrosis
C	Impaired Conduit Function
	LA dilation and deformation

CLINICAL MANIFESTATIONS OF LA FAILURE

LA failure has various clinical consequences, such as suboptimal LV filing, AF resulting from atrial failure which progress to pulmonary hypertension, global heart failure (HF), and increased thrombogenicity.[3] Calenda, et all. suggest that atrial myopathies may initiate atrial substrate, which causes increased thrombogenicity.[36] Moreover, the MESA population study also produced the same conclusion.[37] Numerous studies have shown the correlation between atrial remodeling and myopathies with increased risk of stroke.[38,39] Alteration of sympathetic activation leads to LA endothelial dysfunction and fibrosis and, furthermore, is associated with incidents of stroke.[40]

Atrial Fibrillation

LA enlargement is one of the atrial structural changes that can lead to atrial dysfunction. A cardiovascular health study showed that the risk of new AF is increased by 4 times when the

LA diameter > 0.5 mm.[41] Impaired LA reservoir function also increases the risk of first-time AF, independent of clinical risk factors, LA volume, LV ejection fraction, and diastolic function.[42]

Stroke

Risk of stroke in patients with atrial failure can be related to atrial fibrillation. A study on patients with AF referred for catheter ablation showed that LA structural remodelling is associated with an increased risk of stroke and that LA fibrosis severity (quantified using late gadolinium enchancement-cardiac magnetic resonance imaging) is associated with increased major adverse cardiovascular and cerebrovascular events (MACCE).[43] In elderly patients without AF, the association of LA size with stroke was studied. The study found that a LA volume index (LAVI) ≥32 mL/m^2 was independently predictive of a first ischemic stroke.[44] Leong et al. studied the role of LA dysfunction in the pathogenesis of cryptogenic stroke. This study showed that the LA reservoir strain was significantly lower, indicating LA dysfunction in patients who experienced cryptogenic strokes.[45]

Heart Failure with Preserved Ejection Fraction (HFpEF)

HFpEF is a common condition and patients with HFpEF are more likely to have LV hypertrophy, LV diastolic dysfunction, and LA enlargement. Recent studies have indicated a correlation between LA dysfunction and HFpEF. A study by Santos et al. found that worse LA strain was associated with a higher risk of HF hospitalization in HFpEF patients, independent of other potential clinical confounders, but not independent of LV systolic deformation and diastolic filling pressure.[46] Khan et al. also showed that all LA volumetric and strain parameters are significantly reduced in HFpEF patients compared to healthy controls. Impaired LA function causes atrial compliance to decrease, thus lowering the pressure gradient in the left-side of the heart during early diastole and decreasing the LV filling.[47]

CONCLUSION

Cardiologists have long believed LA failure to be a consequence of LV disfunction. However recent studies have been open to the new possibility of the LA as a potential new source of HF incidents. LA failure is defined as isolated failure of the LA without prior LV or mitral valve abnormalities. There are several etiologies of LA failure and they precipitate heart conditions related to HF, independent of LV involvement. LA failure may also have clinical significance.

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Liver Cirrhosis in Woman with Ciliopathy Syndrome

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ABSTRACT

Ciliopathy syndrome is a congenital abnormality of structure and/or function of cilia, which causes pleiotropic disorder, including liver cirrhosis. This study aimed to describe a unique case of liver cirrhosis with possible aetiology of ciliopathy syndrome. A 44 year-old woman with chief complain of hematemesis had diabetes mellitus, obesity, dyslipidaemia, amenorrhoea and often became unconscious. We found short stature, brachydactyly, hyperpigmented maculae in trunk and four limbs, and hepatosplenomegaly. The laboratory results showed: haemoglobin 7.4 g/dl; albumin 2.42 g/dl; urea 84.8 mg/dl; creatinine 2.4 mg/dl; prolactin 138.8 ng/ml, while HBsAg was negative and anti-HCV was non-reactive. Abdominal ultrasonography showed liver cirrhosis; endoscopy showed grade 3 oesophageal varicose; FibroScan® showed 75 kPa; liver biopsy showed hydropic degeneration and cirrhosis; and head CT scan showed chronic lacunar infarction of corona radiata and mega cisterna magna occipital. We reported female with oesophageal varicose rupture, short stature, brachydactyly, obesity, diabetes mellitus, dyslipidaemia, hyperpigmented maculae, liver cirrhosis and mega cisterna magna, which was likely to suffer from ciliopathy syndrome.

***Keywords**: short stature, brachydactyly, insulin resistance, cirrhosis, ciliopathy.*

INTRODUCTION

Rare aetiologies of liver cirrhosis include disorders.[1] One of the most rare aetiologies of liver cirrhosis associated with genetic disorders is the entity of cilia abnormality. Ciliopathy involves a variety of anatomical abnormalities.[2] Unfortunately, genetic examination is still out of reach due to lack of diagnosis tools in our country. Ciliopathy is rarely discussed in daily clinical practice because of its rarity, and will be discussed in this case report.

CASE ILLUSTRATION

A 44 year old unmarried woman from Javanese ethnic, worked as a tailor, was referred from private hospital to our hospital due to main complaint black tarry stool and bloody vomiting. She passed black tarry stool and vomited blood 2 days before admission, 3-4 times a day about a half glass volume each vomit. She did not feel any pain at her stomach, only bloating sensation and dizziness. She had similar condition one year earlier, had been hospitalised and got transfusion with packed red blood cells at that time. Since one year ago, she suffered enlarged abdomen, and after treated with medication, known by her as diuretics and some other drugs, her abdomen circumference gradually reduced. The patient never experienced a yellowish skin and tea like colour urine. Patient experienced itchy skin almost the entire body, especially her arms and legs; she often scratched it and leaving black spots on it.

She had suffered from diabetes mellitus since for four years before, and took oral anti-diabetes. She also often had high levels of blood

cholesterol. She complained temporary blurred vision and sometimes experienced fainting with unknown causes, and did not remember the incident before it. Several times she woke from fainting in various rooms at home including bathroom without anyone knowing and helping. Her menstrual period was irregular, and the last four months she did not have menstruation. She was short statured since childhood, and other family members who had short stature was her older sister. She was obese, but her weight slowly decreased since the last two years. She never consumed herbs, analgesics, alcohol and smoking.

At admission, she was moderate ill, fully conscious, blood pressure was 130/70 mmHg, pulse rate 70 beats per minute regular, respiratory rate 20 times per minute, axillary temperature 36 °Celsius, weight 50 kilograms, height 145 centimetres, and body mass index 24 kilograms/centimetres^2 (overweight). She looked anaemic and had asymmetric facial expression which we concluded as paresis of right nerve VII upper motor neuron type (**Figure1**). We found her had hepatomegaly with liver span of 14 cm, splenomegaly Schuffner 2, and shifting dullness test was positive which we concluded as ascites. She had short fingers and toes (*brachydactyly*) (**Figure 2**) and multiple hyperpigmented macules in the upper and lower extremities (**Figure 3**).

Abnormal laboratory findings were haemoglobin 7.4 g/dl, albumin 2.42 g/dl, urea 84.8 mg/dl, creatinine 2.4 mg/dl, prolactin 138.8 ng/ml, while HBsAg was negative and anti-HCV was non reative. Abdominal ultrasonography showed liver cirrhosis (**Figure 4**) and FibroScan® showed 75 kPa (F4). We conducted endoscopy, and it showed grade 3 oesophageal varicose (**Figure 5**). Liver biopsy showed hydropic degeneration and cirrhosis. We also conducted head CT scan with contrast because the patient had neurological deficits and often fainted, and it showed a chronic lacunar infarction at right corona radiata and mega cisterna magna occipital (**Figure 6**). We consulted the patient to Neurology Department to evaluate neurological problem. They diagnosed her with benign peripheral positional vertigo.

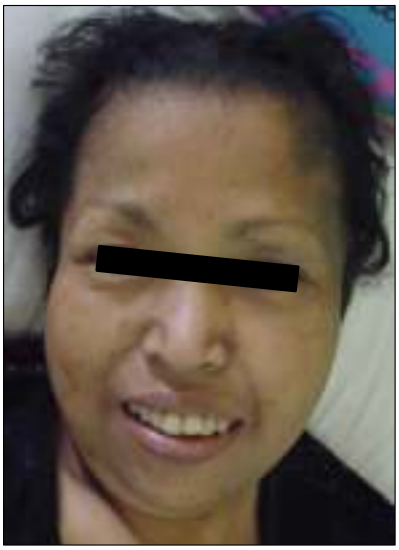

Figure 1. The patient's face looked asymmetry, concluded as paresis of right nerve VII upper motor neuron type.

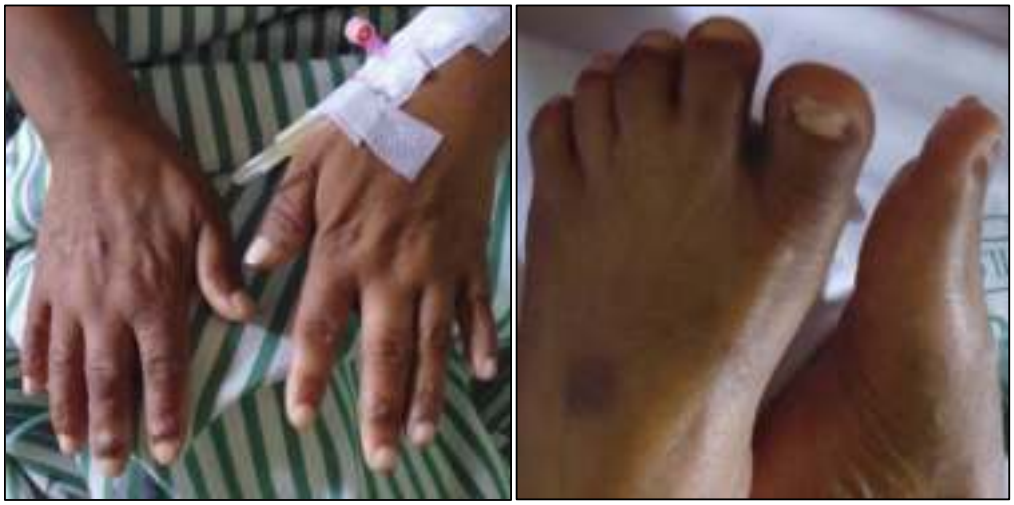

Figure 2. The fingers and toes of the patient were short (*brachydactyly*). The patient's stature is also short and obese.

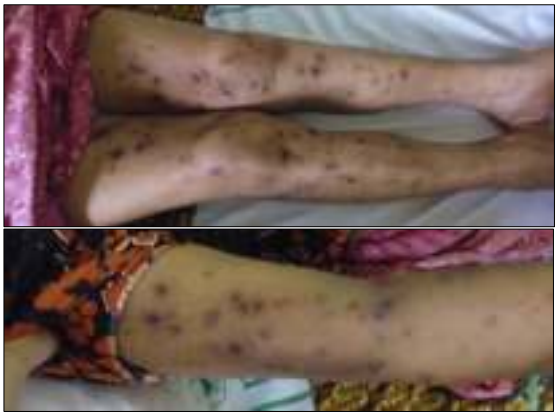

Figure 3. Upper and lower extremity of the patient had multiple hyperpigmented macules.

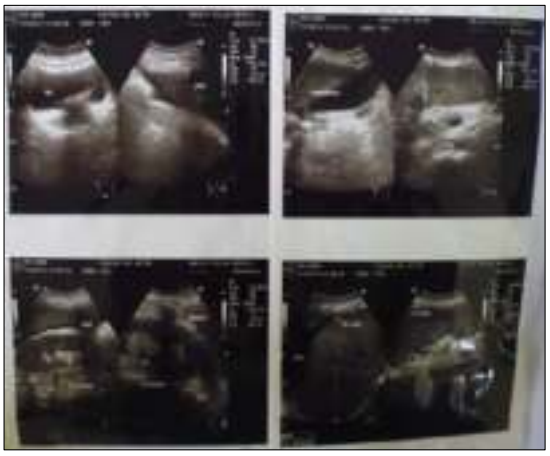

Figure 4. Abdominal ultrasonography showed liver cirrhosis with ascites.

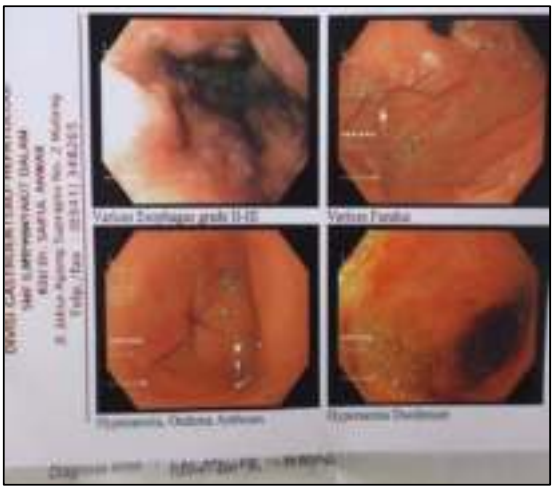

Figure 5. Endoscopy showed grade 3 oesophageal varicose.

We treated the patient with octreotide bolus 50 mcg iv, continued with drip 50 mcg/ hour, lansoprazol 30 mg iv every 12 hours, metoclopramide 10 mg iv every 8 hours, ceftriaxone 1 gram iv every 24 hours, insulin long acting 10 units subcutaneous at bed time, lactulose 1 table spoon 3 times a day, betahistine mesylate 24 mg pro re nata, PRC transfusion and albumin transfusion. After the hematemesis was resolved, we gave her propranolol 20 mg every 8 hours and spironolactone 100 mg a day. With several anatomical abnormality in this patient, we suspected her to had ciliopathy syndrome, but definitive diagnosis, gen abnormality, could not be performed due to lack of diagnostic tool in our hospital.

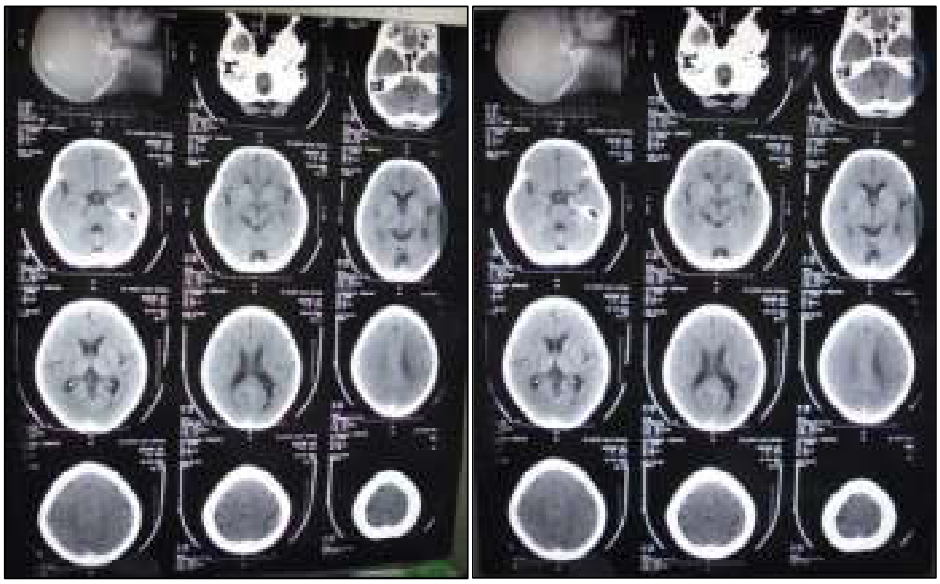

Figure 6. Head CT scan showed a chronic lacunar infarction at right corona radiata and mega cisterna magna occipital

DISCUSSION

Patient presented with hematemesis and melena. We found hepatosplenomegaly, abdominal ultrasonography described liver cirrhosis and FibroScan® showed high degree liver fibrosis. Generally, in hepatic cirrhosis, we find liver in smaller size. However, there are several conditions of liver cirrhosis with hepatomegaly, such as cardiac cirrhosis (Laennac cirrhosis) and fatty liver as seen in non-alcoholic fatty liver disease (NAFLD) that develops to liver cirrhosis.[3] Endoscopy revealed that she had grade 3 oesophageal varicose as the cause of hematemesis. This supported diagnosis of liver cirrhosis. Liver biopsy result was hydropic degeneration which was usually caused by hepatic cell injury. Chronic liver injury can cause cirrhosis and it is important to determine the specific aetiology given its implications in patient management and its long-term outcomes. Certainly, if the aetiology of a disease remains unknown, effective therapy can not be performed. We had excluded common aetiology of liver cirrhosis in the patient, such as viral hepatitis infection and autoimmune hepatitis. Epidemiologically, the most common aetiology of liver cirrhosis other than hepatitis viral infection is NAFLD, which usually revealed fatty degeneration on liver biopsy, but we found no fatty degeneration in the patient.[4,5] However, we considered that she had diabetes, dyslipidaemia and obesity. NAFLD is associated with metabolic syndrome and insulin resistance.[5,6] Obesity is common and well-documented risk factor for NAFLD.[4,5,6] There is a very high prevalence of NAFLD in individuals with type 2 diabetes mellitus. High serum triglyceride levels and low serum HDL levels are very common in NAFLD patients.[7] The prevalence of NAFLD in individuals with dyslipidaemia is estimated to be 50%.[8,9]

Although metabolic disorders such as obesity and type 2 diabetes are increasing in global pandemics, their pathophysiology and molecular basis are not fully understood.[8,9] The cause of obesity is complex, because many confusing genetic and environmental factors are not obviously affecting it.[6] In addition, metabolic disorders involve interconnected disease which can be exemplified by the association of obesity with insulin resistance, leading to the development of type 2 diabetes.[6,7] The genetic factors for obesity are poorly understood. Genomic association studies support the idea that some genes, tissues, and pathways, contribute to this disease. An interesting gene subsets associated with obesity are caused by primary ciliary dysfunction, resulting in a rare pleiotropic disorder in humans called ciliopathy syndrome.[8]

Primary cilia may act as sensory cell antennae, coordinating intercellular communications via receptor clustering and signalling.[10] Bardet-Biedl syndrome (BBS) is the archetypical example of a ciliopathy with profound appetite dysregulation. BBS children are unable to resist the drive to eat, becoming massively obese at an early age, and about half develop type 2 diabetes mellitus and metabolic syndrome. Another childhood obesity syndrome that may be ascribed to a ciliopathy is Alström syndrome (AS).[10,11] In addition to their respective specific features (skeletal, retinal, renal and hepatobiliary fibrocystic abnormalities, hearing defects and infertility), BBS and AS are both associated with hyperphagic obesity, early onset of insulin resistance, type 2 diabetes mellitus and (best described for AS) severe fatty liver disease leading to cirrhosis.[12] An additional exciting finding is that pre-adipocytes also express primary cilia, and these play a role in their capacity to differentiate and form triglyceride-storing adipocytes and secrete adiponectin.[11] The mice carrying a gene mutation for the basal body protein of cilia underwent NAFLD.[12,13] This is very appropriate with the condition of our patient who had characteristics of ciliopathy syndrome and also suffer from diabetes mellitus and history of obesity and dyslipidaemia.

