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Chronic Kidney Disease Care in Indonesia: Challenges and Opportunities

*Ni Made Hustrini**

Division of Nephrology and Hypertension, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

Corresponding Author:
Ni Made Hustrini, MD. Division of Nephrology and Hypertension, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital. Jl. Diponegoro no. 71, Jakarta 10430, Indonesia. Email: madekum99@gmail.com.

The burden of chronic kidney disease (CKD) is a significant global health concern. Previous study reported that the CKD incidence reached 200 cases per million per year in many countries with the prevalence 11.5% (4.8% in stages 1-2 and 6.7% in stages 3-5).[1] Other study further reported that the estimate prevalence of CKD was 15% higher in low- and middle-income countries compared to the high-income countries.[2] However, there are limited statistics available on the epidemiology of CKD in Indonesia. According to the Basic Health Research (Riset Kesehatan Dasar [Riskesdas], 2018], the prevalence of CKD in Indonesia increased from 0.2% in 2013 to 0.3% in 2018.[3-4] These results may, however, understate the true prevalence of CKD in our population. Despite the limited data on the CKD prevalence, the number of patients receiving kidney replacement treatment (KRT), primarily in the form of hemodialysis, is rapidly rising (i.e., more than 132.000 in 2018).[5] Chronic hemodialysis therapy results in extremely high expenses, correspond to the Indonesian Health Profile 2021 report, where kidney failure spending coming in fourth overall after heart disease, cancer, and stroke. However, the report also showed that the current health financing model places more emphasis on the curative or treatment area, while promotive and preventive measures merely occupy a very small portion (i.e., 0.3%).[6]

Addressing nephrology care, especially in developing country such as Indonesia, is not an easy task. In addition to the size of Indonesian population, which is expected to reach 271 million in 2021, we also have a shortage of nephrology professionals (doctors, nurses, technicians) and medical facilities. Meanwhile, the triple burden of disease continuously becoming our national health challenge. This is the result of inadequate control of infectious, re-emerging, and new emerging diseases; a demographic and nutritional transition which causing chronic diseases to be the top five list of catastrophic disorders; and the steady rise in the number of injuries and traumas.[7] Moreover, the prevalence of metabolic diseases associated with the progression of CKD increased over time, according to data from the National Basic Health Survey (Riskesdas, from 2007 to 2018) [e.g., diabetes mellitus 8.3% to 10.9%; hypertension 25.8% to 34.1%; stroke 7% to 10.9%; obesity 26.6% to 34.6%; and cancer 1.4% to 1.8%].[3-4,8] Also, our previous study demonstrated that hypertension prevalence among adults >18 years is as high as 41%, of which only 36.2% subjects treated with anti-hypertensive with less than a third (21.7%) of subjects consumed the medication regularly.[9]

A comprehensive nephrology referral system is also a challenge. We can argue this statement with evidence from the tertiary care, where it was reported that most kidney failure patients (83%) commenced dialysis with an urgent start, along with late referral to nephrologist (90%), started dialysis with temporary catheter

(95.2%), and the median eGFR to start dialysis was 5.3 (range: 0.6 – 14.6) ml/minute/1.73 m^2.[10] However, individual awareness, as well as an effective screening and prevention program for high-risk group are also a significant hurdles. Meanwhile, screening program to identify kidney diseases among population will cause a massive economic burden, thus specific CKD risk-factors should be known for Indonesian unique population. Our study was able to identify CKD risk factors that are significantly different from those in western countries (i.e., hepatitis [OR 3.406; CI 2.496-4.64]). This study underlined that Indonesia might need a different approach to CKD prevention program, that is not only focusing on the traditional risk factors (i.e., diabetes, hypertension, etc.) but also to include the communicable diseases, such as hepatitis.[9]

Since 2022, the Ministry of Health has initiated a health transformation program to improve the health system, to address health disparities, both within the country and between countries. There are six pillars of health transformations. First is transformation of primary services, which put more emphasis on promotive and preventive efforts. This aims to provide education related to disease prevention, and also to increase the capacity and capability of health workers in primary care. Second is transformation of referral services, focuses on increasing access and equitable distribution of health services in all regions in Indonesia. Third, the health resilience system which includes efforts to increase resilience in medical response and strengthen resilience during health crises. Fourth, transformation of health financing system to develop health financing regulations with the aim of building equity, easy accessibility for the community, and sustainability of financing allocations. Fifth, transformation of health human resources, to improving the quality of human resources ensuring and ensuring an even distribution of health workers in all over Indonesia. Lastly, transformation of health technology to encourages technological development and digitization in the health sector.[11]

One of the health transformation programs which specify in nephrology care is the implementation of the Uro-Nephrology Support Program (Program Pengampuan Uro-Nefrologi), with the aim to strengthen services, provide equal distribution of services and increase the latest technology for the diagnosis and treatment of urology/nephrology diseases in Indonesia. This program included secondary and tertiary care to improve the extent and quality of care to slowing the CKD progression, improving kidney replacement therapy (hemodialysis, peritoneal dialysis, and kidney transplant) access and treatment, as well as to provide dialysis training program for health care workers.

Providing high-quality nephrology care that all Indonesians can access is challenging. Yet, steps have already been taken in the direction of service enhancement. Thus, there is hope for better kidney health in Indonesia. Governments, academic medical centres, nephrology societies, as well as the citizen will all need to work together and take consistent effort to make a sustainable and comprehensive kidney care.

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Five-Year Survival Rate of Patients with End-Stage Renal Disease on Continuous Ambulatory Peritoneal Dialysis (CAPD) at Malang CAPD Center, Indonesia

Atma Gunawan[1], Pandu Tridana Sakti[2]*

[1]Division of Renal and Hypertension, Departement of Internal Medicine, Faculty of Medicine, Brawijaya University - Dr. Saiful Anwar Hospital, Malang, Indonesia.
[2]Departement of Internal Medicine, Faculty of Medicine, Brawijaya University, Malang, Indonesia.

**** Corresponding Author:***
Atma Gunawan, MD., PhD. Division of Renal and Hypertension, Departement of Internal Medicine, Faculty of Medicine, Brawiijaya University. Jl. JA. Suprapto no. 2, Malang, Indonesia. Email: atma_gunawan.fk@ub.ac.id.