It was very interesting that in this case, the patient also had body dysmorphism. She had asymmetrical facial expression, short stature and brachydactyly. Short stature and brachydactyly was also found in her older sister. She also suffered oligomenorrhea and even amenorrhoea in the last 4 months, had high level of serum prolactin and mega cisterna magna in the brain. We suggested that these abnormalities were related to one disease entity or syndrome and

associated with her liver cirrhosis. When the structure or function of the cilia is defective, it affects most of the body's organs such as kidneys, brain, limbs, eyes, ears, liver and bones.[11,13,14] The unique characteristics of ciliopathy show a broad phenotypic spectrum determined by the degree of damage to the affected cilia and tissue specificity. The symptoms of ciliopathy vary greatly depending on the affected genes and their role in ciliogenesis and ciliary function.[10,11]

Cilia falls into two broad categories: motile and immotile.[10] Primary cilia are typically immotile and consist of nine peripheral doublet microtubules; while motile cilia, in addition, contain a central pair of singlet microtubules ("9+2" arrangement) to which they are connected by the radial spike proteins. Immotile cilia are characterised by the absence of the central pair of singlet microtubules ("9+0" arrangement).[11] Motile cilia are distinguished from primary cilia by their ability to beat rhythmically, an activity that is powered by adenosine triphosphate (ATP), hydrolysed by dynein proteins, which are anchored to the inner and outer aspects of peripheral doublet microtubules.[11,12] Motile cilia are utilised in both unicellular and multicellular organisms for locomotion. Primary cilia have chemosensory, osmosensory and phototransduction functions.[10,11,12]

As cilia are a component of almost all vertebrate cells, ciliary dysfunction can manifest as a constellation of features include congenital fibrocystic diseases of the liver and pancreas, diabetes, obesity and skeletal dysplasia.[10,11] Phenotypically heterogeneous, ciliopathic features can manifest from variation at a single locus while mutations affecting a number of different loci can, at the same time, result in similar phenotypes. Within each organ, diseases can be developmental phenotypes presenting at birth or later in childhood.[13] Often this may depend on the severity of the underlying mutation in addition to the number of defective proteins encoded where more than one mutation in a ciliary gene occurs.[14] Ciliary membranes contain receptors and ion channel proteins mediating cell signalling, including roles for Sonic Hedgehog (SHH), Wnt and PDGFa signalling pathways that control diverse processes (e.g., cell differentiation, migration, axonal path finding, and planar cell polarity).[14] The SHH pathway is important for dorsal-ventral patterning of the neural tube and, later, for proliferation of cerebellar granule cells. Defects of this pathway can cause anomalies of the cerebral commissures.[15,16]

Intact cilia-based signalling is required for normal development of the biliary and portal system in the liver. The majority of diseases manifesting with hepatic fibrocystic pathology are caused by defective ciliary proteins.[12] Congenital hepatic fibrosis is a histopathological diagnosis with three main components; that is, defective remodelling of the ductal plate; abnormal portal veins; and progressive fibrosis of the portal tracks. The major morbidity associated with congenital hepatic fibrosis is portal hypertension.[12,13] Congenital fibrocystic diseases of the liver are a heterogeneous group of disorders that are characterised by a spectrum of biliary dysgenesis that includes congenital hepatic fibrosis, bile duct dilatation and cyst formation. Defects in cholangiocyte ciliary structure and/or their integrated transducing function lead to a decrease in intracellular calcium and increased cAMP, causing cholangiocyte hyperproliferation, abnormal cell matrix interactions and altered fluid secretion/absorption, which can result in hepatic cystogenesis.[13]

Emerging data indicate that hedgehog signalling, one of signalling pathway in primary cilia, mediates both adaptive and maladaptive responses to liver injury, depending upon the balance between its actions as a regulator of progenitor cell growth and its ability to promote liver inflammation and fibrogenic repair.[14] Synthesis of hedgehog ligands is stimulated by diverse factors that trigger liver regeneration, including both liver cell mitogens and liver cell stressors. These Hh ligands, in turn, are released from ligand-producing cells into the local environment where they engage receptors on Hh-responsive cells. The latter include progenitor cells, hepatic stellate cells, sinusoidal endothelial cells and certain types of resident hepatic immune cells. In general, Hh ligands function as trophic factors and promote the viability of Hh-target cells.[13,14] This enhances the outgrowth

of liver progenitor populations, triggers tissue remodelling, and promotes liver regeneration. However, Hh ligands also stimulate certain cell types (e.g., hepatic stellate cells, immature liver epithelial cells) to acquire a less epithelial and more mesenchymal state during which such cells generate inflammatory mediators and scar tissue, therefore, induces liver fibrogenesis. Hence, excessive or persistent Hh pathway activity actually aborts successful regeneration of damaged liver tissue and contributes to the pathogenesis of liver fibrosis.[14]

Findings of skin disorders in this patient led us to make the differential diagnosis of neurocutaneous disorder due to ciliopathy. Several case reports also presented skin disorders in patients with ciliopathy syndromes in the form of hyperpigmentation macules and sometimes also in the form of multiple nevus.[16,17,18] These skin disorders often coincided with pigmented abnormalities in the patient's cerebral meninges.[17,18] We tested the patient for serum prolactin level because of amenorrhoea. The condition of hyperprolactinemia in her could be caused by liver cirrhosis or stood alone. Hyperprolactinaemia could also be caused by her obesity.

Management of liver cirrhosis in patient with ciliopathy syndrome is same with that of other aetiology. The aetiology of cirrhosis in ciliopathy is related to development of NASH; so, the management of metabolic condition related to obesity and insulin resistance and dyslipidaemia should be optimized.

CONCLUSION

We reported woman with hematemesis, short stature, brachydactyly, hyperpigmented maculae, liver cirrhosis, and mega cisterna magna, which was likely to suffer from ciliopathy syndrome; however, genetic tests has not been performed on the patient yet. Management of patients with this syndrome is same as liver cirrhosis caused by other aetiology. Follow-up related to other organ abnormalities in the future, is necessary. Appropriate genetic counselling and family member screening should be performed. The definitive diagnosis necessarily requires chromosome and gene analysis, which is not available here.

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Successful Treatment Bilateral Panuveitis with Multiple Systemic Infection in HIV/AIDS Patient: A Case Report

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ABSTRACT

There is an increasing number of HIV/AIDS patients in Indonesia, starting from <0.1% in 2010 to 0.4% in 2012, which warrants awareness of ocular manifestation in HIV. This might appear in 70-100% of patients with HIV. A 47 years old man came to the infection and immunology clinic with blurry vision on both eyes. He had been treated before but there was no clinical improvement. Examination showed both eyes had vitreous haziness. Visual acuity was 1/60 in both eyes with appearance of flare and cells within +3. Uveitis workup showed positive results for HIV, HSV and syphilis. Patient was given 100 mg of doxyciclin two times daily and fixed dose tablet which contains the combination of antiretroviral. Three months later, final acuity was 6/10 on the right eye and 6/18 on the left eye. Prompt diagnosis and treatment warrant good prognosis including multidisciplinary approach by ophthalmologist, clinical allergist and immunologist, and dermato-venerologist.

Keywords**:** *HIV/AIDS, syphillis, ocular manifestation in HIV, bilateral panuveitis.*

INTRODUCTION

Increasing number of HIV/AIDS patients in Indonesia, from <0.1% in 2010 to 0.4% in 2012, warrants awareness of ocular manifestations of HIV. Ocular manifestation of HIV might appear in 70-100% patients. In some cases ocular manifestations might be the initial presenting clinical finding in patients with HIV/AIDS.[1] Various causative agents might cause uveitis in these population, such as CMV, syphilis, toxoplasma, herpes, and tuberculosis.[1-3]

In this case report we present a successful management of panuveitis in HIV patients. The aim of this report is to demonstrate the management of uveitis in HIV patients and highlighting the importance of prompt and accurate diagnosis as well as proper treatment.

CASE ILLUSTRATION

A 47 years old man came with complaint of blurry vision for 2 months. One year before the patient complaint of having blurry vision and floaters on his left eye. Two months before, the vision worsen accompanied with red eyes. The patient was initially treated with prednisolone acetate eye drop. There was history of promiscuity and tattoo.

Visual acuity was 1/60 in both eyes with cells +3 and flare. The vitreous was hazy, the optic nerve can not be examined in detail, exudate was found, other details was hard to be evaluated. The patient was initially assessed with panuveitis on both eyes caused by toxoplasma dd/ cytomegalovirus and AIDS without ARV treatment. The patient was planned to have

fundus photograph taken, uveitis workup. And was treated with by the attending ophthalmologist with trimetripim + sulfametoxazole 2 x 960 mg.

Complete uveitis workup was done to the patient to rule in or out the possibility of infectious disease as the cause of uveitis, and the patient return three days later with the result. Uveitis workup showed positive HIV; positive IgG and IgM for anti-HSV 2; reactive VDRL (1/512) and TPHA test (>1:5120); positive IgG for anti-CMV. CD4+ lymphocyte count was 9% and absolute count was 261 cells/mcL. Other uveitis screening workup such as tuberculin test for tuberculosis, IgM and IgG for toxoplasma, showed negative result excluding the diagnosis. Chest X-Ray showed no signs infiltrate in both lungs.

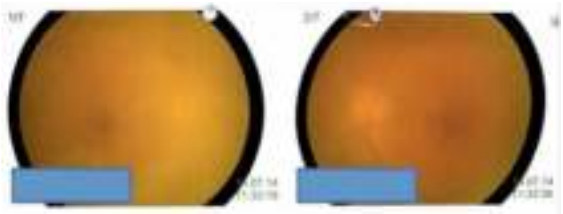

Figure 1. The first fundus photograph showing he optic nerve head was hazy and other details were hard to be evaluated

The patient was treated as syphilitic panuveitis. Because the patient was allergic to penicillin, he was treated with doxyciclin 2 x 100 mg. The HIV infection was treated with fixed dose tablet consisting zidovudine 300 mg and lamivudine 150 mg and efavirenz 600 mg once daily. Because negative result test for toxoplasma, the treatment trimetropim and sulfametoxazole was stopped.

Two weeks later the patient's acuity was 6/20 on the right eye and 6/18 on the left eye. Anterior segment was quiet. Posterior segment shows vitreous cells +1, with normal appearing optic nerve head and retina (**Figure 2**).

Three months after treatment for syphilis, the patient's visual acuity was 6/10 in the right eye and 6/18 in the left eye. Anterior segment and posterior segment were within normal limit. His final titer for VDRL and TPHA was 1:128.

1.

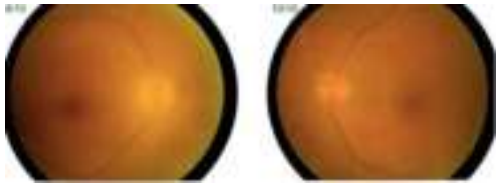

Figure 2. Second fundus and photograph taken 2 weeks later. Optic nerve head was round with clear margin, artery to venous ratio was 2/3, with cup to disc ratio 0.3-0.4, no hemorrhage or exudate can be found on both eyes.

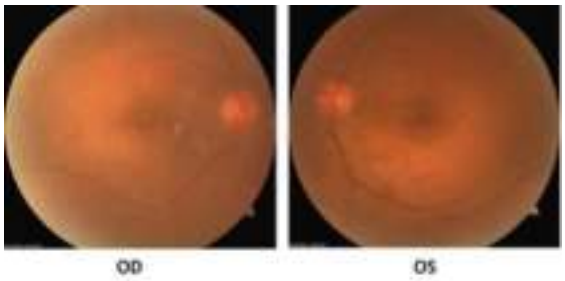

Figure 3. Final fundus photographed. Optic nerve head was round with clear margin, artery to venous ratio was 2/3, with cup to disc ratio 0.3-0.4, no hemorrhage or exudate was found.

DISCUSSION

Most common ocular manifestation in HIV/AIDS patients includes HIV retinopathy, opportunistic infections, Kaposi sarcoma, and adnexal disease, and might occur in 70-100% patients in this population.[1] Uveitis might be the first manifestations of AIDS stage of HIV infections. In patients with HIV/AIDS the course of the uveitis tend to be severe and produce more sequelae.[1-3]

Uveitis in patient with HIV/AIDS might be caused by various etiology. Study in Taiwan in patients with uveitis as initial manifestations of AIDS, showed CMV, syphilis, and toxoplasmosis is the leading pathogen.[2] In HIV patients with CD4+ lymphocyte count >200/mcL, one study showed infectious uveitis is mostly caused by syphilis and herpes virus.[3]

The diagnosis of syphilitic uveitis requires thorough history taking, clinical findings and established through serological test. The most common finding in the fundus are multifocal chorioretinitis associated with vitritis or severe vitritis alone but, necrotizing retinitis, retinal vasculitis, exudative retinal etachment, isolated papilitis and neuroretinitis can also be found.

Panuveitis is not a rare manifestation of syphilitic uveitis, comprising of 40% cases in one study.[4] Two type of serological test are available for syphilis classified as non-treponemal, (e.g. VDRL test) , and treponemal test (e.g. TPHA, FTA-ABS or MHA-TP). Current CDC recommendation is to test patient suspected of having syphilis with treponemal test, then if positive, should be tested for non-treponemal test.[5,6]

Because of the wide possibility of the cause of uveitis in patients with HIV, our patients was tested with various tests to confirm the causative agent. The most common viral infection of uveitis in patient with HIV is cytomegalovirus, thus testing for this disease is very important. Other important causative agents are varicella zoster virus and herpes simplex virus, which, unlike CMV that cause slowly progressive disease, causes rapidly developing and confluent retinitis. Ocular toxoplasmic retinochoroiditis is also one of the most common ocular manifestation in patients with HIV, thus testing for toxoplasma infection is very important in these cases. Infectious agents that can be commonly seen in HIV-negative uveitis patients such as syphilis and tuberculosis should also be tested since it these infection is commonly found in HIV positive patients.[(2-,4)]

The treatment for ocular syphilis is the same with syphilis with neural involvement. CDC only recommended aqueous penicillin G 18-24 MU/d given IV as 3-4 MU every 4 hours for 10-14 days.[5] European guidelines recommend the administration of oral doxycyclin 2 x 200 mg daily in cases of penicillin allergy, but the evidence is very weak (graded IV C).[7] There is one case series that reports the improvement of clinical findings and visual acuity in one patients with ocular syphilis and penicillin allergy.[8]

Our patient was a 47 years old HIV positive male with remarkable risk factors for sexually transmitted diseases. Patients with HIV with history of promiscuity is at risk for other sexually transmitted infection. Examination showed dense vitritis hampering proper examination of retina, and serological test showed positive test for both treponemal and non-treponemal test for syphilis, establishing the diagnosis of syphilitic uveitis. Test for other diseases should be done, because the dense vitritis hampers the evaluation of the retina, making the diagnosis for disease that might cause retinal necrosis worth being considered. Although test for HSV-2 IgM and IgG also showed positive result, clinical examination that support acute retinal necrosis or progressive outer retinal necrosis can not be found, thus excluding this diagnosis. But the serological test is still justifiable because on initial examination dense vitritis hamper the examination of the fundus. Because our patient was diagnosed with syphilitic uveitis in both eyes as well as HSV-2 infection, we advise to cooperate with other fields of expert in order to properly manage this patient.