ABSTRACT

Background: *Continuous Ambulatory Peritoneal Dialysis (CAPD) is an alternative therapy for renal replacement in patients with kidney failure in developing countries such as Indonesia. The CAPD program in Malang Indonesia has been running since 2010. Until now, there has been little research on the mortality of CAPD therapy in Indonesia. We aimed to provide report on the characteristics and 5-year survival of CAPD therapy in patients with end-stage renal disease (ESRD) in developing countries like Indonesia.* ***Methods:*** *We conducted a retrospective cohort study involving 674 patients with end-stage renal disease receiving CAPD therapy from the medical records of the CAPD Center RSUD Dr. Saiful Anwar from August 2014 to July 2020. The 5-year survival rate was analyzed using Kaplan-Meier, and the hazard ratio was analyzed using Cox regression.* ***Results:*** *Of 674 patients with end-stage renal disease who underwent CAPD, 63.2% survived up to 5 years, with general survival rates at 1, 3, and 5 years of 80%, 60%, and 52%, respectively. The 3-year survival rate for patients with end-stage renal disease and comorbid hypertension was 80%, while it was 10% for patients with comorbid hypertension and type II diabetes mellitus. The hazard ratio for patients with end-stage renal disease who had comorbid hypertension and type II diabetes mellitus was 8.4 (95% CI = 6.36-11.21).* ***Conclusion:*** *Patients with end-stage renal disease who receive CAPD therapy have a favorable 5 years survival rate. Patients with end-stage renal disease on CAPD therapy who have comorbid hypertension and type II diabetes mellitus have a lower survival rate than patients with comorbid hypertension alone.*

Keywords: S*urvival, end stage renal disease, continuous ambulatory peritoneal dialysis.*

INTRODUCTION

Continuous Ambulatory Peritoneal Dialysis (CAPD) may be the primary option for efficient and cost-effective renal replacement therapy for end-stage renal disease in developing countries such as Indonesia. Despite the fact that CAPD is better tolerated in patients with end-stage renal disease, it is more cost-effective than hemodialysis (HD).[1] The annual cost of HD per patient in Indonesia is approximately 12,000 USD, while the cost of CAPD is approximately 6,000 USD. Furthermore, CAPD has fewer requirements in terms of medical personnel and facilities than HD. Therefore, the use of CAPD in developing countries can help to bridge the gap between the demand and supply of renal replacement therapy (RRT) for patients with end-stage renal disease.

The number of active patients with end-stage renal disease in Indonesia until 2018 was 132,142

patients (499 patients per million population), while new cases of end-stage renal disease in 2018 were 53,940, with hypertensive kidney disease (36%) and diabetic nephropathy (27.8%) being the most common etiologies. East Java Province has the highest number of active CAPD patients in Indonesia, with 561 patients, 75% of whom came from the CAPD center of RSUD Dr. Saiful Anwar Malang.[2]

In Indonesia, hypertension (42%) and diabetes mellitus (16%) are the most common comorbidities in patients with end-stage renal disease.[2] CAPD also has serious complications, such as peritonitis, which can progress to systemic infection.[3] These comorbidities and complications can increase the mortality of CAPD patients and decrease their survival rate. There are currently no reports on the therapeutic success and survival analysis of patients with end-stage renal disease who undergo CAPD in developing countries like Indonesia. Patients with end-stage renal disease and hypertension alone or in combination with diabetes mellitus still have a low survival rate. We aimed to analyze the five-year survival of CAPD patients with end-stage renal disease caused by hypertension alone or in combination with diabetes mellitus at one of Indonesia's CAPD centers.

METHODS

The study design was a retrospective cohort study. The research was conducted at the CAPD unit of RSUD Dr. Saiful Anwar Malang, using medical record data of CAPD patients. The target population of this study was patients with end-stage renal disease who were undergoing CAPD therapy. Data is collected using medical records and telephone interviews for additional data not found in the medical record. The inclusion criteria of the study were patients with end-stage renal disease who underwent CAPD installation surgery at Dr. Saiful Anwar Malang from August 2014 to July 2020. The exclusion criteria included CAPD drop-out patients for reasons other than death and CAPD patients traveling to other CAPD centers.

This study was approved by the Health Research Ethics Commission dr. Saiful Anwar Hospital, Malang, Indonesia (Reference no. 400/083/K.3/102.7/2022).

The patient's comorbidities were the confounding variable in this study. The sample size required to determine the difference in survival rates of patients with end-stage renal disease who had comorbid hypertension alone versus hypertension and type 2 diabetes mellitus was 190 samples with a 0.05 significance level and 80% study power with a 0.15 effect size rate.

The SPSS 25 program was used for the survival analysis. Kaplan-Meier analysis was used to distinguish the 5-year survival rate between patients with end-stage renal disease who had comorbid hypertension alone and those who also had hypertension and type 2 diabetes mellitus. Cox regression analysis was used to determine the adjusted hazard ratio (HR) with a 95% confidence interval (CI). The p-value of <0.05 indicates a significant value.

RESULTS

During the period of August 2014 to July 2020, there were 840 CAPD operations at the CAPD Center of Dr. RSUD. Saiful Anwar Malang. From the total of 840 cases, 166 cases (19.5) dropped out of CAPD (due to mechanical problems, recurrent peritonitis, switching to other renal replacement therapies, or traveling to another CAPD center) were excluded from this study. As a result, this study involved 674 patients. Within 5 years, there were 248 deaths (29.5%) with cardiovascular disease being the leading cause of death (79.8%). The sample in this study was male (58.8%), the highest age group when starting CAPD therapy was 46-59 years (42.6%), and hypertension was the most common comorbid condition (57.7%).

Table 1. Characteristics of CAPD Patients

Characteristic	N=674 n (%)
Gender	
Men	389 (58.8)
Women	285 (41.2)
Age	
12-25 years	61 (9.1)
26-45 years	195 (28.9)
46-59 years	287 (42.6)
>60 years	120 (17.8)

Marital status	
Single	61 (9.1)
Married	613 (90.9)
Smoker	
Yes	287 (42.6)
No	287 (57.4)
History of Hypertension Medication	
Calcium channel blocker	195 (28.9)
Angiotensin-converting enzyme inhibitor	287 (42.6)
Angiotensin receptor blocker	192 (28.48)
Comorbidity	
Hypertension	381 (57.7)
Hypertension and Diabetes Mellitus	278 (42.3)
Cause of Mortality	
Cardiovascular Disease	198 (79.8)
Cerebrovascular Disease	8 (3.2)
Sepsis (peritonitis)	40 (16.1)
Other	2 (0.8)

In general, the survival rate of patients with end-stage renal disease who were receiving CAPD therapy within 12 months (1 year) was 80%, 36 months (3 years) was 60%, and 60 months (5 years) was 52%, with a mean survival rate of 42.4 months (95% CI = 40.3-44.5). Survival rates between 1 and 3 years of patients with end-stage renal disease who had comorbid hypertension and type 2 diabetes mellitus who received CAPD therapy were 61% and 10%, respectively, with a median survival of 16 months (95% CI = 13.6-18.3) and comorbid hypertension alone were 90% and 80%, respectively, with a mean survival of 49.9 months (95% CI = 47.9-51.8). The hazard ratio of patients with end-stage renal disease who had comorbid hypertension and type II diabetes mellitus was 8.4 (95% CI = 6.36-11.21), p-value <0.001.

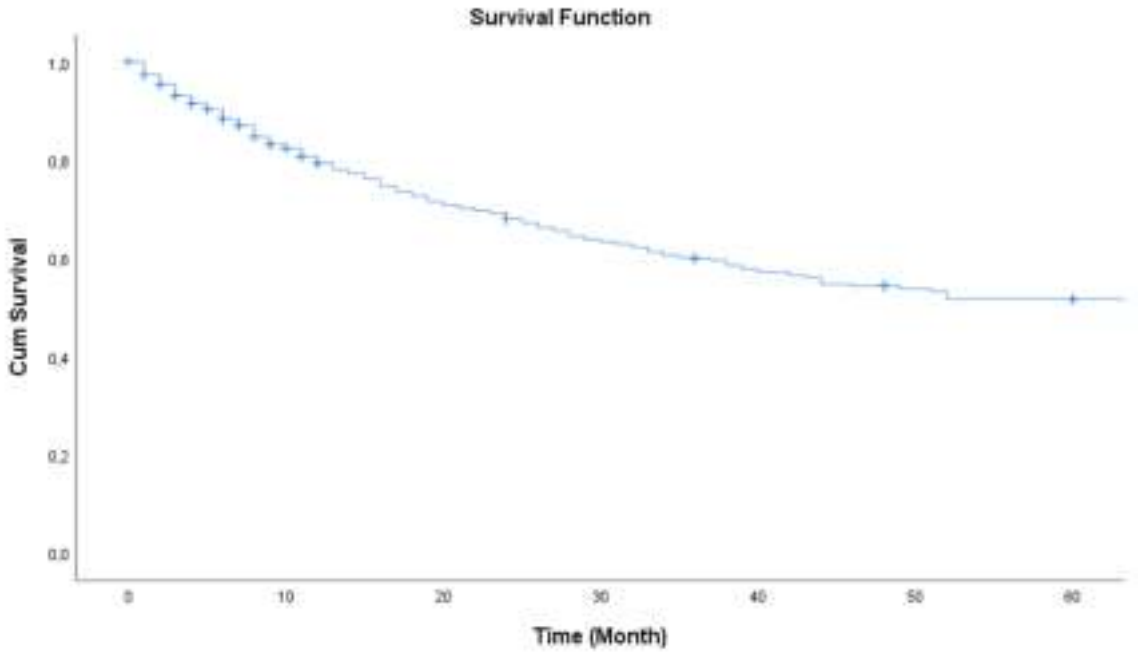

Figure 1. Kaplan-Meier survival curve of patients with the end-stage renal disease treated with CAPD therapy.

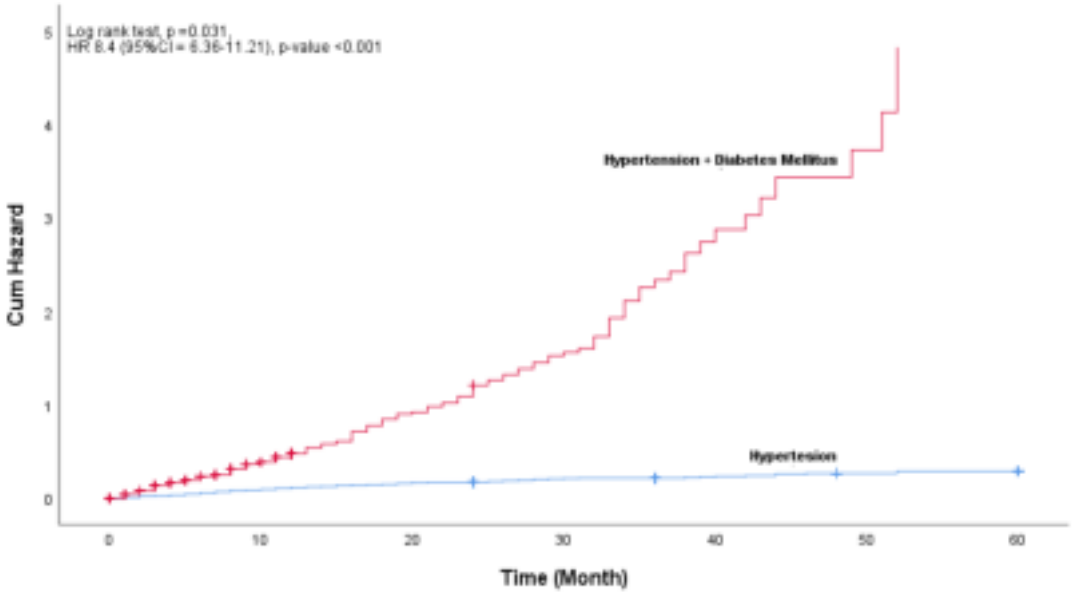

Figure 2. Kaplan-Meier survival curve comparison of CAPD patients with end-stage renal disease who had comorbid hypertension and those who had hypertension and type II diabetes mellitus.

DISCUSSION

The survival rate of end-stage renal disease patients undergoing CAPD therapy in this study was 80%after one year, 60% after three years, and 52% after five years, with a mean survival rate of 42.4 months (95%CI = 40.3-44.5), the same as in several other studies. Research in Hong Kong from 2002 to 2006 found that the patients survived for one year (90.8%), three years (68.2%), and five years (48.4%).[4–6] In Thailand, the 2012 survival rates of CAPD patients were one year (79.2%), three years (66%), and five years (57%).[7–9] In China, the 2006 survival rates for CAPD patients were one year (94%), three years (81%), and five years (64%).[10] In continental Europe, the 2012 survival rate of CAPD patients was two years (81.7%).[11] In the United States, the 2013 survival rates for CAPD patients were one year (90%), two years (79%), and five years (50%).[11] In Canada, the 2013 survival rates for CAPD patients were one year (94%), three years (73%), and five years (55%).[12] In Latin America, the 2000 survival rates for CAPD patients were one year (91%), three years (77%), and five years (58%).[12] These data show that both developing and developed countries have nearly the same 5-year survival rate of CAPD, which is above 50%. Recent research on CAPD mortality showed that 248 patients (36.8%) died, while 426 patients (63.2%) survived for five years. CAPD peritonitis is the second leading cause of 40 (16.1%) mortality cases after cardiovascular disease. The most common bacteria causing CAPD peritonitis was coagulate-negative *Staphylococcus,* with as many as 15 (37.5%) cases.[13]

The survival analysis of patients with end-stage renal disease on CAPD therapy with comorbidities revealed that survival at 1 and 3 years was 61% and 10% for hypertensive patients with type 2 diabetes mellitus, respectively, and 90% and 80% for patients with only comorbid hypertension, respectively. According to a 2013 research conducted in Australia in, the survival rate of CAPD patients with comorbid diabetes was 1 year (89%), 3 years (61%), and 5 years (39%).[14] This is similar to the 2013 New Zealand study, which found that CAPD patients survived for one year (89%), three years (59%), and five years (34%).[14] These data show that patients with end-stage renal disease who have comorbid diabetes have a lower survival rate.

Hyperfiltration in diabetes is caused by efferent arteriolar vasoconstriction due to an activated renin-angiotensin-aldosterone system (RAAS). However, it has become increasingly

evident over the years that hyperglycemia is not the sole cause of nephropathy, despite inarguably playing a major role. Several pathophysiologic pathways are involved in the development of nephropathy, and this review will attempt to elucidate those pathways and, hopefully, shed some light on therapeutic options that may one day play a role in halting the nephropathy epidemic and suppressing the progression to ESRD.[15] In clinical practice, physicians should pay special attention to CAPD patients with diabetes mellitus and hypertension comorbidity.