Our patient was allergic to penicillin, and given doxycycline 100 mg orally twice daily for two weeks. In spite of not adhering to CDC recommendation, and low evidence based on European Guideline it is interesting to note that our patient had dramatic clinical improvement.

The prognosis of patient with ocular syphilis is favorable if treated promptly. One research study showed that the final BCVA for patients who had been treated with penicillin regimen ranging from 20/50 – 20-20 and no recurrences in 2 years. [9] Another report stated that treatment more that 28 days since symptoms occur is a risk factor for poor prognosis.[10]

CONCLUSION

Prompt diagnosis and treatment warrant good prognosis in these population. Our patient came with poor visual acuity and severe inflammatory reaction, but with proper antibiotic and anti-retroviral treatment improve the visual acuity of our patient improve dramatically. The treatment involving multidisciplinary approach by ophthalmologist, clinical allergist and immunologist, and dermato-venerologist is required in these cases.

ACKNOWLEDGMENTS

Address for reprints and sources of support that require acknowledgement is none.

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Sight-Threatening Condition in Severe Thyroid Eye Disease: How We Should Manage

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ABSTRACT

Thyroid eye disease (TED) is an autoimmune disorder that is associated with thyroid gland dysfunction which causes muscle and orbital fat enlargement. This case report is aimed to present a case of sight-threatening TED and how we should manage this condition. We present a case of patient with chief complaint of vision loss and prominent eyes for 5 months prior to the visit to our eye hospital. Patient had sought advice from an ophthalmologist and internist. TED was eventually diagnosed 2 months after consulted with an ophthalmologist in the rural area. According to EUGOGO guidelines, TED with sight-threatening condition should be treated with glucocorticoid IV 500-1000 mg for 3 days consecutively. Although the patient was already given steroid injection for the initial treatment, the dosage was inadequate. After the inflammation process was reduced, the patient was reluctant to have an orbital decompression that was suggested. Hence, TED progressed continuously besides sight-threatening complications arising. He indeed underwent fat decompression and tarsorrhaphy as eyelid surgery to prevent corneal exposure. In follow-up, both visual acuity and corneal improvement were finally achieved. In the management of TED, collaboration between ophthalmologist and internist, who may be specialized in endocrinology, is imperative. They should be able to manage TED promptly and correctly, hence sight-threatening and other complications can be prevented and satisfactory results are achieved. Fat decompression should be considered as a good help to improve visual acuity nevertheless orbital decompression cannot be done.

Keywords: *grave's disease, thyroid eye disease, dysthyroid optic neuropathy, orbital decompression, fat decompression.*

INTRODUCTION

Graves' disease is an autoimmune disease that leads to a generalized overactivity of the entire thyroid gland (hyperthyroidism). It is the most common cause of hyperthyroidism in the United States. Generally occurring in patients with hyperthyroidism, sometimes thyroid-associated ophthalmopathy or thyroid eye disease (TED) occurs in patients with euthyroid or hypothyroid chronic autoimmune thyroiditis. The condition has an annual adjusted incidence rate of 16 women and 3 men per 100.000 population.[1]

TED is an autoimmune disorder that is associated with thyroid gland dysfunction which causes muscle and orbital fat enlargement. TED generally accompanies Graves hyperthyroidism where the course of eye disease does not always parallel with the activity of thyroid gland. The onset of TED can precede, together, or after the onset of hyperthyroidism.[2-5]

TED pathophysiology is complex and the treatment has not fully focused on the pathogenesis of the disease. Although the majority of TED cases are mild, around 3-7% of patients develop vision-threatening complications such as corneal damage or dysthyroid optic neuropathy (DON). The strategy for handling this disease consists of various methods such as medical therapy, surgery, and radiotherapy.[4-6]

The ability of ophthalmologists and internists to diagnose and assess the activity and severity of this disease correctly is expected to help determine the right treatment for patients. In an active TED, proper handling can improve vision, appearance, and quality of life. Therefore, TED is a challenge for ophthalmologists and internists both in diagnosis and appropriate management.[4-6] This case report discussed severe TED with corneal damage and DON. Eventually, TED was diagnosed, yet the treatment given was not according to guidelines, so the patient was deteriorating and in high risk of losing his sight.

CASE ILLUSTRATION

A 64-year-old male was referred to our eye hospital presented with complaint of vision loss, protrusion and soreness on both eyes. Five months prior, he complained of redness, swelling, and blurred vision. There was no other sign of systemic infection, previous illness, and family illness. All of these conditions lead his first-seen-ophthalmologist to diagnose his red eyes with an eye infection. Two months later, he went to an internist with a complaint of trembling, sweating, weight loss, heart palpitations, and sleep deprivation. There was no data about physical examination because he was seeking the internist in another hospital in rural area. The laboratory tests supported the diagnosis of hyperthyroidism and type two diabetes mellitus because he has high level of Total T3 (5.15 ng/mL), high level of Total T4 (20,7 μg/dL) and low level of TSHs (<0,02 μIU/mL). There was no data on blood glucose level. He was a heavy-smoker although he had already been advised to quit smoking. The patient was given Thiamazole 3x10 mg, Glimepiride 1x2 mg, and Univoxy® 1x1 tablet per day from the internist.

One month later, both of his eyes became protruded. Right eye (RE) was blind and left eye (LE) was otherwise normal. Clinically suspected with pseudotumors, the doctor evaluated him with orbital Computed Tomography (CT) Scan, but neither retrobulbar nor pseudotumor was found. This patient was then referred to ophthalmologist at another hospital in urban area.

One month later, he felt his LE vision began to be blurred. Orbital CT-scan depicted an orbital muscle enlargement with a coke-bottle sign and proptosis in both eyes, hence the patient was eventually diagnosed with TED and received steroid injections twelve times for three days (divided doses of steroid) of hospitalization consecutively from the first ophthalmologist. **(Figure 1)** The patient did not remember the dosage of steroid injection that was given by the ophthalmologist. In spite of the inflammation process that was subsided, one week later, swelling of both eyes became recurrent and the visual acuity of his LE got worsened until he lost his vision. The laboratory tests revealed a hypothyroid condition with low FT4 (0.28 ng/dL), high TSHs (28.834 μIU/mL) and low Total T3 (0.46 ng/mL). Hence, the internist in the second hospital reduced the dosage of Thiamazole to 1x10 mg. This patient did not have a regular schedule of follow up for thyroid and diabetes mellitus.

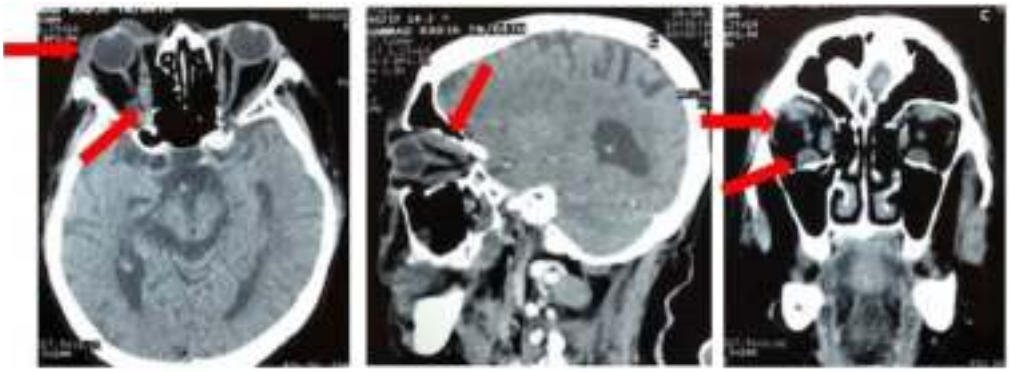

Figure 1. Axial, sagittal, and coronal CT-scan showed proptosis and extraocular muscles enlargement (coke-bottle sign).

The patient came to our eye hospital. On examination, there was no light perception (NLP) for the RE and light perception (LP) for the LE. He has 30° exotropia. Both eye movements were -4 in all directions (fixed eyeball). There were proptosis, edema on upper and lower eyelids, retraction of upper and lower eyelids, lagophthalmos, severe chemosis, and caruncular edema in both eyes. Corneal ulceration in the RE and corneal infiltrate in the LE were also recognized. Pupillary light reflexes were reduced for both eyes, so relative afferent pupillary defect (RAPD) was difficult to be assessed. Cataract could also be identified in both eyes. The result of Hertel exophthalmometers on each eye was 25 mm. Diabetes mellitus was again confirmed by laboratory tests with high level of HbA1c (NGSP 6.4% and IFCC 46 mmol/mol). At the same time, his hypothyroidism condition was supported by laboratory tests such as low FT3 (1.55 pg/mL), low FT4 (0.68 ng/dL), high TSHs (0.375 µIU/Ml), low Total T3 (0,51 ng/mL), low Total T4 (4,49 µg/dL), high TRAb (15,74 IU/L), and low Anti-TPO (<0.5 IU/mL).

Based on all manifestations and work-up studies, this patient had complex problems due to the complications of Grave's disease, such as bilateral proptosis, RE corneal ulcer, LE exposure keratitis, bilateral dysthyroid optic neuropathy (DON), and bilateral immature cataracts. He was hospitalized and treated with Methylprednisolone intravenous (IV) 500 mg for three consecutive days, Chloramphenicol eye ointment 3 times/day, Citicoline 2x500 mg, Mecobalamin 2x500 µg, Gatifloxacin eye drops every 3 hours, and Solcoseryl® eye gel every hour. After the methylprednisolone IV administration, patient's blood glucose level increased to 355 mg/dL indeed. He was given insulin (Lantus 1x14 unit and Novorapid 3x10 unit) and Thiamazole 1x10 mg from the internist.

His LE vision was improved to 2/60 on his fourth day of hospitalization. However, he still had DON and at high risk for corneal perforations on both eyes, hence he was suggested to undergo an orbital decompression. As he was being reluctant to proceed, he underwent fat decompression and blepharorrhaphy. The procedures included chemosis incision, eyelid fat removal, upper and lower blepharotomy, amniotic membrane transplant, bandage contact lenses, and blepharorrhaphy. Two consecutive days after the surgery, he was given an infusion of 250 mg MP per day. The blepharorrhaphy was then released after 3 days. Central corneas were healed but left scars at the inferior corneas of both eyes.

During hospitalization, intraocular pressures (IOP) were high (20.7 mmHg for RE and 34.3 mmHg for LE). He was given Latanoprost-Timolol Maleate eye drops 2 times/day, Acetazolamide 3 x 250 mg, Potassium L-Aspartate 3 times/day, Calcium-Vitamin D_3 2 x/day, Acetylsalicylic Acid 2 x 160 mg, Tobramycin-Dexamethasone eye drops 1 x/day, Gatifloxacin 4 x/day, Solcoseryl® 4 x/day, Selenium 1 tablet/day, Vitamin C 500 mg IV, Ciprofloxacin 2 x 500 mg and Mefenamic Acid 2 x 500 mg.

On the 11th day of hospitalization, his eye movements improved to -3 in all directions for both eyes and his dosage of Methylprednisolone was increased to 500 mg. He was finally allowed to go home on the 12th day of hospitalization. **(Figure 2, Figure 3)**

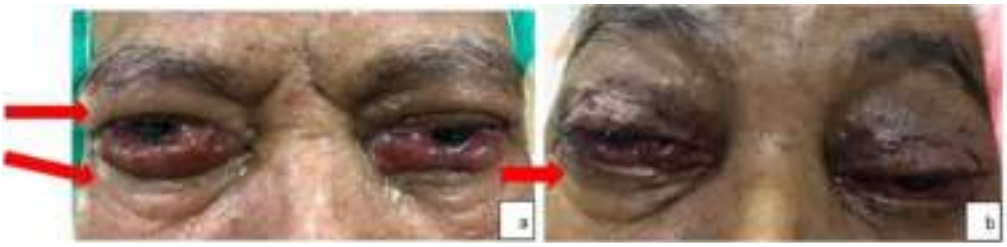

Figure 2. a) before surgery, there were eyelid retraction on the RE and chemosis on both eyes; b) eye condition after fat decompression and blepharorrhaphy was released. Chemosis was gradually diminished but caruncular oedema was still recognized. On the LE, eyelid oedema was slowly subsided.

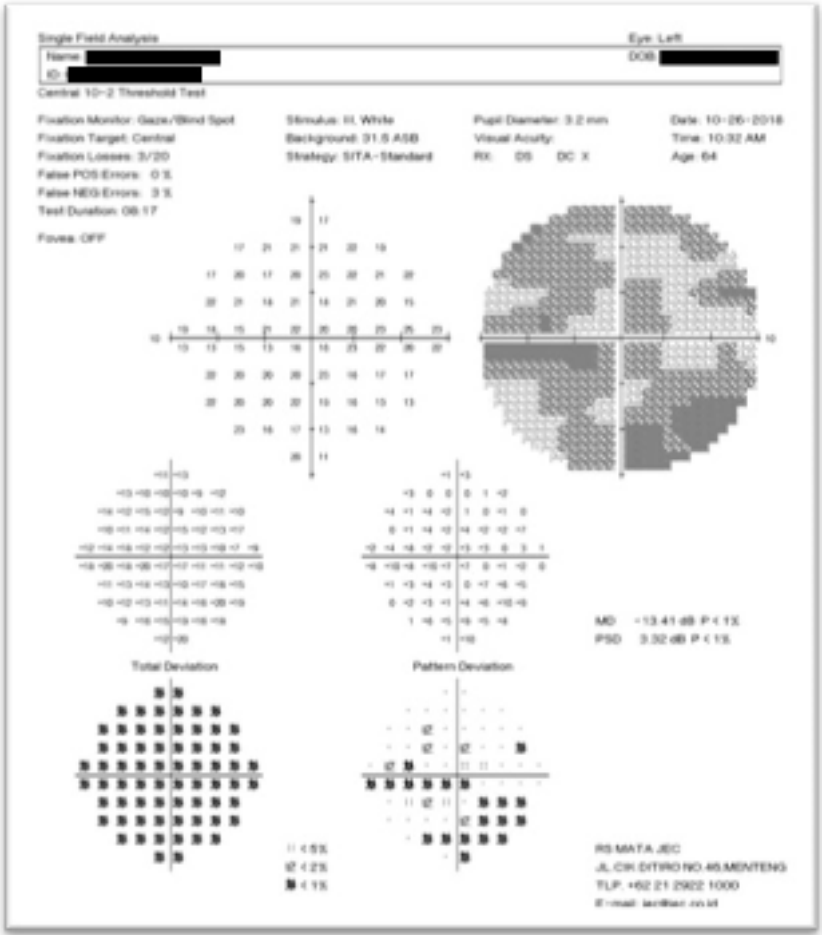

Figure 3. Perimetry of the LE demonstrated a visual field defect.

On the last visit, his eye pain was alleviated, vision of the RE remained NLP and vision of the LE improved to 0.1. Exotropia improved to 15º and both eye movements were improved in all directions as well. In spite of eyelid oedema, caruncular oedema and conjunctival chemosis that were still found, his eyes were generally improved compared to the initial condition. RAPD was positive in the RE. Ishihara test for the LE showed total colour blindness. **(Figure 4)**

DISCUSSION

The assessment recommended by The 2018 European Thyroid Association (ETA) guidelines for suspected Grave's hyperthyroidism is Thyroid-Stimulating Hormone (TSH), free T3 (fT3), free T4 (fT4), TSH Receptor Antibodies (TRAb), and thyroid gland ultrasound examination. TSHs should be used as an initial screening test because it has the highest sensitivity and specificity for evaluating suspected Grave's hyperthyroidism.