There were several limitations to this study, including the fact that there were 15 patients who did not have comorbid hypertension and diabetes mellitus, so we did not analyze them and only made an analysis for the survival rate of patients who did. Because the retrospective data for the last 5 years recorded in medical records is insufficient, including data on the etiology of ESRD, data on whether or not hypertension and diabetes mellitus are controlled, and data on other characteristics and comorbidities, we cannot include them in the sample characteristics. The inability to determine the survival status of some patients in the study population had a negative impact on the internal and external validity of the study's main result - the cumulative survival probability -as well as the ability to correlate the study's independent variables to patient survival. A template or format may be used to improve the quality of documentation in the future. At least two phone numbers should be requested from the patient to ensure adequate communication. It is also recommended that similar research be conducted on a regular basis for CAPD patients. This may be addressed by improving community awareness of the disease, introducing self-examination concepts, and providing screening services.

CONCLUSION

This study was conducted to improve understanding of the CAPD patients in Malang. CAPD patients have a survival rate of 80% within 12 months (1 year), 60% within 36 months (3 years), and 52% within 60 months (5 years). Diabetes was found to have a negative impact on the survival of CAPD patients. The survival rate of end-stage renal disease with diabetes and hypertension is lower than hypertension alone.

CONFLICT OF INTEREST

The author declares no conflict of interest or funding in this research.

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Factors Associated with 30-day Major Adverse Cardiovascular Event in Acute Coronary Syndrome Patients with Non-Dialysis Chronic Kidney Disease: A Retrospective Cohort Study

Eka Ginanjar[1], Ni Made Hustrini[2], Arif Mansjoer[1], M. Shiddiq Al Hanif[3]*

[1]Division of Cardiovascular, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

[2]Division of Nephrology-Hypertension, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

[3]Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

Corresponding Author:
Eka Ginanjar, MD., PhD. Division of Cardiology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo General Hospital. Jl. Diponegoro 71, Jakarta 10430, Indonesia. Email: ekaginanjar.MD@gmail.com.

ABSTRACT

Background*: Acute coronary syndrome (ACS) accounts for the majority of ischemic heart disease-related deaths. It is known that ACS patients with chronic kidney disease (CKD) tend to have worse clinical outcomes, including major adverse coronary events (MACE) compared to patients without CKD. Some studies suggested that several determinant factors may be involved in this condition. Until now, research on determinant factors of MACE in ACS patients with CKD in Indonesia is still limited. Thus, we aimed to investigate the relationship of various factors to MACE in ACS patients with non-dialysis CKD who underwent percutaneous coronary intervention (PCI), in the form of neutrophile leukocyte ratio (NLR) as a factor describing chronic inflammation, left ventricular hypertrophy (LVH) as a factor describing cardiac remodeling, Gensini score may represent coronary severity, whereas GRACE was used to evaluate the severity and clinical risk of ACS patients.* ***Methods:*** *This study is a retrospective cohort study using secondary data from the medical records of 117 ACS patients who underwent percutaneous coronary intervention (PCI) at Cipto Mangunkusumo General Hospital Jakarta from January 2018 to June 2018 . Patients were classified based on the stage of CKD and assessed for 30-day MACE. Data were recorded on GRACE score, Gensini score, LVH, and neutrophil-lymphocyte ratio (NLR). Analysis of the relationship between these factors was carried out using the chi-square test.* ***Results****: Of the 117 patients, 62.3% were STEMI. At the end of hospital treatment, 67.5% were in the normal-stage 2 CKD group, 17.1% in the CKD stage 3a-3b group, and 15.4% in the CKD stage 4-5 group. MACE occurred in 47 (40.2%) patients with 17 (14.5%) dying. There was a significant relationship between GRACE scores and MACE (54.8% MACE at high GRACE scores vs. 32% MACE at low-moderate GRACE scores, p = 0.016, OR: 2,57 CI 95%, 1,18-5,59), while no significant relationship was found for the Gensini score, LVH, and NLR scores even though there was an increase in the proportion of MACE.* ***Conclusion:*** *The incidence of MACE is higher than in the previous studies conducted in the same place, i.e. Cipto Mangunkusumo General Hospital, no significant relationship is found in NLR, LVH, and Gensini score with the 30-day MACE of ACS patients with non-dialysis CKD, meanwhile the GRACE score correlates with the 30-day MACE of ACS in non-dialysis CKD patients as is the known theory regarding this score.*

Keywords*: ACS, CKD, MACE, GRACE score, Gensini score, LVH, NLR.*

INTRODUCTION

Acute coronary syndrome (ACS) and sudden death account for the majority of ischemic heart disease-related deaths with at least 1.8 million deaths caused by ACS every year.[1] The American Heart Association (AHA) estimates that one person has a heart attack every 41 seconds.[2] In Indonesia, cases of heart disease reached 1,017,290 with a mortality percentage of 35% of the total.[3] Similar to ACS, the prevalence of chronic kidney disease (CKD) increases and causes global health problems.[4] It is reported that 14.9% of the adult population in the United States in 2015-2018 suffered from CKD. In Indonesia, there are 132.142 patients with CKD undergoing hemodialysis in 2018.[5]

Several studies are showing an association between ACS and CKD. A study found that the risk of ACS increased linearly, and patients with CKD stages 3a to 4 had a 2-3 times chance of cardiovascular mortality compared to patients without CKD.[6] Major Adverse Cardiovascular Event (MACE), a term used to describe important poor clinical outcomes in ACS patients[7], showed an increased by one year post percutaneous coronary intervention (PCI) in CKD patients compared to ACS patients with normal renal function.[8] Another study also showed that during follow-up, patients with severe renal dysfunction were an independent risk factor for MACE, and were associated with poor prognosis.[9]

Experts have investigated the relationship between ACS and CKD through the determinant factors involved in MACE. A study found that the chronic inflammatory process through increased chemotactic activity, tumor necrosis factor-alpha (TNF-α), interleukin 1 beta (IL-1β), and interleukin 6 (IL-6) in the arteries of CKD patients resulted in endothelial damage to blood vessels, thereby accelerating the process of atherosclerosis.[10,11] This finding is also supported by other studies that prove that patients with CKD stage 1-4 with a high neutrophil-lymphocyte ratio (NLR) have a worse outcome than patients with low NLR.[11] In addition, the high prevalence of congestive heart failure in CKD patients is associated with cardiac remodeling to the occurrence of left ventricular hypertrophy (LVH). A population study based on autopsy data found that lower glomerular filtration rate (GFR) was associated with greater left ventricular wall thickness,[12] whereas in the 4D study, the presence of LVH was associated with a nearly doubled risk of sudden cardiac death in CKD patients.[13]