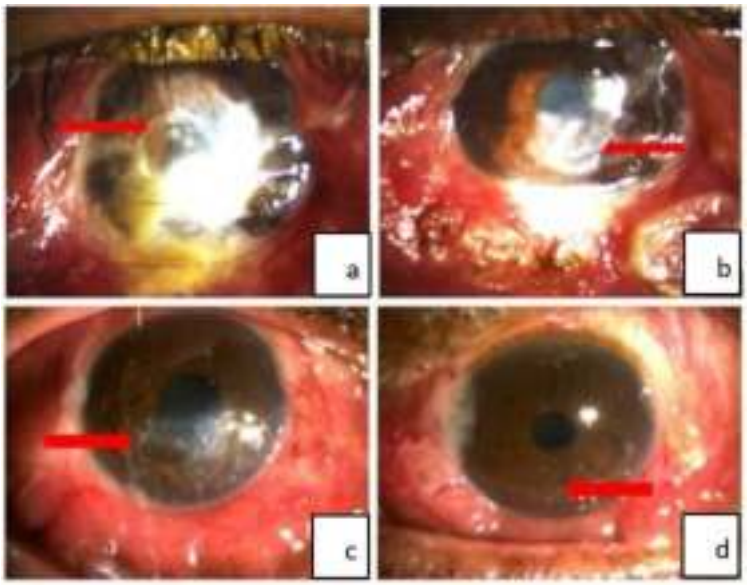

Figure 4. a) corneal ulcer on the RE; b) corneal infiltrate was seen on the LE; c) corneal leucoma (improvement on the RE); d) corneal nebula (improvement on the LE).

Serum TSH levels are more sensitive than thyroid hormone (T3, T4) tests for assessing thyroid hormone excess. Diagnostic accuracy improves when both serum TSHs and fT4 are assessed at the initial evaluation. TRAb measurements are also sensitive and specific in evaluating suspected Grave's hyperthyroidism. TRAb is a specific biomarker for the extrathyroidal manifestations of Grave's disease and correlates with TED activity and severity.[7-10] In Grave's hyperthyroidism, 30-50% of patients will have ocular involvement. In one-third of TED cases, symptoms and signs appear altogether with hyperthyroidism.

Diagnosis of TED is made when 2 of the following 3 signs are present: (1) typical orbital signs (1 or more of the following): unilateral/bilateral eyelid retraction with typical temporal flares (with/without lagophthalmos), unilateral/bilateral proptosis, restrictive strabismus, compressive optic neuropathy, eyelid oedema/erythema, or caruncular oedema/chemosis; (2) immune-related thyroid dysfunction (1 or more of the following): Grave's hyperthyroidism, Hashimoto thyroiditis, or presence of thyroid antibodies without a coexisting dysthyroid state (TSH-receptor antibodies, thyroid-binding inhibitory immunoglobulins/TBII, thyroid-stimulating immunoglobulins/TSI, anti microsomal antibody); (3) radiographic evidence of TED (enlargement of 1 or more of the following): inferior/medial/superior/lateral rectus muscle or levator palpebral muscle.[1-3]

In this patient, the diagnosis of TED was made based on ophthalmology examination, laboratory tests, and orbital CT-scan findings. Orbital CT-scan of this patient demonstrated an extraocular muscle enlargement with tendon sparing and optic nerve compression at the orbital apex.[11,12] Prior ophthalmologists were unable to recognize the disease despite all orbital signs, laboratory findings and CT-scan that supported the diagnosis of TED. As a result, the patient did not get the proper treatment immediately. Even after the patient was eventually diagnosed with TED, he was not given an appropriate treatment according to The European Group on Grave's Orbitopathy (EUGOGO) guidelines.

This patient had a poor prognosis because of several risk factors of TED including old age, smoking habits, and diabetes mellitus. Furthermore, the patient came to us when he already had DON. Therefore, these conditions deteriorated and lead his eyes to a severe sight-threatening TED consequently. In the age of 64 years old, he was considered as in the peak incidence of TED (age 45-49 years old and at 65-69 years old in men). DON generally

occurs at older ages (over age 60 years old).[2,13] Hyperthyroid patient who smokes is five times higher to suffer from TED and will suffer more severe form than non-smokers. The risk of TED in an active smoker is related to the number of cigarettes smoked per day which causes the progression of TED. Smokers have a poor response to immunosuppressant therapy, hence, the patient should be advised to taper and stop smoking.[2,5,6] Diabetes mellitus (DM) is also an important risk factor for the occurrence of DON. The incidence of DON in patient with DM is greater (15-35%) than without DM (3-4%) due to vasculopathy. Visual improvement is not significant in DON, smoker and diabetic patients.[4,5,14]

In accordance to EUGOGO guidelines and Italian consensus, the priorities in TED cases are prompt correction of thyroid dysfunction and stable maintenance of euthyroidism, smoking cessation, conservative therapy for eyes, and assessing the activity and severity of TED. Based on 7/7 Clinical Activity Score (CAS), the patient was indicated as an active TED.[2,4,6,15] It is imperative to determine TED activity because high-dose glucocorticoid is only effective in an active phase. Severity assessment is also important to decide whether it is worth running the risk of high-dose glucocorticoid or it is preferable to limit the therapeutic intervention to local and preventive measures.[3,6,7]According to EUGOGO guidelines, the severity of TED in this case is categorized as sight-threatening (very severe) TED due to corneal damage and DON in both eyes. Both of these conditions are emergencies that must be treated immediately.[4,6,7]

Until the third day of hospitalization, even though the patient already received an infusion of methylprednisolone 500 mg/day for 3 consecutive days, both eyes were still protruded, eyelids were still retracted, and lagophthalmos were still present. Thus, the risk for corneal perforation in both eyes was still inevitable. We decided to do chemosis incision and fat decompression, blepharotomy, amniotic membrane transplant for corneal ulceration, bandage contact lenses, and partial blepharorrhaphy in both eyes. This appears in line with EUGOGO recommendations that stated severe corneal exposure should be treated immediately, medically or with surgery, to avoid corneal perforation. After blepharorrhaphy was released, both corneas were healed and the vision of the LE could finally improve.

In this case, methylprednisolone IV was continued until the second week with total of 3 grams. Vision of the RE did not improve but vision of the LE improved from LP to 2/60. The pupillary light reflexes of both eyes were still decreased, so DON has not been resolved. Therefore, it was required to do orbital decompression surgery for the LE. Instead, the patient was reluctant to undergo such surgery. We decided to continue giving Methylprednisolone IV, corresponding to EUGOGO guidelines for severe TED treatment (infusion of Methylprednisolone 500 mg/week for 6 weeks, followed by infusion of Methylprednisolone 250 mg/week for 6 weeks, with the total limit should not exceed 8 grams).[6] On the last day of treatment, RE vision remained NLP and LE vision improved to 0.1.

Surgery for TED is not advised until a euthyroid state is maintained and it has been in the stable phase for at least 6-9 months. Exceptions include DON or corneal damage, in which cases urgent surgical intervention is warranted.[18,19] Studies from Kahaly *et al* and Vaphiades *et al* revealed case series of 3 patients who had DON with NLP vision and onset of 5 days to 3 months, showed a return of vison following orbital decompression surgery suggesting that axonal death may be delayed by months after total nerve function loss. Orbital decompression surgery may still be effective in reversing compressive optic neuropathy in patients with NLP vision of up to 3 months.[7,20]

The TED course is conceptualized by the Rundle's Curve. TED consists of 2 phases, the inflammatory/active phase which lasts for 6-24 months, followed by the inactive phase where fibrotic changes occur.[4-6] TED treatment during the active phase aims to reduce the immune and inflammatory reactions and to limit destructive consequences. In our case, the active phase had occurred 5 months before the patient was treated with methylprednisolone infusion. Referring to Rundle's Curve, it was more difficult to shift this

disease to be inactive. Therefore, TED is indeed a challenge for ophthalmologists and internists, both in the diagnosis and management. It is crucial to be able to diagnose TED immediately because early initiation of therapy lead to a better prognosis.[4,5,6]

CONCLUSION

The ability to diagnose TED promptly and manage this disease correctly is importantly needed to prevent sight-threatening complications and to achieve satisfying results. A collaboration between internist, who may be subspecialized in endocrinology, and ophthalmologist should be established to control and treat the associated systemic disease.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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Atrial Failure: What Do We Know About It?

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ABSTRACT

Heart failure is the end of all pathological conditions in the heart. Most accepted paradigms in heart failure are always preceded by left ventricle disfunction. Currently, there are several clinical studies that show that heart failure may occur without prior left ventricular dysfunction. Left atrial dysfunction may play a more important role in heart failure than previously expected. Failure of the left atrium can exist independently of left ventricle dysfunction and mitral valve abnormalities. Atrial failure, just like left ventricular failure, can lead to global heart failure. Etiology, pathomechanism and clinical symptoms of atrial failure are complex and not well understood. This review will explain atrial failure.

Keywords*: atrial dysfunction, failure, etiology, pathomechanism, clinical symptoms*

INTRODUCTION

To date, heart failure (HF) has been a never-ending story for cardiologists. Despite many attempts to untangle its complexity, there is no single conceptual paradigm that can rationalize this precise event. HF syndrome is defined as the inability of the heart to provide adequate blood to the body and is classified according to whether it is systolic and/or diastolic, acute/ chronic, compensated or uncompensated, and uni- or bi-ventricular.[1] Many cardiologists consider neurohormonal factors and ventricular function to be one the key player in the pathomechanism of HF and under-estimate the role of the atria.[2] HF is initiated when either the cardiac myocytes are damaged or myocardium are altered while generating force. As a consequence, the heart is not able to contract normally. Recently, through the development of cardiac imaging modalities, many clinicians have agreed that the atria play a more critical role than previously expected. Alteration of atria function arises as a result of alteration of its mechanical or hemostatic function, or electrical physiology of the ventricle.[3]

HF has an extensive variety of clinical symptoms, although in left ventricle (LV) dysfunction, asymptomatic conditions may occur and there is no rigid explanation to explain this phenomenon.[2] However, many scientists believe that neurohormonal and cytokine interactions result in heart remodeling, including the atria chambers.[2-3] The left atrium (LA) is not only involved in LV filling, but also has a bigger role via its multiple mechanisms, such as its endocrine function (atrial natriuretic peptide/ ANP) and regulator function (regulation of the autonomic nervous system and antidiuretic

hormone/ADH).[4] Recently, concerns have risen considering atrial failure to be a new separate entity, which may reduce heart function without significant valvular or ventricular abnormalities.[3] Failure of the LA may trigger neurohumoral overactivity, vasoconstriction, and volume overload.[5]

STRUCTURE AND FUNCTION OF THE LEFT ATRIUM

McAlpine classifies the muscular wall of the LA into the superior, posterior, left lateral, septal/ medial, and anterior regions, especially for interventionist purposes.[6] Nevertheless, the thickness of the muscular LA wall is varied, and the anterior part is especially thin near the vestibule of the mitral annulus and is defined as the "unprotected" area by McAlpine, in that it has a greater risk of perforation.[6-7] Posteriorly, the area around the orifices of the left and right pulmonary veins tend to be thinner and also border with the vagal nerve.[6] Muscle sleeves that spread from the left atrium to the outer aspects of the venous wall are considered to be important in electrical heart activity, especially due to their association with focal activity which initiates atrial fibrillation (AF).[8] The epicardial fat pads at the veno-atrial junction contain autonomic nerve bundles and intrinsic ganglia.[6]

Anatomically, the pulmonary vein (PV) is a varied anastomosis connected to the left atrium and found at the posterior aspect of the LA.[9] At the veno-atrial junction, there are no clear separating structures between the atrium and vein. The atrial musculature extends to the pulmonary vein and acts as a sphincter avoiding reflux during atrial systole. It has been associated as a source of ectopic beats.[8] Moreover, PV attachment also favors early diastolic LV filling and avoiding blood stasis.[10] In the LA wall, there is an infolding that protrudes to the external part of the heart called the left atrial appendage (LAA). It is small, narrow, and tubular in shape, and the left appendage mirrors the right appedage.[6] A postmortem study showed that the atrial appendages from patients with atrial fibrillation had 3 times the volume of those with a normal heart beat.[11] Several investigations also concluded that the LAA is associated with atrial fibrillation and thrombus formation.[11,12]

The LA mechanically consist of three phases; the filling phase, passive emptying phase and active emptying phase.[3,13] The LA stretches during the filling phase and blood flows from the PV into the LA chamber. The filling phase is followed by passive emptying, which is marked by the opening of the mitral valve and the blood flowing passively downstream from the atria to the ventricle. The filling phase is affected by the size, function, relaxation and stiffness of the LA.[13,14] Then, the muscle in the LA is immediately shortened (active emptying) to ensure that the entire volume of the LA is transferred into the ventricle chamber. This process is referred to as the atrial systolic or LA booster pump function. The LA's systolic function is affected by the diastolic myocardial length, afterload, and myocardial contractility.[14] This can be seen in **Figure 1**.

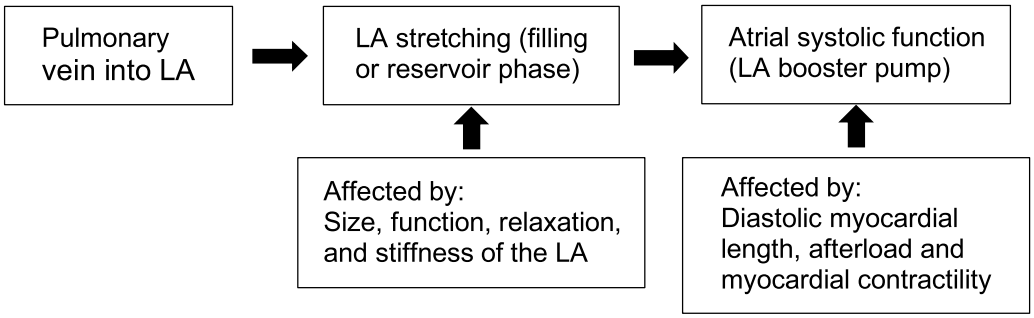

Figure 1. LA mechanism. LA: left atrium

LEFT ATRIA FAILURE

Recent studies have shown LA dysfunction without prior LV disfunction and/or mitral valve abnormalities.[3,15-17] Bisbal, et al. suggest that atria failure be defined as any dysfunction (anatomical, mechanical, electrical, and/or rheological, including blood stasis) that could alter heart performance and symptoms, and worsen quality of life or life expectancy, in the absence of significant valvular or ventricular abnormalities.[3] Additionally, another study defines LA dysfunction as an LAA peak emptying velocity of < 40cm/s, or the presence of spontaneous echo contrast, and/or thrombus in the LA/LAA detected by transesophageal echocardiography (TEE).[16]

Several anatomical spots in atria can initiate their own (ectopic) rhythm.[8,11] Electrical conduction problems in the atria, such as atrial fibrillation (AF), are a common rhythm problem encountered by cardiologists. As a result, LA pumping may be altered and the LA chamber dilatated, which could result in LA failure.[5] A cohort of studies found a correlation between LA disfunction in AF patients, despite having recovered for 3 months.[16] The alteration of LA function in sinus rhythm patients who have previously been diagnosed with AF conditions may occur as a result of mechanical and neurohormonal remodeling, leading to atrial failure.[16,18] Moreover, in subpopulations that receive different types of drugs (beta blockers, angiotensin converting enzyme inhibitors, angiotensin receptor blockers/ARB, anti-arrhythmic drugs, digitalis, diuretics, and calcium channel blockers/CCB), the incidence of LA dysfunction was not significantly different. The LA dimension chamber was significantly larger in patients with LA dysfunction compared to the control group (40±6mm vs 36±8mm, p=0.018).[16] Atrial Fibrillation Investigators concluded that there are several clinical risk factors independently associated with LV dysfunction, such as being aged >65 years old, or having a history of hypertension, diabetes mellitus, coronary artery disease (CAD), and previous TIA or stroke.[19] Other than AF, distortion of the atrioventricular (AV) conduction system and atrial dyssynchrony may also trigger atrial failure.[3]

Cardiomyopathy of the atria, caused by isolated primary or secondary atria pathology, may lead to atrial failure.[3,6] In recent consensus, cardiomyopathies have been described as any complex structural, architectural, contractile, or electrophysiological changes affecting the atria with the potential to produce clinically relevant manifestations.[20] The isolated etiologies of atria cardiomyopathies other than AF are genetic (amyloidosis), infective (myocarditis), infiltrative, inflammatory, and toxin causes.[21] Any specific causes that affect the atrial chamber lead to tachyarrhythmias and result in impairment of atrial systolic contraction and further atrial dilatation, which can persist even after the heart's rhythm returns to normal.[22] Atrial cardiomyopathies can progress into atrial fibrosis, electrical dysfunction, or a procoagulant state, which can worsen the preexisting condition (**Figure 2**).[20,23]

The third mechanism of atrial failure is atrial remodeling.[3] Left atrial remodeling consists of a spectrum of structural and electrical alterations, which lead to atrial dilatation and disrupt atrial function.[6] The problem with atrial remodeling is that it is caused by volume/pressure overload, although not exclusively. Other clinical factors predisposing remodeling are obesity, exercise, obstructive sleep apnea, and modifiable atherosclerosis.[24] Maladaptive responses of atria cells in high stress conditions (such as volume or pressure overload) are myocyte growths, hypertrophy, necrosis, apoptosis, alteration of the extracellular matrix (ECM), recalibration of energy production and expenditure, and changes in the expression of cellular ionic channel and atrial hormones.[6,25] Maladaptive responses result in atrial fibrosis and can lead to shortening of atrial refractoriness, re-entrant wavelengths, and create local conduction heterogeneities (arrhythmias).[26] The connection between electrical arrhythmias and cardiac remodeling remains poorly understood, but the complexity of pathogenesis may involve multiple agents, such as oxidative stress, calcium overload, atrial dilatation, micro-RNAs, inflammation, and myofibroblast activation.[27] These changes are the underlying mechanisms behind atrial remodeling.

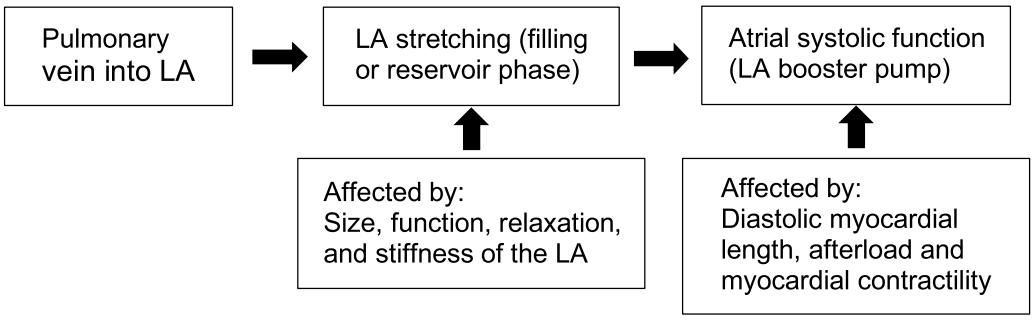

Figure 2. Concept of atrial cardiomyopathy[20-23]

DETERIORATION MECHANISM IN LA FAILURE

After several etiologies, LA function finally fails (Table 1). Fat pads around the ostium of the PV consist of ganglionated plexus (GP) and are innervated with both adrenergic and vagal nerve endings.[28] A volume receptor reflex is activated by a mechanical stretching at the pulmonary venous-atria junction. Over expansion of the blood volume leads to an inhibition of renal sympathetic nerve activity, increasing diuresis, and affecting the heart rate (Bainbridge and reverse Bainbridge reflexes).[29] On the other hand, lack of blood volume leads to thirst and vasopressin hormone release.[30] LA failure downregulates sympatho-inhibition and, unfortunately, upregulates sympatho-excitatory.[6] A recent study conducted by the New York Heart Association (NYHA) was successful in finding a correlation between sympathetic over-activation and functional capacity and prognosis in heart failure patients.[31] As a result, alteration of sympathetic regulation in LA failure can worsen the atria's condition and, in turn, overall heart performance.

The natriuretic peptide (NP) family consists of atrial -type, brain -type, and c-type peptides with their own receptors. Atrial natriuretic peptides (ANP) are stored inside the atria and appendages, and are released during disruption to the LA wall.[31] A precursor for atrial natriuretic peptides is proANP with 126 amino acids that are stored in secretory granules of atrial cardiomyocytes. The proANP secreted from the atria has 3 major forms: proANP with 126 amino acids, proANP with 98 amino acids N terminal peptide (NT-proANP), and pro ANP with 28 amino acids C terminal (ANP) which hormonally actives.[33] ANP plays an important role in cardioprotective mechanisms through several functions.[34,35] However, when LA failure occurs, ANP processing becomes defective and desensitized.[6]

Tabel 1. Causes and triggers of atrial failure

A	Electrical dyssynchrony:
	Atrioventricular dyssynchrony
	Atrial dyssnchrony
B	Booster-Pump and Reservoir Dysfunction
	Fast/disorganized atrial activation
	Extensive atrial fibrosis
C	Impaired Conduit Function
	LA dilation and deformation

CLINICAL MANIFESTATIONS OF LA FAILURE

LA failure has various clinical consequences, such as suboptimal LV filing, AF resulting from atrial failure which progress to pulmonary hypertension, global heart failure (HF), and increased thrombogenicity.[3] Calenda, et all. suggest that atrial myopathies may initiate atrial substrate, which causes increased thrombogenicity.[36] Moreover, the MESA population study also produced the same conclusion.[37] Numerous studies have shown the correlation between atrial remodeling and myopathies with increased risk of stroke.[38,39] Alteration of sympathetic activation leads to LA endothelial dysfunction and fibrosis and, furthermore, is associated with incidents of stroke.[40]

Atrial Fibrillation

LA enlargement is one of the atrial structural changes that can lead to atrial dysfunction. A cardiovascular health study showed that the risk of new AF is increased by 4 times when the

LA diameter > 0.5 mm.[41] Impaired LA reservoir function also increases the risk of first-time AF, independent of clinical risk factors, LA volume, LV ejection fraction, and diastolic function.[42]

Stroke

Risk of stroke in patients with atrial failure can be related to atrial fibrillation. A study on patients with AF referred for catheter ablation showed that LA structural remodelling is associated with an increased risk of stroke and that LA fibrosis severity (quantified using late gadolinium enchancement-cardiac magnetic resonance imaging) is associated with increased major adverse cardiovascular and cerebrovascular events (MACCE).[43] In elderly patients without AF, the association of LA size with stroke was studied. The study found that a LA volume index (LAVI) ≥32 mL/m^2 was independently predictive of a first ischemic stroke.[44] Leong et al. studied the role of LA dysfunction in the pathogenesis of cryptogenic stroke. This study showed that the LA reservoir strain was significantly lower, indicating LA dysfunction in patients who experienced cryptogenic strokes.[45]

Heart Failure with Preserved Ejection Fraction (HFpEF)

HFpEF is a common condition and patients with HFpEF are more likely to have LV hypertrophy, LV diastolic dysfunction, and LA enlargement. Recent studies have indicated a correlation between LA dysfunction and HFpEF. A study by Santos et al. found that worse LA strain was associated with a higher risk of HF hospitalization in HFpEF patients, independent of other potential clinical confounders, but not independent of LV systolic deformation and diastolic filling pressure.[46] Khan et al. also showed that all LA volumetric and strain parameters are significantly reduced in HFpEF patients compared to healthy controls. Impaired LA function causes atrial compliance to decrease, thus lowering the pressure gradient in the left-side of the heart during early diastole and decreasing the LV filling.[47]

CONCLUSION

Cardiologists have long believed LA failure to be a consequence of LV disfunction. However recent studies have been open to the new possibility of the LA as a potential new source of HF incidents. LA failure is defined as isolated failure of the LA without prior LV or mitral valve abnormalities. There are several etiologies of LA failure and they precipitate heart conditions related to HF, independent of LV involvement. LA failure may also have clinical significance.

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Thyroid Abscess as a Clinical Manifestation of Papillary Thyroid Carcinoma

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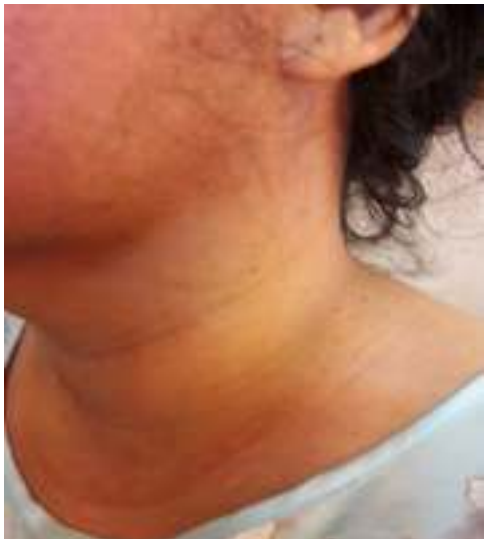

Figure 1. Lateral view of the neck in a patient with a thyroid abscess at the left lower pole

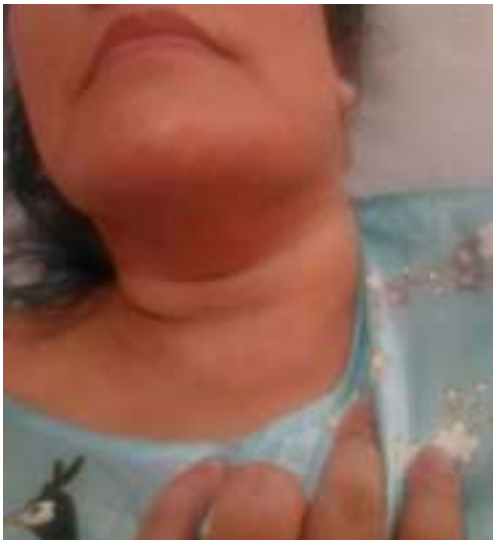

Figure 3. Anterior view of the neck in a patient with a thyroid abscess at the left lower pole

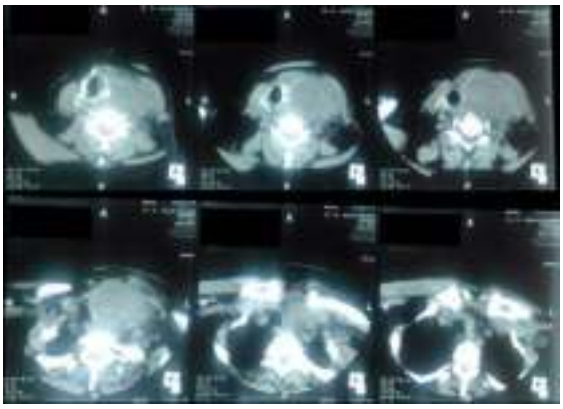

Figure 2. Computed tomography imaging of the neck

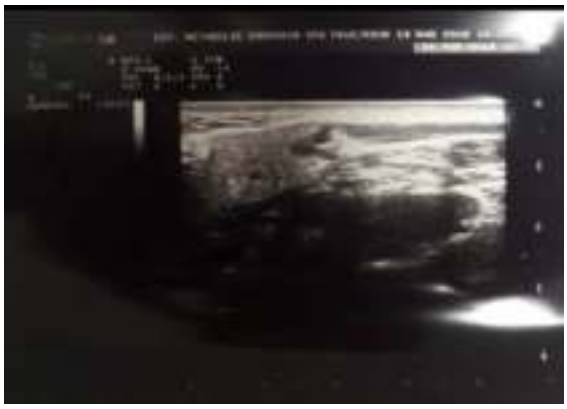

Figure 4. Neck ultrasound

Thyroid abscess is a relatively uncommon condition. It accounts for less than 1% of all thyroid diseases and less than 0.7% of surgical pathology in the thyroid gland. The thyroid gland has protective factors, including high iodine content that acts as a bacterial effect, well-developed capsule, adequate lymphatic drainage, a plentiful blood supply, and hydrogen peroxide production within the gland, which inhibits infection development in the thyroid tissue. Predisposing factors that may increase the susceptibility of the thyroid gland to infection include pyriformis sinus fistulae, thyroid nodule or cancer, or immunocompromised patients. Around 5-10% of the population is estimated to have a palpable nodule, but with ultrasound, it could increase to 50-60% and approximately 5% of the detected nodules are malignant. Therefore, a thorough examination of the thyroid nodule is essential in order to rule out thyroid cancer. Usually, thyroid cancer is present with a mass. However, it is rare for thyroid cancer to present with a thyroid cyst or thyroid abscess, even with infection symptoms.

Numerous reports indicate that females with a pre-existing thyroid nodule had a higher incidence of thyroid abscesses. Although thyroid abscesses are uncommon, they can result in significant morbidity and mortality. Therefore, once a thyroid abscess is diagnosed, aggressive management should be initiated to avoid dangerous complications. The majority of thyroid abscesses are successfully treated with a combination of antibiotics and surgery. Percutaneous drainage and intracavitary antibiotic injection are conservative and is a less invasive alternative management strategy.

A 50-year-old woman presented with a 7-day history of an enlarged, painful, and warm neck mass resulting in swallowing difficulty, as well as a high fever and headache. She had no history of upper respiratory tract infection or neck trauma but had previously been diagnosed with a thyroid nodule a year prior without being treated.

She was found critically ill with a temperature of 38^0C and a heart rate of 100 beats per minute. Other vital signs were within normal limits. Her left anterolateral neck was swollen (10x10x5cm), warm and tender, and was reddish. Due to neck tenderness, cervical lymphadenopathy was difficult to assess. The white blood cell count was 16,800/uL with an elevated neutrophil count (78%), ESR of 120 mm/h (0-20), procalcitonin of 0.09 ng/mL (<0.05), and quantitative CRP of 86.0 mg/L (<5.0). TSHs were within the normal range (0.580 uIU/mL, reference 0.270-4.200), free T4 was 1.390 ng/dL (reference 0.930-1.700), random blood glucose was 161 mg/dL (<140), electrolytes were normal, and both anti-HIV and HbsAg were non-reactive.

Chest X-ray revealed a soft tissue mass in the left inferior neck, displacing the trachea to the right between the C6 and T1 levels. A computed tomography scan of the neck revealed a 7.2x5.4x7.1cm cystic lesion in the left lobe with a thick wall, dislodging the glottis and trachea to the right and extending inferiorly to the supraclavicular, consistent with a left thyroid abscess. The patient was diagnosed with thyroid abscess and treated with ceftriaxone at a dose of 2 grams per day and metronidazole for 500 mg three times daily.

Neck sonography revealed a cystic lesion in the left lobe, surrounded by a thick wall and contain a ruptured lateral capsule.

Ultrasound-guided aspiration of this lesion was performed for diagnostic purposes and to alleviate pain; 100 cc of thick yellow-brown liquid was aspirated from the lesion. Following aspiration, the cavity was twice injected with 50 cc of metronidazole. The patient was scheduled for left isthmolobectomy due to pus expanding into the tissue from a ruptured capsule. Histopathological examination revealed that the specimen was composed primarily of thyroid tissue that had partially formed a cyst cavity. Granulation tissue lined with necrotic tissue and inflammatory cells formed the cyst wall. The pericystical area contained thyroid tissue with epithelium-lined follicles with round nuclei, clear chromatin, and nuclear groove. This finding confirmed the presence of a thyroid abscess associated with a follicular variant of papillary thyroid carcinoma with no extrathyroidal extension.

All symptoms were relieved following surgery on the eighth day of admission, including the neck tenderness that was the patient's chief

complaint. The WBC count was reduced to 7,950/uL in the laboratory, and no bacteria were detected in blood culture or fluid specimens. She was then discharged and prescribed ampicillin sulbactam 375 mg twice daily for three days.

Thyroid abscesses are uncommon, even in patients with compromised host defenses.[1-3] It is more common in women aged 20-40 and affects the left lobe more than the right.[3-5] It accounts for less than 1% of all thyroid diseases and less than 0.7% of surgical pathology in the thyroid gland.[1-3] It is even more uncommon to see thyroid carcinoma presenting as thyroid abscess.

The patient is a 50-year-old woman who has had an untreated thyroid nodule for a year. The patient was then admitted to the hospital with acute symptoms of an enlarged, painful, and warm mass on the left side of the neck that caused difficulty swallowing and was accompanied by a high fever and headache. At first, we suspected it purely as an infection of thyroid abscess. She had no history or laboratory evidence of respiratory infection or immunodeficiency; however, she had a history of thyroid nodule since a year ago. The patient's initial laboratory test results revealed an elevated WBC count of 16,800/uL, an elevated neutrophil count (78 %), ESR of 120 mm/h, and CRP of 86.0 mg/L, but normal TSH and free T4 levels. It is common to see increased non-specific markers of infection such as leukocyte and ESR with normal thyroid function tests. However, hyperthyroidism or hypothyroidism may be present in some cases of thyroid abscess.[3,4,9]

Ultrasound and computed tomography (CT) scans are highly sensitive for the detection of abscess collection.[9] Ultrasound examinations provide sufficient information about intra- or extra-thyroid abscesses, solid or mixed lesions, and the thyroid gland's echostructure and vascular flow. A CT scan can be used to pinpoint the abscess's location and any adjacent organs or structures.[4] When a pyriform sinus fistula is suspected, barium swallow has a high sensitivity for detecting it. This test, however, should be done after the infection has been resolved.[8] In our case, the CT scan revealed the abscess and its relationship to neighboring structures. It revealed a cystic lesion in the left lobe measuring 7.2x5.4x7.1cm with a thick wall, dislodging the glottis and trachea to the right and extending inferiorly to the supraclavicular, consistent with a left thyroid abscess. At the same time, neck sonography revealed a cystic lesion in the left lobe that was encased in a thick wall and contained a ruptured lateral capsule.