As a method that attempts to quantify the overall coronary atherosclerosis burden[14], it was found that the Gensini score in CKD patients was higher.[8] Similar to this finding, CKD patients also have worse coronary artery stenosis and higher three-vessel disease incidence compared to patients with normal kidney function and CVD problems.[8] This condition may occur due to several mechanisms, including microinflammation, oxidative stress, endothelial dysfunction, impaired lipid metabolism, uremic toxins such as homocysteine, glycation end products, protein oxidation end products, parathyroid adenine, abnormal calcium, and phosphorus metabolism, asymmetric dimethylarginine, hyperuricemia, and other factors that can cause damage to the vascular endothelium thereby increasing the occurrence of chronic renal insufficiency, activation of the renin-angiotensin-aldosterone system and hypertension.[8,15,16] Additionally, the researchers observed that CKD shared both traditional (such as hypertension, diabetes, and dyslipidemia) and non-traditional (such as homocysteinemia, calcium and phosphate disorders, elevated serum uric acid levels, C-reactive protein, and other oxidative stress as well as inflammatory markers levels) risk factors that have multiple or superimposed effects on atherosclerosis and vascular endothelial damage.[8,16,17]

To date, research on determinant factors of MACE in ACS patients with CKD in Indonesia is still limited. Thus, this study aimed to analyze the role of 30-day MACE factors in ACS with non-dialysis CKD patients by assessing its determinants including NLR, LVH, Gensini score, and GRACE score.

METHODS

The study was a retrospective cohort study performed at Cipto Mangunkusumo General Hospital Jakarta as the national referral hospital in Indonesia.

Study Population

This study analyzed secondary data from the study entitled "Effect of Beta2-Microglobulin and Fibroblast Growth Factor 23 on Coronary Severity and Major Adverse Cardiac Events in Patients with Acute Coronary Syndrome Having Chronic Kidney Disease".[18] Patients undergoing treatment at the intensive cardiac care unit (ICCU) Cipto Mangunkusumo General Hospital Jakarta with a diagnosis of ACS and coronary angiography conducted between January 2018 and June 2018 were considered for this study. All adult patients (> 18 years old) admitted to the hospital, diagnosed with ACS with non-dialysis CKD, undergoing coronary angiography, and hospitalized in Cipto Mangunkusumo General Hospital between January 2018 and June 2018, including patients with CKD stage 5 who did not undergo dialysis procedure were eligible for inclusion. Exclusion criteria were CKD patients undergoing hemodialysis, and patients with severe comorbidities, including acute stroke, hepatic cirrhosis, chronic inflammatory disease, sepsis, autoimmune, and malignancy. Pregnant women and breastfeeding mothers were excluded from this study. Incomplete medical record data such as incomplete variable data were also excluded.

Data Collection

Data for this study were collected using consecutive sampling methods collected from the previous study data that was taken from the ICCU and internal medicine ward at Cipto Mangunkusumo General Hospital. Case definitions were based on clinical diagnosis. The diagnosis of ACS was based on clinical symptoms, electrocardiogram, echocardiography, and elevated cardiac enzymes.[2,19] The diagnosis of CKD was established previously based on the serial examination of creatinine serum. The first examination is at the time of admission and the next examination is at the time the patient is discharged from the hospital. This examination was conducted to distinguish between CKD and acute kidney injury due to cardiorenal-related conditions. Additionally, we also defined CKD by the elevation of creatinine serum, decreased estimated GFR, and evidence of kidney damage (albuminuria).[20] The non-dialysis CKD was defined as patients with CKD who did not undergo dialysis procedures. GRACE score and NLR were calculated based on clinical condition and laboratory data during admission and the Gensini score was calculated based on the result of coronary angiography during hospitalization. LVH was calculated based on echocardiography during hospitalization. Patients were followed from admission to 30 days after hospitalization to determine MACE.

Data Analysis

Identified data were further analyzed with STATA. Each variable was analyzed to determine the distribution and percentage. Furthermore, categorical data were presented in the table and numerical data in mean (SD) or median (IQR) depending on the data distribution. The bivariate analysis was carried out to find out the association between the independent variables (NLR, LVH, GRACE score, and Gensini score) with the dependent variable (30-day MACE) using the chi-square test.

Ethical Approval

This study was approved by the institutional review board of the Faculty of Medicine Universitas, Indonesia with the number KET-110/UN2.F1/ETIK/PPM.00.02/2021. The consent was waived by the ethics committee due to the negligible risk nature of data collection by retrospective datasets already on electronic health records.

RESULTS

117 samples from previous studies were included (**Figure 1**). The characteristics of the patients are shown in **Table 1**. The proportion of men was 77.8% with the mean age of the patient being 57.79 years. The most common risk factors were hypertension, smoking, and diabetes mellitus, respectively. The median NLR was 4.83 with the lowest value of 1.25 and the highest value of 29.63. To determine the NLR cut-off, a receiving operating curve (ROC) analysis was performed on MACE, and the cut-off was determined to be 4.8. The median GRACE score was 110 with 64.1% of patients having a category score ≤ 128. The results of echocardiography showed that 53.1% of the subjects had LVH

with the predominantly eccentric type (73.3%). Coronary angiography found that 65.8% had multivessel disease with a median Gensini score of 50. At the end of hospitalization, the mean eGFR was 68.5 mL/min/1.73m^2 with a median of creatinine 1.1 mg/dL, with the lowest value of 0.5 mg/dL and the highest value of 11.5 mg/dL. Based on the stage of CKD, the highest proportion was found in stage 2 (37.6%), subjects who had eGFR less than 60 ml/min/1.73m^2 38 (32.5%). To simplify the analysis, we divide CKD categories into stages 1-2, 3a-3b, and 4-5 with the highest proportion found in stages 1-2 (67.5%). After 30 days of follow-up, MACE occurred in 47 patients (40.2%) (**Table 2**).

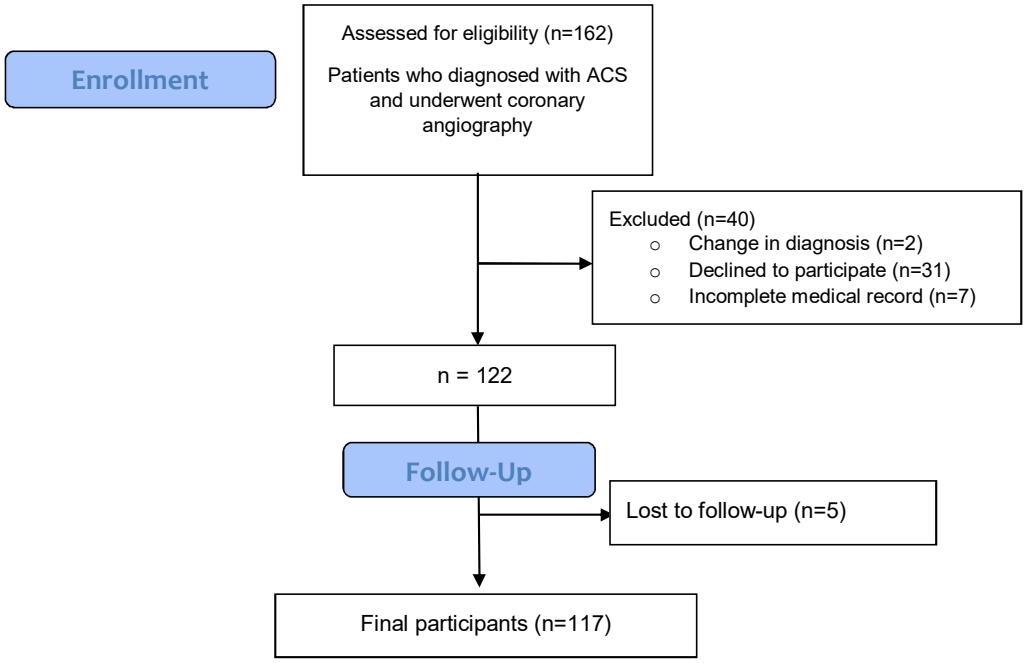

Figure 1. Flowchart of sample selection.