Although the incidence is rare, thyroid abscesses may lead to significant morbidity and mortality, especially if left untreated. Thyroid storm, airway obstruction due to laryngeal oedema or tracheal compression, tracheal and esophageal perforation, descending necrotizing mediastinitis, internal jugular vein thrombosis, and generalized sepsis are all severe complications of thyroid abscess that can occur at any time.[7,9,11] FNA (fine needle aspiration) is a minimally invasive procedure that can be used to diagnose a thyroid abscess, differentiate benign from malignant thyroid nodules, and obtain culture specimens of the causative organism. Thus, a more targeted antibiotic therapy can be prescribed.[4,10] Staphylococcus aureus is the most frequently implicated causative organism in thyroid abscesses. However, other organisms, such as oropharyngeal anaerobes or Gram-negative aerobes, are also involved.[7] Mycobacterium tuberculosis, Candida albicans, and Brucellosis, all of which are mixed flora, have been reported occasionally.[3,4,8,12] As a result, this should be considered when initiating empiric antibiotic therapy in the absence of a culture result.[9]

As no organism grew in the culture of blood and fluid specimens in this patient, antibiotics were given empirically in combination with percutaneous drainage and intracavitary antibiotic injection to resolve the symptoms. However, because the thyroid capsule has ruptured and the abscess has spread to adjacent tissue, the isthmolobectomy procedure is necessary in this case. Surgical intervention and dual antimicrobial therapy were used to resolve the thyroid abscess. Histological examination confirmed thyroid abscess with a follicular variant of papillary thyroid carcinoma without extrathyroidal extension.

The management of a thyroid abscess is appropriate systemic antibiotics and abscess drainage. Aspiration of the abscess may resolve

in cases with small abscesses, although a larger one will need partial or total thyroidectomy.[7,11] Percutaneous image-guided drainage of thyroid abscesses with catheter irrigation and intracavitary antibiotics also have been reported as conservative management. Yeow, et al. recommended needle aspiration drainage for small lesions (<3 cm) and catheter drainage for lesions larger than 3 cm or with the involvement of thyroid or parotid gland. Percutaneous needle drainage was performed twice in each case and antibiotics were injected inside the cavity after pus evacuation and rinsing the lesions with 0.02% chlorhexidine gluconate.[5] In the presence of underlying pathology such as the pyriformis sinus tract, operative management is recommended to achieve definite control and prevent a recurrence.[9]

We presented a rare case of thyroid cancer presenting as thyroid abscess. A year before, the patient was diagnosed with an unevaluated thyroid nodule, which could be a malignant lesion, while benign or malignant nodule is a risk factor of thyroid abscess. The patient was initially planned to be treated conservatively by intravenous and intracavitary antibiotic injections. Ultrasound-guided aspiration was conducted for diagnostic and to relieve pain. However, surgical intervention was needed due to the rupture of the capsule and the expansion of the abscess to the surrounding tissue. The histopathological findings from the surgery confirmed thyroid abscess with a follicular variant of papillary thyroid carcinoma. In this case report, this rare finding (cancer presenting as an abscess) explained that physicians must be aware of thyroid abscess in acute tender neck swelling, as it may present as thyroid cancer.

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Clinical Outcome Following Prolonged Neoadjuvant Chemotherapy and Delayed Surgery in Osteosarcoma Patients: An Evidence-based Clinical Review

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ABSTRACT

Background: *The incidence of osteosarcoma reached 16.8 cases annually at dr. Cipto Mangunkusumo Hospital in 1995-2008. Previous studies suggested that prolonged neoadjuvant chemotherapy followed by delayed surgery improves the clinical outcome. Prolonged neoadjuvant chemotherapy followed by delayed surgery commonly occurs in Indonesia, as diagnostic imaging and surgery waiting list will delay the surgery. The aim of this study is to observe the survival rate and the event-free survival rate of osteosarcoma patients with prolonged neoadjuvant chemotherapy and delayed surgery.* ***Methods:*** *This review included randomized controlled trials (RCTs), cohort studies, retrospective cohort studies, clinical trials, and reviews. Literature search was conducted through MEDLINE (PubMed search engine), Cochrane Central Register of Controlled Trial, and Scopus. The studies were screened and selected according to inclusion criteria by author and contributors independently.* ***Results:*** *Six studies were included in the qualitative synthesis of this study. Overall survival rate, event-free survival rate, histological response and recurrence as well as neoadjuvant chemotherapy duration, cycle and regiment were assessed in this study.* ***Conclusion:*** *Prolonged neoadjuvant chemotherapy and delayed surgery results in 5-years survival rate of 43.2% to 96.6% and 5-years event-free survival rate of 35.7% to 86.4%.*

Keywords**:** *prolonged chemotherapy, neoadjuvant chemotherapy, delayed surgery, osteosarcoma.*

INTRODUCTION

Osteosarcoma is a malignant bone tumor of mesenchymal origin produced in the bone stroma characterized histologically by spindle cells and osteoid production.[1,2] Osteosarcoma most frequently develops from the epiphyseal growth plate in distal femur or proximal tibia, where rapid bone growth occurs.[1] The alteration of mesenchymal stem cell differentiation to osteoblast is considered the most probable cause of osteosarcoma.[1] The definite cause of osteosarcoma has not been elucidated.[3] Several authors suggested that the germline mutation of TP53 gene in chromosome 17p13C in Li-Fraumeni syndrome (LFS) plays a role as a predisposing factor for osteosarcoma.[3] Microdeletion germline loss of RB1 gene in chromosome 13q14 in retinoblastoma is also associated with osteosarcoma in some degree.[3,4]

Osteosarcoma is the most common primary bone tumor with the global incidence of 2-3 cases/million/year in the general population, 8-11 cases/million/year in adolescent, and 2.5-5 cases/million/year in elderly.[1,5] The incidence of

osteosarcoma has bimodal distribution across age groups with the first peak in children and adolescents (<24 years old) and the second peak in the elderly (>60 years old) and it is commonly related to Paget's disease.[3,5,6] The incidence of osteosarcoma in Asia, including Indonesia, shows a similar epidemiological pattern with the incidence of 2.5-4.1 cases/million/year in adolescents and 2.4-3.1 cases/million/year in elderly.[5] A study conducted in Jakarta, Indonesia showed the incidence of osteosarcoma reached 16.8 cases annually at dr. Cipto Mangunkusumo General Hospital in 1995-2008.[7] Furthermore, in Indonesia the osteosarcoma patients usually receive delayed proper therapy due to social-economical condition, low education, strong belief in traditional medicine, geographical factor, long administrative process, and scarcity of oncologic orthopedic surgeons. Delayed treatment in osteosarcoma could lead to significant morbidity and mortality.[8] Previous study shows significant lower overall survival and event free survival in patients with delayed chemotherapy initiation and completion.[9]

Advancement of chemotherapy, surgery, and reconstruction options improves the clinical and functional outcomes of osteosarcoma in the last 50 years.[10] A study conducted in 1982 showed increased survival rate with preoperative chemotherapy (neoadjuvant) compared to immediate surgery.[3] Currently, osteosarcoma is treated by neoadjuvant chemotherapy followed by wide surgical resection and adjuvant chemotherapy.[10] Even with chemotherapy, the previous study showed that the survival rate of osteosarcoma had plateaued around 60% in 5-years survival.[11] However, a study in China suggested that a prolonged neoadjuvant chemotherapy followed with delayed surgery showed an improved clinical outcome with 2-years survival of 74.2%.[12] Moreover, prolonged neoadjuvant chemotherapy followed by delayed surgery commonly occurs in Indonesia, as diagnostic imaging and surgery waiting list will delay the surgery. Further investigation of prolonged neoadjuvant chemotherapy followed by delayed surgery is necessary to improve the clinical outcome of osteosarcoma. Reviewing the outcome of prolonged neoadjuvant chemotherapy followed by delayed surgery is important as a potential treatment to improve clinical outcomes in osteosarcoma patients..

The aim of this evidence-based clinical review is to observe and evaluate the clinical outcome of prolonged neoadjuvant chemotherapy and delayed surgery in osteosarcoma patients. This review will answer these following questions: 1). What are the survival rate and the event-free survival rate of prolonged neoadjuvant chemotherapy and delayed surgery in osteosarcoma patients? 2) What is the ideal duration for neoadjuvant chemotherapy in osteosarcoma patients?

METHODS

In this review, we only included randomized controlled trials (RCTs), cohort studies, retrospective cohort studies, clinical trials, and reviews. Case reports, case series, and commentary were not included in this review. The characteristics of population in this review are patients with osteosarcoma in any age with or without metastatic disease. The interest of this study is interventions addressing prolonged neoadjuvant chemotherapy followed by delayed surgery defined as preoperative chemotherapy with three or more cycles of any chemotherapy regimens followed by surgical resection and adjuvant chemotherapy. There was no restriction by type of comparison of intervention in this review. The primary outcomes for this study were outcomes related to the survival of osteosarcoma patients such as overall survival rate and event-free survival rate. Other outcomes affecting the survival including histopathological pattern and recurrence rate were considered as a secondary outcomes. Primary and secondary outcomes were collected as reported from each study. The outcome were analyzed and graded.

Information Sources

Literature search strategy were developed using medical subject headings (MeSH) terms and any text words related to prolonged neoadjuvant chemotherapy in osteosarcoma cases. Literature search were conducted through MEDLINE (PubMed search engine), Cochrane Central Register of Controlled Trial, and Scopus

from 1970-2020. The literature search was not limited by language. Reference lists of included studies and relevant reviews provided by the electronic database were scanned to ensure literature saturation.

Search Strategy

Quantitative studies and qualitative studies were included in this study. An electronic database search engine was used to search the literatures. Specific search keywords were created by the primary author with input from other contributors. Search keywords were (((((osteosarcoma) OR osteogenic sarcoma) OR bone sarcoma)) AND ((((neoadjuvant chemotherapy) OR neo-adjuvant chemotherapy) OR preoperative chemotherapy) OR pre-operative chemotherapy)) AND ((((delayed surgery) OR prolonged chemotherapy) OR long course chemotherapy) OR multiple course chemotherapy) for MEDLINE and Cochrane database. Search keywords of (Osteosarcoma) AND (prolonged neoadjuvant chemotherapy) AND (delayed surgery) were used for Scopus database. Literature searching was conducted in January 24th 2020.

Study Records

Study titles and abstracts were screened and selected according to the inclusion criteria by author and contributors independently. Selected studies were matched among the contributors. Full-text articles were downloaded and reviewed by the author and contributors. Additional information from the study was sought if necessary. Disagreement among authors and contributors was solved by discussion.

Risk of Bias

To ascertain the validity of the selected studies, a pair of independent reviewers were selected to determine the randomization adequacy, blinding, concealment of data, data collection, drop-out subjects, and outcome assessment. Cochrane risk of bias for cohort study was used as risk of bias assessment tool. Reviewers were then concluded the risk of bias from the selected studies.

RESULTS

Systematic literature searching identified 130 relevant titles and abstracts with 109 articles from MEDLINE, 18 articles from Cochrane and 3 articles from Scopus. Eight abstracts were excluded after duplication screening of the titles. One-hundred twenty-two abstracts were reviewed and 98 abstracts were excluded due to study population, study design, and intervention incompatibility. Twenty-four full-text articles were reviewed and analyzed. Eighteen full-text articles was excluded from this study due to intervention incompatibility (neoadjuvant chemotherapy regimen shorter than 3 cycles, combined with radiotherapy, regional therapy, and early surgical intervention), unrelated outcomes (did not include overall survival rate nor event-free survival rate), and other considerations (no full-text available). Therefore, six studies were included in the qualitative synthesis of this study. A complete flowchart of the systematic literature search is outlined in **Figure 1**.

The characteristics of the selected studies are outlined in **Table 1**. The studies were published between 1979-2019. The number of subjects in the study ranged from 31 to 300 osteosarcoma patients. Two of the studies obtained were published in China, two studies were published in the United States, and two studies was published in Europe. Five studies were retrospective studies and only one study was a prospective study. The neoadjuvant chemotherapy in these studies was followed by surgical intervention and various adjuvant chemotherapy.

Cycle and Duration

All studies had at least 3 cycles of neoadjuvant chemotherapy ranging from 3 – 10 courses of chemotherapy. One study administered 3 courses of chemotherapy with 36 days duration. One study administered 3-5 courses of chemotherapy with 13 weeks duration. Other studies administered 3-6 courses and 6 courses of chemotherapy with a duration of 52 and 50 weeks, respectively. Two studies administered 4 and 6-10 courses of chemotherapy without describing the duration of the therapy.

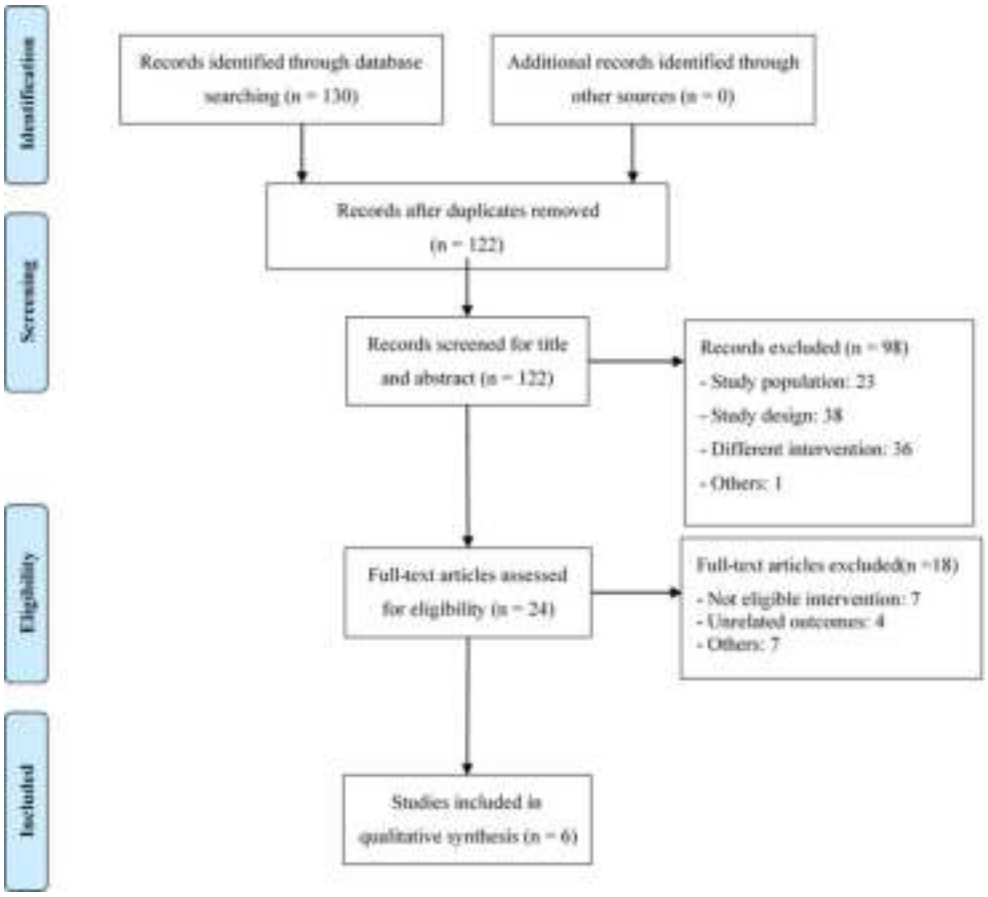

Figure 1. A flowchart of the study.

Regimen

Five of six studies included high-dose methotrexate (HD MTX) in the combination of neoadjuvant chemotherapy regimen, including HD MTX + ADR + IFO, HD MTX + DOX + IFO + CDDP, HD MTX + DOX + CDDP, HD MTX + CFR + ADR +VcR, and HDMTX + CDDP + ADR. Another study administered chemotherapy regimen without HD MTX with DOX + IA CDDP, and CDDP + ADR + IFO. We found no study administering monotherapy as prolonged neoadjuvant chemotherapy.

Overall Survival Rate

Survival analysis was obtained in the forms of overall survival rate and event-free survival rate. Three of the studies reported 5-years survival rate ranging from 43.2% to 96.6%. Two studies reported 10-years survival rate ranged from 36.6% to 93.2%. One study reported 4-years survival rate of 77% and two studies did not report the overall survival rate. Event-free survival rate was reported in 5 studies within 5-10 years follow-up. Four studies reported 5 years of event-free survival rate ranging from 35.7% to 86.4%. One study reported 8-years event-free survival rate of 59% and two studies reported a 10-years event-free survival rate of 26.8% to 86.4%.

Histological Response and Recurrence

The histological response was reported in 5 of 6 studies included. Four studies reported good histological response as >90% tumor cell necrosis and one study reported good histological response as >90% reduction in tumor neovascularity. Studies reported >90% tumor cell necrosis ranged from 8% to 86% and reduction in tumor neovascularity of 87%. Local recurrence was described in 5 studies ranging from 0% to 41% local recurrence.