Table 1. Characteristics of the patients.

Characteristic	All Subject	MACE	
	(n=117)	Yes (%)	No (%)
Gender, n (%)			
Men	91 (77.8)	39 (42.9)	52 (57.1)
Women	26 (22.2)	8 (30.8)	18 (69.2)
Age, mean (SD), years	57.8 (10)		
Risk Factor, n (%)			
Diabetes mellitus	46 (39.3)	16 (34.8)	30 (65.2)
Dyslipidemia	35 (29.9)	12 (34.3)	23 (65.7)
Hypertension	76 (65)	34 (44.7)	42 (55.3)
Obesity	15 (12.8)	8 (53.3)	7 (46.7)
Chronic kidney disease (anamnesis)	8 (6.8)	3 (37.5)	5 (62.5)
Cigarette smoking	73 (62.4)	29 (39.7)	44 (60.3)
Family history CHD, n (%)	4 (3.4)	1 (25)	3 (75)
NLR, median (min-max)	4.83 (1.25-29.63)		
NLR Classification, n (%)			
NLR < 4.8	51 (49)	20 (39)	31 (61)
NLR ≥ 4.8	53 (51)	24 (45)	29 (55)

Diagnosis ACS, n (%)			
STEMI	73 (62.3)	25 (34.2)	48 (65.8)
NSTEMI	21 (18)	9 (42.9)	12 (57.1)
UAP	23 (19.7)	12 (54.5)	10 (45.5)
GRACE score, mean (SD)	119 (40.8)		
GRACE score classification, n (%)			
Low-Medium (≤128)	75 (64.1)	24 (32)	51 (68)
High (> 128)	42 (35.9)	23 (54.8)	19 (45.2)
Systolic function, n (%)			
Normal, ≥ 50%	63 (55.8)	20 (32.3)	42 (67.7)
Decrease	53 (46.9)	24 (47.1)	27 (52.9)
LVH, n (%)			
Yes, IVS or LVPW > 12 mm	60 (53.1)	26 (43.3)	34 (56.7)
No	53 (46.9)	18 (34)	35 (66)
Gensini score, median (min-max)	50 (0-132)		
Gensini score classification Gensini score, n (%)			
Low < 18	17 (14.5)	6 (35.3)	11 (64.7)
Medium 18-41	33 (28.2)	14 (42.4)	19 (57.6)
High > 41	67(57.3)	27 (40.3)	40 (59.7)
Vessel Disease, n (%)			
0	6 (5.1)	2 (33.3)	4 (66.7)
1	34 (29.1)	15 (42.9)	20 (57.1)
2	34 (29.1)	11 (31.4)	24 (68.6)
3	35 (29.9)	19 (46.3)	22 (53.7)
Left Main Disease	8 (6.8)	5 (62.5)	3 (37.5)
GFR, mL/min/1.73m^2, mean (SD)	68.57 (29.78)		
Creatinine, median (min-max)	1.1 (0.5 – 11.5)		
CKD stage, n (%)			
1	35 (30)	11 (31.4)	24 (68.6)
2	44 (37.6)	15 (34.1)	29 (65.9)
3a	13 (11.1)	5 (38.5)	8 (61.5)
3b	7 (6)	5 (71.4)	2 (26.8)
4	8 (6.8)	5 (62.5)	3 (37.5)
5	10 (8.5)	6 (60)	4 (40)
CKD stage, n (%)			
Stage 1 – 2	79 (67.5)	26 (33)	53 (67)
Stage 3a – 3b	20 (17.1)	10 (50)	10 (50)
Stage 4 – 5	18 (15.4)	11 (61)	7 (39)

Table 2. Characteristics of MACE.

Characteristics of MACE	Incidence (n=117) n (%)
MACE	
Yes	47 (40.2)
Mortality	17 (14.5)
Stroke	6 (5.1)
Cardiogenic shock	6 (5.1)
Heart Failure	34 (29)
Arrhythmia	9 (7.7)
Recurrent miokardial infarction	17 (14.5)
Time to Event	
1 day	23 (48.9)
2 day	4 (8.5)
3 day	5 (10.6)
4 day	4 (8.5)
5 day	2 (4.3)
10 – 20 day	4 (8.5)
21 – 30 day	5 (10.6)
No	70 (59.8)

Bivariate analysis results showed that there was a significant relationship between the GRACE score and MACE (54.8% MACE at high GRACE score vs. 32% MACE at GRACE score low-medium, p = 0.016, OR: 2,57 CI 95%: 1,18-5,59), while no statistically significant relationship was found with the Gensini, LVH, and NLR scores even though an increase in the number of MACE along with an increase in the degree of the Gensini score (6,14, and 27), more MACE in LVH (43.3%) than without LVH (34%), and a higher proportion of MACE in NLR 4.5 than MACE at NLR < 4.8 (45% vs. 39%) was observed (**Table 3**).

Table 3. Bivariate analysis for MACE.

Independent Variables	MACE (n=117)	
	OR (CI 95%)	P value
GRACE score	2.57 (1.18-5.59)	0.016
Gensini score	-	0.888
LVH	1.48 (0.69-3.19)	0.308
NLR	1.283 (0.588-2.799)	0.531

DISCUSSION

This study was a retrospective cohort study of ACS patients with non-dialysis CKD who underwent coronary angiography between January 2018 to June 2018 in Cipto Mangunkusumo General Hospital. The findings of this study showed that the incidence of MACE was 40.2%. It was higher than the incidence of MACE from previous studies at Cipto Mangunkusumo General Hospital in 2013 (14%) and 2016 (19.2%).[21,22] This increased incidence of MACE may be related to the characteristics of patients who were diagnosis specifically with non-dialysis CKD and it is also influenced by the tiered referral system in Indonesia which makes patients come with more severe conditions to Cipto Mangunkusumo General Hospital because this hospital is a national referral hospital.

Another finding in this study was the patients with MACE at NLR ≥ 4.5 showed no statistically significant compared to MACE at NLR < 4.8 (45% vs. 39%, p = 0.531). This may be due to the amount and distribution of data and NLR data collection. The NLR data was taken when the patient was admitted to the hospital or in the initial condition of the ACS attack, the ACS condition itself is a condition of physical stress that will trigger an increase in inflammation, which can affect the picture of chronic inflammation as a baseline for CKD patients. However, a previous study found that ACS patients with a high NLR value > 4.5 had higher mortality compared to ACS patients with NLR < 1.5.[23] Another study reported that there was a significant difference in ACS patients with NLR > 6.52 compared to NLR < 3.4 for 1 year-MACE, 1 year-mortality, in-hospital MACE, and in-hospital mortality.[24]

We also found that the condition of LVH was reported in 53.1% of patients dominated by the eccentric type (73.3%) and 46.9% of patients experienced a decrease in systolic function with a median ejection fraction of 54%. Several studies have found that CKD is associated with cardiac remodeling, which is mostly manifested by LVH and increased fibrosis.[25] LVH was also observed to be more common in individuals who started renal replacement therapy and dialysis procedure.[26] Meanwhile, some recent studies indicated that LVH begins to occur in the early stage of CKD patients.[13] The prevalence of LVH in individuals with GFR > 30 mL/min/1.73 m^2, before starting renal replacement therapy, and after starting dialysis was 16-31%, 60-75%, and 90%, respectively.[27] As a result of LVH, myocardial apoptosis, and intermyocardial fibrosis, decreased myocardial capillary density concomitantly with diastolic and systolic dysfunction, impaired intraventricular conduction, and dilatation of cardiac chambers occur. Then progressively, compensatory hypertrophy, dilatation, and cardiac dysfunction (uremic cardiomyopathy) also occurs.[28] The severity and persistence of LVH are strongly associated with the risk of death and cardiovascular events in CKD patients. Several studies revealed that a 10% reduction in LV mass was associated with a 28% reduced risk of cardiovascular death in the group of patients undergoing hemodialysis.[29,30] Whereas in the 4D study, the presence of LVH was associated with twice the risk of sudden cardiac death.[31]

The median Gensini score was found of 50, with a minimum value range of 0 and a maximum value range of 132, and 57.3% of the individuals fell into the high Gensini score category. Several studies have linked an increase in the Gensini score with an increase in the stage of CKD. For example, a study found that patients with CKD stage 5 had the highest Gensini score and that there was a significant difference in patients with normal renal function to CKD stage 2 vs. CKD stage 3, CKD stage 3 vs. CKD stage 4, and in CKD stage 4 vs. CKD stage 5.[15] Despite an increase in the number of MACE with increased Gensini scores, no significant association was found in this study's analysis of the relationship between Gensini scores and 30-day MACE. However, a previous study discovered a significant difference in the mean Gensini scores of survivors and patients who died in hospital in the study of the

association between Gensini scores and mortality in STEMI patients in Turkey.[32] In China, a study found that there was a significant correlation between Gensini scores and all one-year causes of death in patients with myocardial infarction with a mean Gensini score higher in patients who experienced death compared to survivors.[33] However, both studies merely investigate the death as an outcome and did not look in detail at how it relates to impaired renal function.

Moreover, the median GRACE score was 110 with 45.3% of subjects in the moderate GRACE score category and 35.9% in the severe category. Based on the stage of CKD, the highest proportion was found in stage 2 (37.6%), followed by stage 3 (17.1%), while patients with GFR < 60 mL/min/1.73m^2 were 32.5%. A significant correlation was found between the stage of CKD and the degree of GRACE score ($p = 0.002$). It was also revealed that CKD stage 4-5 obtained more than a large proportion of high GRACE scores compared to low-medium GRACE scores (66.7% vs. 33.3%) and this finding was not found in the CKD stage 1-2 (25.3% vs. 74.7%). Aside from creatinine levels, CKD may be linked to an increase in the value of other assessed variables in the GRACE score. A previous study conducted using GRACE found that patients with moderate or severe renal impairment have older age-related features and had more comorbidities than patients with normal or mildly impaired renal function.[34] It was also found that patients with impaired and severe renal function are twice and four times as likely to die compared to normal and mildly impaired renal function, respectively.[34] This is consistent with the findings of this investigation. Another study found that the GRACE score in patients with end-stage renal disease and acute myocardial infarction who died in the hospital was significantly higher than in patients who survived, and the AUC of the GRACE score to predict mortality hospitalization in patients with end-stage renal disease and AMI was 0.754.[35] Coronary angiography revealed that 65.8% of the subjects had multivessel disease. Another study supported this finding by revealing that CKD (GFR 60 mL/min/1.73 m^2) is associated with a 2.9-fold increased risk of multivessel coronary artery disease.[36] Although the exact mechanism is unclear, CKD is thought to hasten the progression of atherosclerosis through traditional i.e., hypertension and diabetes mellitus and non-traditional risk factors such as mineral and bone disorder disorders (CKD-MBD), anemia, inflammation chronic disease, and hyperhomocysteinemia, as well as dialysis-related factors in CKD patients on dialysis.[8,16,17]

In this study, we took secondary data from previous research as well as from medical records due to insufficient data such as data related to specific pathophysiology in other CKD, for example, CKD-MBD (phosphate, calcium, and parathyroid) and homocysteine data. Moreover, design sampling in this study was carried out consecutively. Additionally, the number of samples is not too large because the distribution of some data is not normal. However, statistical calculations indicate that the number of samples (n=117) meets the requirements. This potentially affects some of the results of the analysis which are not significant. The research was only conducted in Cipto Mangunkusumo General Hospital thus the possibility of different clinical characteristics of the patient might occur compared to other hospitals.

CONCLUSION

The incidence of MACE is higher compared to the previous studies conducted in Cipto Mangunkusumo General Hospital**.** The GRACE score has a statistically significant relationship with the 30-day MACE in ACS patients with non-dialysis CKD patients, meanwhile, the Gensini score, LVH, and NLR score had no statistically significant.

CONFLICT OF INTEREST

There is no conflict of interest.

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Efficacy and Safety of Clopidogrel in the Prevention of Primary Failure of Arteriovenous Fistula in Patients with End-Stage Renal Disease: A Systematic Review

Wahyudi[1,2], Dono Antono[3], Pringgodigdo Nugroho[4], Ikhwan Rinaldi[5,6], Ika Prasetya Wijaya[3], Hamzah Shatri[6], Em Yunir[7], Lusiani Rusdi[3]*

[1] Department of Internal Medicine, Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[2] Department of Internal Medicine, Faculty of Medicine, Andalas University - RSUP M Djamil, Padang, Indonesia.
[3] Division of Cardiology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[4] Division of Kidney and Hypertension, Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia – Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[5] Division of Hematology and Medical Oncology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[6] Clinical Epidemiology Unit, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[7] Division of Endocrine and Metabolic, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

Corresponding Author:
Wahyudi, MD. Department of Internal Medicine, Faculty of Medicine, Andalas University - RSUP M Djamil. Jl. Perintis Kemerdekaan, Padang, Indonesia. Email: wahyudi@med.unand.ac.id.