Risk of Bias Assessment

The risk of bias concluded from the assessment tool showed low risk of bias for study by Ferrari (2001). Moderate risk of bias were concluded from studies by Xu (2014), Xu (2018), Wilkins (2005), and Bacci (1993). Serious risk of bias was found from study by Rosen (1979) with poor confounding bias consideration and analysis method. Complete risk of bias assessment is presented in **Table 2**.

Table 1. Characteristics of included studies

Reference (year)	Study design	Cycle	Neoadjuvant chemotherapy duration	Neoadjuvant chemotherapy regimen	Surgical procedure
Xu et al.[13] (2014)	Retrospective study	6-10 courses	Not described	MMIA protocol (HD-MTX, ADR, IFO) and DIA protocol (CDDP, ADR, IFO)	Tumor resection and prosthetic replacement OR Tumor resection and autograft implantation OR Marginal tumor resection
Xu et al.[14] (2018)	Retrospective study	3-6 courses	1 year	DOX, HD MTX, IFO, CDDP	Not described
Ferrari et al.[15] (2001)	Retrospective study	4 courses	Not described	HD MTX, DOX, CDDP	Limb salvage, amputation, rotationplasty
Rosen et al.[16] (1979)	Retrospective study	6 courses	50 weeks	HD MTX, CFR, ADR, VcR	En bloc resection
Wilkins et al.[17] (2005)	Prospective study	3-5 courses	13 weeks	IA DOX, IA CDDP	Wide resection and endoprosthetic replacement
Bacci et al.[18] (1993)	Retrospective study	3 courses	36 days	HD MTX, CDDP, ADR	Limb salvage, amputation, rotationplasty

HD MTX: High-dose methotrexate, ADR: Adriamycin, IFO: Ifosfamide, CDDP: Cisplatin, DOX: Doxorubicin, CFR: Citrovorum factor, VcR: Vincristine, IA DOX: Intra-arterial Doxorubicin, IA CDDP: Intra-arterial Cisplatin

Table 2. Data extraction

Reference (year)	Cycle	Neoadjuvant chemotherapy regimen	Overall survival rate	Event-free survival rate	Histologic response	Recurrence
Xu et al.[13] (2014)	6-10 courses	MMIA protocol (HD-MTX, ADR, IFO) and DIA protocol (CDDP, ADR, IFO)	5-years survival rate = 61.8%	5 years = 57.7%	100% in 27 subjects (54%) >90% in 16 subjects (32%) 50-90% in 7 subjects (14%)	8.5% local recurrence
Xu et al.[14] (2018)	3-6 courses	DOX, HD MTX, IFO, CDDP	5-years survival rate = 43.2% 10-years survival= 36.6%	5 years = 35.7% 10 years = 26.8%	>90% in 4 subjects (8%) 60-90% in 23 subjects (45%) <60% in 24 subjects (47%)	30% local recurrence
Ferrari et al.[15] (2001)	4 courses	HD MTX, DOX, CDDP	Not described	8 years = 59%	>90% in 203 subjects (68%)	41% local recurrence
Rosen et al.[16] (1979)	6 courses	HD MTX, CFR, ADR, VcR	4-years survival rate = 77%	Not described	Not described	Not described
Wilkins et al.[17] (2005)	3-5 courses	IA DOX, IA CDDP	5- years survival rate = 96.6% 10-years survival rate = 93.2%	5 years = 86.4% 10 years = 86.4%	>90% in 54 subjects (87%)	No local recurrence
Bacci et al.[18] (1993)	3 courses	HD MTX, CDDP, ADR	Not described	5 years = 63.1%	>90% in 117 subjects (71.3%)	3% local recurrence

HD MTX: High-dose methotrexate, ADR: Adriamycin, IFO: Ifosfamide, CDDP: Cisplatin, DOX: Doxorubicin, CFR: Citrovorum factor, VcR: Vincristine, IA DOX: Intra-arterial Doxorubicin, IA CDDP: Intra-arterial Cisplatin

Table 3. The risk of bias in cohort studies assessment tool

Reference	Bias due to confounding	Bias in selection of participant into the study	Bias in classification of interventions	Bias due to deviation from intended interventions	Bias due to missing data	Bias in measurement of the outcome	Bias in selection of the reported result	Conclusion
Xu et al. (2014)	Moderate risk	Low risk	Low risk	Low risk	Moderate risk	Low risk	Moderate risk	Moderate risk of bias
Xu et al. (2018)	Moderate risk	Low risk	Low risk	Low risk	Moderate risk	Low risk	Low risk	Moderate risk of bias
Ferrari et al. (2001)	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk of bias
Rosen et al. (1979)	Serious risk	Moderate risk	Low risk	Low risk	Low risk	Low risk	Low risk	Serious risk of bias
Wilkins et al. (2005)	Moderate risk	Low risk	Low risk	Low risk	Moderate risk	Low risk	Low risk	Moderate risk of bias
Bacci et al. (1993)	Moderate risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Moderate risk of bias

DISCUSSION

Nowadays, neoadjuvant chemotherapy followed by wide surgical resection and additional adjuvant chemotherapy has been the main treatment of osteosarcoma.[10,19,20] Studies showed that administration of neoadjuvant chemotherapy has increased the 5-years survival rate of osteosarcoma to 50%-60% compared to 20% with surgery alone.[10,21] Furthermore, neoadjuvant chemotherapy theoretically could optimize the surgical result by treating micro-metastases early, allowing histological assessment of chemotherapy, and adjusting the adjuvant chemotherapy.[21] Common chemotherapeutic agents used as neoadjuvant chemotherapy are high-dose methotrexate (HD MTX), Doxorubicin (DXR), Cisplatin (CDDP), and Ifosfamide.[21,22] Other chemotherapeutic agents such as Vincristine (VcR), Bleomycin, and Dactinomycin have also been used for neoadjuvant chemotherapy.[22] Recent trials still used HD MTX as the backbone of neoadjuvant chemotherapy.[19] However, 5-years survival rate of osteosarcoma has remained stagnant in the past 25 years despite various attempt to improve survival.[23]

Prolonged neoadjuvant chemotherapy and delayed surgery have been around since the discovery of neoadjuvant chemotherapy for osteosarcoma patients.[14] Prolonged neoadjuvant chemotherapy aims to improve the histological response leading to the improvement of survival in osteosarcoma patients. Previous studies have shown a significant increase of event-free survival rate and overall survival rate in osteosarcoma patients with good histopathological response following neoadjuvant chemotherapy.[19,24,25] Prolonged neoadjuvant chemotherapy could also be used as a waiting therapy for osteosarcoma patients with delayed surgery in the case of long waiting time for prosthetic development. However, studies have shown various outcomes following prolonged neoadjuvant chemotherapy and delayed surgery.

Overall survival rate and event-free survival rate of most studies included in this review showed similar survival to previous studies with standard neoadjuvant chemotherapy, in which the 5-years survival rate ranged from 50%-60%.[19,22,23] The longest course of neoadjuvant chemotherapy cycle (6-10 cycles) shown by Xu et al. in 2014 reported 5-years survival rate of 61.8%.[12] However, a study conducted by Wilkins et al. in 2005 showed surprisingly high 5-years and 10-years survival rate with good histological response.[17] Direct evaluation of histological response (reduction in neovascularity) by using angiography in this study could be one of the factors improving the overall survival rate.[17] This could also explain the high rate of histological response reported in this study. On the contrary, a study by Xu et al. in 2018 showed particularly low overall survival rate and histological response to prolonged neoadjuvant

chemotherapy.[14] A long interval of neoadjuvant chemotherapy (up to 1 year) and higher risk of bias in this study could affect the outcome.[14] However, this study included an older patient with high infiltration rate to local tissue which could affect the outcomes.[14] The incidence of local recurrence in the studies included showed various results of up to 41%. Older age, tumor volume, aggressive tumor, no surgical resection, metastasis, poor histological response, and poor postoperative chemotherapy compliance are the prognostic factors for worse outcomes in osteosarcoma patients.[26–30] However, the chemotherapy regimen does not associate with the outcomes in osteosarcoma patients.[26]

The histological response of prolonged neoadjuvant chemotherapy showed various results ranging from 8% to 86% of >90% local tumor necrosis and 87% of >90% reduction of tumor neovascularity.[14,17,18] As aforementioned above, the histological response to neoadjuvant chemotherapy is one of the most important prognostic factors in osteosarcoma patients.[24,25,31–33] A previous study showed that a good histological responder to neoadjuvant chemotherapy had long-term survival of 70% to 80% compared to that of 15% in poor responders.[25,31] Studies included in this review shows a similar tendency towards high overall survival rate in good histological response. The poor histological response shown by Xu et al. (2018) translates to low overall survival rate compared to other studies with better histological response.[14]

The histological response of neoadjuvant chemotherapy in osteosarcoma depends on several factors, e.g. osteosarcoma type, various chemotherapy regimens, and drug resistance. The degree of necrosis in response to neoadjuvant chemotherapy varies between osteosarcoma type.[25] Telangiectatic osteosarcoma generally shows a good response to neoadjuvant chemotherapy (80-90%).[31] Incompatibility to chemotherapy regimen and prolonged waiting duration could also affect the histological response of osteosarcoma.[14] High-intensity neoadjuvant chemotherapy also shows a significant association with the histological response but not with overall survival rate in osteosarcoma patients.[34] High-intensity neoadjuvant chemotherapy might not increase the histological response high enough to affect the overall survival rate in osteosarcoma.[34] Furthermore, increased risk of adverse effects in high-intensity neoadjuvant chemotherapy, e.g. thrombocytopenia and mucositis, might also affect the overall survival rate.[34] Chemotherapy drug-resistant clone has been described in a previous study with poor response to neoadjuvant chemotherapy and increased risk of metastasis.[32]

Local recurrence after complete therapy in osteosarcoma patients is identified as a poor prognostic factor, especially the early local recurrence and positive margin at the time of initial surgery.[35–38] In this study, local recurrence ranged from 0% to 41% local recurrence.[15,17] A previous study showed 6% to 9% local recurrence with 60% of them occurring early (24 months).[39] Prognostic factors for local recurrence are good quality of surgical margin and good histological response to chemotherapy.[37] Improved survival after local recurrence could be achieved by wide surgical resection of osteosarcoma. Prognostic factors for survival after local recurrence are gender, metastasis, treatment of local recurrence, length of resection margin, alkaline phosphatase level, tumor volume, histologic subtypes, chemotherapy protocol.[36,40–42] Recurrence rate following prolonged neoadjuvant chemotherapy and delayed surgery showed similar recurrence rate, except for two studies with 41% and 30% local recurrence.[14,15] However, as aforementioned above, the local recurrence in osteosarcoma patients is highly affected by surgical procedure and histological response to chemotherapy.[37]

This review describes variable chemotherapy regimen, duration, and survival outcomes following prolonged neoadjuvant chemotherapy and delayed surgery. A limited amount of studies that controlled the duration of neoadjuvant chemotherapy and the quaility of the studies hinder the ability to conclude the optimal duration of neoadjuvant chemotherapy in this study. Therefore, we could not assess the effectivity of prolonged neoadjuvant chemotherapy and delayed surgery for the clinical outcomes nor the ideal duration of prolonged neoadjuvant chemotherapy in osteosarcoma patients. To

further address the outcomes of prolonged neoadjuvant chemotherapy and delayed surgery in osteosarcoma patients, a comparative study with a controlled duration of neoadjuvant chemotherapy models should be considered.

CONCLUSION

Studies have shown that prolonged neoadjuvant chemotherapy and delayed surgery results in 5-years survival rate of 43.2% to 96.6% and 5-years event-free survival rate of 35.7% to 86.4%. The ideal duration of neoadjuvant chemotherapy could not be concluded in this study.

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Management of Cholelithiasis with Concomitant Choledocholithiasis

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ABSTRACT

Cholelithiasis refers to a condition in which hardened deposits exist within the gall bladder. These deposits are also known as gallstones. Among other gastrointestinal diseases, Cholelithiasis is associated with the highest hospital admissions. Those with cholelithiasis are generally asymptomatic. However, symptoms may start to appear in the case of inflammation or the blockage of the bile duct, which occurs in 10-25% of patients with cholelithiasis. The condition in which gallstones are present in the common bile duct is known as choledocholithiasis. Surgery is a curative therapy for cholelithiasis concomitant with choledocholithiasis. Other available options include laparoscopy, endoscopy, percutaneous technique, and open surgery. These methods can be done gradually or in combination. Considering this, there have been controversies about the best management option for the case. Therefore, this article aims to analyze and compare each methods of management.

Keywords**:** *Cholelithiasis, choledocholithiasis, management.*

INTRODUCTION

Cholelithiasis refers to a condition in which the formation of stones occurs within the gallbladder system. These stones are called gallstones.[1] Cholelithiasis has become one of the most common gastrointestinal diseases with the most hospital admissions. Several data have shown that the occurrence of cholelithiases is found in 10-15% of Caucasian population, 70% in American Indian population, and 20% in European population. Asia on its own has a relatively low prevalence of the disease.[2,3] As for now, there is no data found concerning the epidemiology of cholelithiases in Indonesia. However, research by Tuuk *et al*. at the Provincial Public Hospital Prof. Dr. R.D. Kandou Manado revealed that there were 113 cases of cholelithiasis between 2015 and 2016.[4] Another research conducted in Cipto Mangunkusumo Hospital between 2008 and 2010 revealed 63 out of 129 patients with jaundice gone through endoscopic cholangiopancreatography therapy suffered from gallstones.[5]

Generally, patients with cholelithiasis do not show clinical manifestations or are asymptomatic. Nevertheless, around 10-25% patients will develop symptoms if inflammation or obstruction of the biliary duct is involved. The condition in which the common biliary duct is obstructed by gallstones is called choledocholithiasis.[1,2] Therefore, the incidence of cholelithiasis and choledocholithiasis is often diagnosed altogether, which is predicted to be 5-19%.[6] Research conducted by Prasson *et al.* (2016) showed

that the development of choledocholithiasis is approximately around 3-10% of cholelithiasis cases.[7] Memba *et al.* (2019) have specified that generally, choledocholithiasis is unintentionally diagnosed in routine checkups and pre-surgery radiology with incidence up to 5-33%.[8]

Cholesterol stones have become the most frequent cause of cholelithiasis and choledocholithiasis.[2] Several risk factors trigger the formation of the stones. Sex, family history, pregnancy, and age older than 40 are nonmodifiable risk factors. On the other hand, modifiable risk factors include obesity, rapid weight loss, high calorie diet, drugs such as oral contraceptives, type II diabetes history, hemolytic anemia, metabolic syndrome, dyslipidemia, smoking, and sedentary lifestyle.[1]

The management of cholelithiasis with choledocholithiasis has been a topic of discussion. Curative therapy for this case is surgery. Laparoscopic cholecystectomy is considered the gold standard of symptomatic cholelithiasis management. In contrast, the best management choice for choledocholithiasis is yet to be decided. Several choices are available such as laparoscopy, endoscopy, percutaneous technique, and open surgery, whether they are done in combination of gradually.[2] This article aims to discuss management choices for cholelithiasis with choledocholithiasis patients, and compare the methods.

PATHOPHYSIOLOGY

Gallstones are made from the elements in the gallbladder such as cholesterol, bile salts, bilirubin, and phospholipids. Its formation occurs when the concentration of each element is imbalanced and then it settles into a solid compound.[1] There are two types of gallstones, cholesterol stones composed of cholesterol and pigment stones mainly composed of calcium bilirubinate. Pigment stones are further divided into two types, black pigment and brown pigment. Black pigment stones are formed due to polymerization in the gallbladder and usually found in hyperbilirubinemia conditions. Brown pigment stones have a softer texture because they are more often found unpolymerized in the bile ducts. These stones can appear due to anaerobic bacterial infection or due to static flow.[1]

About 80% of the stones found in cholelithiasis are cholesterol stones. These stones form when the concentration of cholesterol is higher than the bile's ability to store them in solution.[1] The formation is triggered by the hormones estrogen and progesterone which play a role in increasing cholesterol secretion, decreasing bile salt secretion, and relaxing smooth muscle which will result in gallbladder stasis. Other conditions such as diabetic neuropathy, elevated levels of non-HDL cholesterol, metabolic syndrome, obesity, and rapid weight loss can also trigger cholesterol stone formation.[1]

Gallstones, especially small ones, can migrate to the common bile duct due to gallbladder contraction.[6] When these stones begin to block the ducts, they can cause hepatocyte damage due to reflux of flow to the liver and damage to the pancreas. The patient will also have biliary colic or pain in the epigastrium.[1]

CLINICAL MANIFESTATIONS AND DIAGNOSIS

Cases of asymptomatic and uncomplicated cholelithiasis patients are estimated to be between 60 and 80% of total cases.[9] However, the risk of becoming symptomatic increases by 1-2.3% annually.[2] The cardinal signs of cholelithiasis or choledocholithiasis are usually fever, jaundice, accompanied by biliary colic. Biliary colic is a constant and sharp pain lasting more than 15 minutes, with irregular intervals in the right upper quadrant or epigastrium.[3] This pain may radiate to the ipsilateral scapula called Collin's sign.[1] Other symptoms may include back pain, nausea, vomiting, dyspepsia, diaphoresis, and abdominal bloating.[1,3] In patients with choledocholithiasis, the stool produced tends to be oily and has a foul odor due to obstruction of fat digestion substance, bile, in the duodenum.[1]

The presence of cholelithiasis can be diagnosed by transcutaneous ultrasonography (US) examination with a sensitivity of 95% and a specificity of 100%. The purpose of this sonography is to completely visualize the gallbladder from various positions along with the appearance of gallstones. According to the National Institute for Health and Care

Excellence (NICE), patients who are suspected of cholelithiasis are advised to undergo US and blood tests to see liver function.[3] In patients with suspected choledocholithiasis, liver function tests such as total bilirubin, gamma-glutamyltransferase (γ-GT), alkaline phosphatase (AP), alanine aminotransferase and aspartate aminotransferase (ALT/AST), and lipase, should be checked together with US.3 Direct and total bilirubin have the highest sensitivity and are therefore considered the most reliable predictors of suspected choledocholithiasis.[2]

US examination sometimes fails to confirm choledocholithiasis. So, further radiological examination with MRI or CT scan is required. Radiological examination with CT scan, in this case, is rarely chosen because of the lower diagnostic power compared to that of MRI and high radiation exposure. In order to confirm further choledocholithiasis, Magnetic Resonance Cholangiopancreatography (MRCP) or Endoscopic Ultrasonography (EUS) are the most common options. NICE confirmed that EUS and MRI are also highly effective in detecting choledocholithiasis.[3] Both EUS and MRCP have similar specificity. For sensitivity, EUS is significantly superior to MRCP, with a percentage of 97% vs. 87%.[3]

The results of clinical, laboratory, and sonographic findings can be used as a guide to establishing the probability of the cholelithiasis patient also has choledocholithiasis complications. The probability is divided into three categories, high, medium, and low.[3] The criteria for each can be seen in **Table 1**.

Patients with a high probability of choledocholithiasis will be treated with Endoscopic Retrograde Cholangio-pancreatography (ERCP). ERCP is very helpful in assisting clinicians, not only as a diagnostic tool but also for therapeutic uses, especially in pancreaticobiliary tract disorders which need stone extraction. Retrospective study by Abdullah *et al.* (2012) in Cipto Mangunkusumo Hospital about the effectiveness of ERCP in the treatment of 53 choledocholithiasis patients showed 81% successful stone removal. Baron TH and Harewoods GC7 also reported 94.3% overall successful stone removal.[5]

If the probability of choledocholithiasis is low or moderate, an EUS or MRCP is recommended to decide whether an ERCP is necessary or not. For patients with a high probability of choledocholithiasis, it is also advisable to check with EUS or MRCP first. This examination is to improve accuracy and allow ERCP-referred patients to avoid unnecessary invasive procedures.[3] Figure 1 shows the diagnosis and treatment algorithm for choledocholithiasis in cholelithiasis patients.

MANAGEMENT

The principle of management of cholelithiasis is based on the presence or absence of symptoms and complications. Management generally includes changes in lifestyle, diet, and pharmacology. In asymptomatic patients diagnosed accidentally, the best treatment that can be done is expectant management. Pharmacological management is usually given to patients with mild symptoms.[1] Pharmacology provided consists of pain control, antiemetic,

Table 1. Criteria for simultaneous choledocholithiasis in a patient with cholelithiasis.

High likelihood of simultaneous choledocholithiasis (> 50%)
- Sonographically widened extrahepatic bile duct (>7 mm) + hyperbilirubinemia + elevation of γ-GT, AP, ALT, or AST or
- Sonographic demonstration of bile duct concrements or
- Clinical and laboratory criteria of ascending cholangitis
Moderate likelihood of simultaneous choledocholithiasis (5–50%)
- Criteria for neither high nor low likelihood fulfilled
Low likelihood of simultaneous choledocholithiasis (<5%)
- Normal bile duct width (up to 7 mm)
- Total bilirubin, γ-GT, AP, ALT, or AST not elevated during current pain episode
- Absence of episodes with biliary pancreatitis, acholic stools, and/or urobilinogenuria or bilirubinuria in recent past

Source: (Gutt *et al.*, 2020)

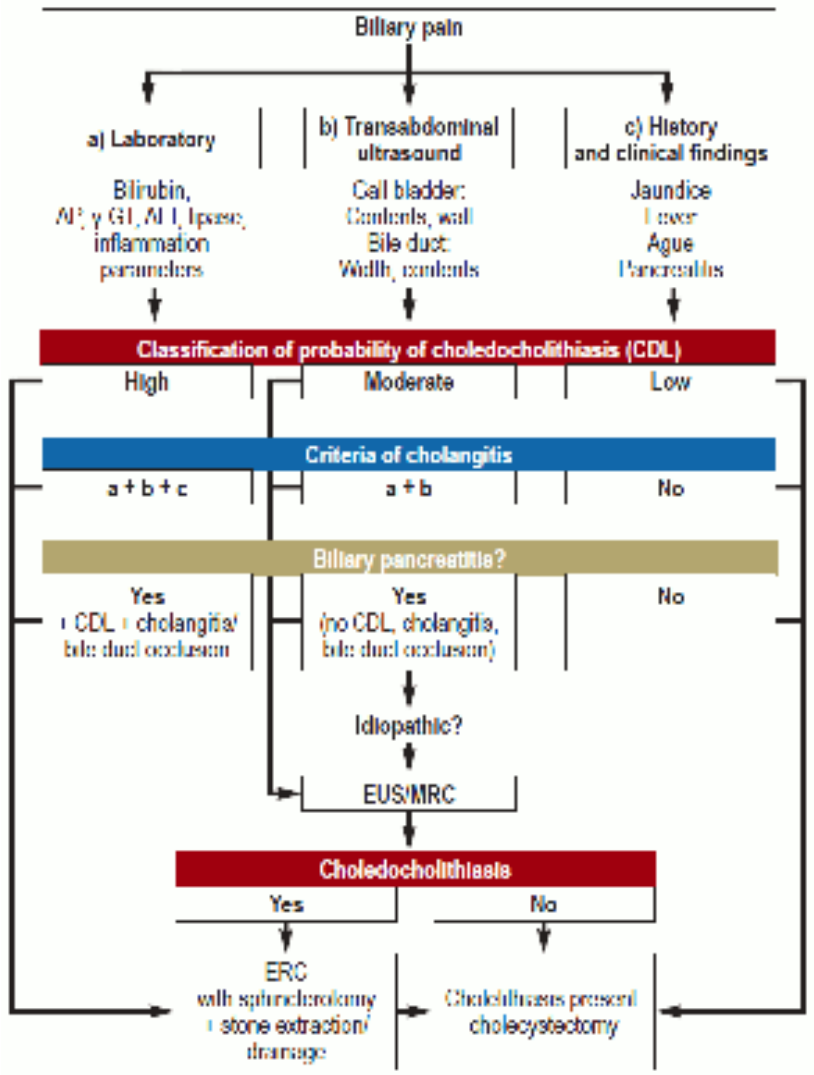

Figure 1. Algorithm for diagnosis and management of choledocholithiasis in cholelithiasis patients. Source: (Gutt et al., 2020)

and dissolution agent if needed. However, administration of a dissolution agent such as UDCA (Ursodeoxycholic Acid) to dissolve gallstones is not routinely recommended in all patients. Administration of UDCA is recommended in risk groups such as patients with rapid weight loss and patients with LPAC (low phospholipid-associated cholelithiasis). UDCA can also be administered to symptomatic patients with small cholesterol stones to avoid surgical intervention.[1,3] Surgical management is indicated in patients with symptomatic cholelithiasis, both complicated and uncomplicated, patients with asymptomatic cholelithiasis with gallstones >3 cm, polyps >1 cm, porcelain gallbladder, and patients with an increased risk of gallbladder cancer.[3]

In cases of cholelithiasis accompanied by choledocholithiasis, surgical intervention is carried out to clear the tract and prevent more severe complications such as cholangitis and pancreatitis due to obstruction.[2,9,10] However, until now the best method for cholelithiasis patients accompanied by choledocholithiasis has not been determined.[6] Laparoscopic cholecystectomy is considered the gold standard for symptomatic cholelithiasis. On the other hand, the best method for choledocholithiasis is still uncertain.[2] Several guidelines such as the German Clinical Practice guideline, EASL, and SAGES provide treatment recommendations for cases of cholelithiasis accompanied by choledocholithiasis.

Management recommendations from each guideline are presented in **Table 2**.

Over time, new surgical methods have emerged with minimal invasion. The management of cholelithiasis with choledocholithiasis has now changed from open surgery to laparoscopy or endoscopic surgery.[11] This new surgical method can be divided into two, namely that which is carried out in stages (two stage treatment or two stages of therapy) and carried out simultaneously (one stage or single stage or one stage therapy).[8] The first category includes preoperative ERCP+laparoscopic cholecystectomy (LC) and postoperative LC+ERCP. The second category includes LC+ Laparoscopic Common Bile Duct Exploration (LCBDE) and rendezvous method.[2] However, open surgery in cases of cholelithiasis accompanied by choledocholithiasis is still performed in approximately 5-20% of cases.[12] The procedure consists of cholecystectomy by making a large incision, open exploration of the common bile duct by choledochoenterostomy, and drainage with a T tube.[2,12] Currently, open surgery is performed when the stone clearance fails or the stone is difficult to extract by laparoscopic and endoscopic methods.[2]

ERCP followed by LC is the two-stage treatment most often chosen. ERCP is performed first to extract stones in the common bile duct using a sphincterotomy technique.[8] The second stage is followed by LC, which is removal of the gallbladder through four small incisions.[13] This second stage is carried out several days to weeks after ERCP depending on the patient's condition.[11] The other two-stage therapy is the reverse of the previous method where the LC is performed first and then post-operative ERCP is followed. However, this method has a lower success rate than preoperative ERCP+LC. So, it is rarely chosen.[2]

In addition to preoperative ERCP + LC, LCBDE is also often recommended nowadays.[6] LCBDE includes single-stage therapy consisting of LC together with exploration of the common bile duct using intra-operative cholangiography (IOC) or ultrasonography (IUS). Stones in the common bile duct are then removed via a transistic approach or choledocotomy under cholescopic direction.[7,8] As for the rendezvous method, LC and intra-operative ERCP were performed simultaneously. In this method, after cystic duct catheterization, cholangiography is performed to confirm the common bile duct stones. If the results are positive, then ERCP is performed, after which the LC is completed. However, the rendezvous method is rarely used because of the minimal availability of tools and experts.[2]

The three surgical methods for the management of cholelithiasis with choledocholithiasis have their respective advantages and disadvantages. Several RCTs and meta-analyses were performed to compare either two or all three methods of stone removal success, morbidity and mortality, complications, length of hospital stay, and cost of care. For comparison, the three methods are

Table 2. Recommendations for the management of cholelithiasis with choledocholithiasis.

Guideline	Treatment strategies in patients with choledocholithiasis with simultaneous cholelithiasis
German Clinical Practice Guideline	Therapeutic separation (pre- or intra-operatively) is recommended for patients diagnosed with choledocholithiasis and concomitant cholelithiasis (grade B recommendation, level of evidence I, strong consensus). After successful endoscopy of the bile ducts, cholelithiasis should be treated by cholecystectomy, ideally within 72 hours. Gallbladder that is still functional after stones are cleared (grade B recommendation, level of evidence I, strong consensus).
EASL	In patients found to have gallbladder and bile duct stones, early laparoscopic cholecystectomy should be performed within 72 hours after preoperative ERCP (moderate evidence, strong recommendation).
SAGES	ERCP with stone extraction that can be performed before, during, or after cholecystectomy, with little difference in morbidity and mortality rates, but the clearance rate is the same as for laparoscopic biliary tract exploration (level of evidence I, grade A recommendation).

Source: (Gutt *et al.*, 2020)

summarized in **Table 3**.

Meta-analysis by Singh and Kilambi (2018) compared the LCBDE+LC method with the ERCP+LC method to see which method is more ideal in cholelithiasis patients with choledocholithiasis.[6] There are several parameters for comparison. From the success rate of stone removal, LCBDE+LC had a higher success rate than ERCP+LC with a percentage of 88.1% and 82.2%, respectively. The failure rate of LCBDE+LC was also significantly lower (OR 0.59, 95% CI (0.38, 0.93), p=0.02). There was no significant difference in mortality and morbidity rates, treatment costs, and stone recurrence rates between the two methods. LCBDE+LC had a shorter hospitalization time than ERCP+LC with a value of 4.9±1.6 days compared to 6.5±3.4 days. This meta-analysis concludes that single-stage therapy with LCBDE+LC is better than two-stage ERCP+LC therapy in terms of the success rate of the procedure and length of hospitalization.[6] Similar results were obtained from a retrospective study in 2015-2016 comparing single-stage rendezvous therapy with two-stage ERCP+LC therapy. It was concluded that patients who underwent one-stage therapy had a shorter hospitalization time than patients who underwent two-stage therapy ($p < 0.026$).[9]

A study by Bayramov and Ibrahimova (2017) compared three procedures, namely open surgery, two-stage therapy, and one-stage therapy in 229 patients diagnosed with cholelithiasis with choledocholithiasis. The comparison parameters were seen from the length of the operation time, the success of removing all stones, the length of hospitalization, and complications. From the length of operation time, single-stage therapy and open surgery are not much different, but two-stage therapy requires a longer time. For successful removal of all stones, single-stage therapy has higher effectiveness than two-stage therapy, and open surgery, with percentages of 97%, 85.7%, and 94.8%, respectively. Open surgery has the longest hospital stay. The highest complications were found in open surgery (52.5%), followed by two-stage therapy (33.3%), and single-stage therapy (19.4%). Increased mortality was also found in open surgery but not in the other two methods. The results of this study suggest that single-stage therapy can be used as the first choice for treating cholelithiasis patients with choledocholithiasis.[12]

Some guidelines such as EASL and SAGES still recommend two-stage therapy as the management of cholelithiasis with choledocholithiasis. This therapy is also still widely used. The advantage of this two-stage therapy is that the procedure is simpler or the tools are commonly available so that it can be carried out in various health facilities, especially with endoscopic facilities.[8] The results of various studies show the safety and effectiveness of this therapy, especially with the presence of MRI or EUS which increases the sensitivity and specificity of therapy in the preoperative diagnosis of choledocholithiasis.[2] The success rate, mortality, morbidity, cost of treatment, and length of treatment time of the two-stage therapy are quite good. However, two-stage therapy requires two separate procedures and two anesthetic procedures. So, it has a higher risk of complications than single-stage therapy.[8] The most common risks of complications are pancreatitis, sphincter of Oddi dysfunction, and duodenobiliary reflux due to retrograde procedures in ERCP.[8,11,12] The lag between the two stages also increases the possibility of the stone re-migrating to the common bile duct

Table 3. Comparison of the three operative methods in cholelithiasis with choledocholithiasis.

Parameter	One-Step Procedure (LCBDE+LC or rendezvous)	Two-Stage Procedure (ERCP+LC or LC+ERCP)	Open Surgery
Stone removal success rate	97%	85,7%	94,8%
Length of stay (days)	2.3 ± 0.65	6.5 ± 1.5*	8.2 ± 2.7*
Mortality rate	0%	0%	3.8%
Complications	19.4%	33.3%*	52.5%*
Operating time (minutes)	123 ± 7	152 ± 8*	121 ± 8

* p≤0.05 compared to single-stage therapy (LCBDE+LC)[11]

before the LC can be performed.[2,8] Therefore, the implementation of LC should not be delayed too long to avoid stone recurrence.

From various comparison results, single-stage therapy has more advantages over the other two methods. Single-stage therapy is believed to be safe, effective, and efficient because two different pathological conditions can be resolved under one anesthesia and surgery. Complications due to two surgeries can also be minimized.[2,6] Despite these advantages, the choice of single-stage therapy needs to be considered. Adequate skills and instruments are required considering this therapy technique is quite complicated.[6]

CONCLUSION

Choledocholithiasis is one of the complications of cholelithiasis. This condition requires immediate management by surgery intervention to avoid serious complications caused by obstruction. There are a variety of surgery methods at the moment, starting from the minimally invasive methods such as one stage and two stage treatment, to the extremely invasive methods such as open surgery. Based on the results of several studies comparing each method, it can be concluded that one stage treatment is significantly more effective in terms of the successful rate of stone removal, complications, and duration of hospitalization, while still considering instrument availability and masterliness. No research has determined the best management method for cholelithiasis with choledocholithiasis cases. Therefore, a subsequent study is required to determine the best method of management for the referred case.

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