ABSTRACT

Background: *Arteriovenous fistula (FAV) is the most widely used vascular access for end-stage renal disease (ESRD) patients undergoing routine hemodialysis in Indonesia. However, FAV can become dysfunctional before it is used for the initiation of hemodialysis, a condition known as primary failure. Clopidogrel is an anti-platelet aggregation that has been reported to reduce the incidence of primary failure in FAV compared to other anti-platelet aggregation agents. Through this systematic review, we aimed to assess the role of clopidogrel to the incidence of primary FAV failure and the risk of bleeding in ESRD patients.* ***Methods:*** *A literature search was carried out to obtain randomized Control Trial studies conducted since 1987 from Medline / Pubmed, EbscoHost, Embase, Proquest, Scopus, and Cochrane Central without language restrictions. Risk of bias assessment was performed with the Cochrane Risk of Bias 2 application.* ***Results:*** *All of the three studies involved indicated the benefit of clopidogrel for the prevention of AVF primary failure. However, all of the studies have substantial differences. Abacilar's study included only participants with diabetes mellitus. This study also administered a combination of clopidogrel 75 mg and prostacyclin 200 mg/day, while Dember's study gave an initial dose of clopidogrel 300 mg followed by daily dose 75 mg and Ghorbani's study only gave clopidogrel 75 mg/day. Ghorbani and Abacilar started the intervention 7-10 days before AVF creation, while Dember started 1 day after VAF creation. Dember gave treatment for 6 weeks with an assessment of primary failure at the end of week 6, Ghorbani's treatment lasted for 6 weeks with an assessment at week 8, while Abacilar gave treatment for one year with an assessment at weeks 4 after AVF creation. In addition, the prevalence of bleeding did not differ*

between the treatment and control groups. ***Conclusion:*** *Clopidogrel can reduce the incidence of primary FAV failure without significant increase of bleeding events.*

Keywords*: arteriovenous fistula, primary failure, clopidogrel, end-stage renal disease, systematic review.*

INTRODUCTION

Arteriovenous fistula (FAV) is the most widely used vascular access by 75% of End Stage Renal Disease patients undergoing routine hemodialysis in Indonesia.[1] It is often used due to the lower risk of dysfunction and infection with long-term use compared to other vascular accesses.[2] However, newly created FAV cannot be used immediately and might become dysfunctional before the initiation of hemodialysis. This condition also known as primary failure is a major problem in ESRD patients undergoing FAV creation. The incidence of thrombosis is found in 65-85% of FAV, hence, it is estimated that thrombosis plays a major role in the incidence of primary failure.[3] The administration of heparin or aspirin reportedly does not reduce the prevalence of primary failure. Meanwhile, clopidogrel is an anti-platelet anti-aggregation that has a pleiotropic effect and reduces FAV primary failure.[4–6] To date, there is no recommendation for the use of clopidogrel in preventing FAV primary failure. Patients with ESRD are also at high risk for bleeding due to disturbances in coagulation factors. Therefore, we aimed to assess the role of clopidogrel to the incidence of FAV primary failure and the risk of bleeding in ESRD patients undergoing FAV surgery.

METHODS

This is a systematic review conducted based on The Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA). We registered our protocol on The International Prospective Register of Systematic Reviews (PROSPERO) with ID CRD42022323934.

Inclusion and Exclusion Criteria

The inclusion criteria were Randomized Control Trials on subjects aged > 18 years, studies using clopidogrel for the treatment arm, with primary FAV failure as the primary outcome, and bleeding as the secondary outcome. The exclusion criteria were active bleeding, known coagulopathy, thrombocyte < 75.000/ mm^3 and pregnancy.

Search Strategy

A comprehensive literature search was conducted by authors (WA and LU) from January until August 2022 using the databases Medline/Pubmed, EbscoHost, Embase, Proquest, Scopus, and Cochrane Library databases as well as manual searches on studies published since 1987 without language restrictions. The search used Medical Subject Heading (MeSH) and a combination of keyword synonyms (Boolean mode) for FAV, primary failure, and clopidogrel. The FAV synonym used was Arteriovenous Fistula OR Arteriovenous Shunt OR Hemodialysis Access OR Dialysis Access, with MeSH namely "Arteriovenous Shunt, Surgical". Meanwhile, the synonym used for clopidogrel was Clopidogrel OR P2Y12 inhibitor OR P2Y12 antagonist OR antiplatelet OR antithrombotic OR anti-aggregation OR antiaggregant, where MeSH is "Clopidogrel". The synonym for primary failure was Patency OR Maturation OR Immature OR Stenosis OR Thrombosis OR Fail, while the MeSH used was "Vascular Patency".

Extraction Data

Data extracted by authors (WA and LU) from the studies included the name of the first author, year of publication, the number of samples, average age, gender, type and dose of treatment given, time of assessment of primary failure, the proportion of primary failure, degree of bleeding and its proportion in each group, mortality, as well as the bleeding time before and after treatment, the data were then summarized in a tabular form.

Risk Assessment Bias

The assessment of the risk of bias was carried out using the Cochrane Risk of Bias 2

Table 1. Keywords for searching the database.

Database	Keywords	Filter	Number of Literature
Pubmed	"Arteriovenous Shunt, Surgical"[Mesh] OR "arteriovenous fistula*"[tiab] OR "arteriovenous shunt"[tiab] OR "hemodialysis access"[tiab] OR "dialysis access"[tiab] AND "Clopidogrel"[Mesh] OR clopidogrel[tw] OR "P2Y12 inhibitor*"[tiab] OR "P2Y12 antagonist*"[tiab] OR "antiplatelet"[tiab] OR "antithrombotic"[tiab] OR "antiaggregation"[tiab] OR "antiaggregant"[tiab] AND "Vascular Patency"[Mesh] OR "patency"[tiab] OR maturation[tiab] OR immature[tiab] OR stenosis[tiab] OR thrombosis[tiab] OR "fail*"[tiab]	Human, age ≥ 19 years old	79
EBSCOhost	(MM "Arteriovenous Shunt, Surgical") OR arteriovenous fistula(ab) OR arteriovenous shunt(ab) OR hemodialysis access(ab) OR dialysis access(ab) AND (MM "Clopidogrel") OR clopidogrel[text] OR P2Y12 inhibitor (ab) OR P2Y12 antagonist(ab) OR antiplatelet(ab) OR antithrombotic(ab) OR antiaggregation(ab) OR antiaggregant(ab) AND (MM "Vascular Patency") OR patency(ab) OR maturation(ab) OR immature(ab) OR stenosis(ab) OR thrombosis (ab)OR failure(ab)"	Human, age ≥ 19 years old	116
ProQuest	(MESH.EXACT("Arteriovenous Shunt, Surgical") OR ab("arteriovenous fistula") OR ab("arteriovenous shunt") OR ab("hemodialysis access") OR ab("dialysis access")) AND (MESH.EXACT("Clopidogrel") OR ft(clopidogrel) OR ab("p2y12 inhibitor") OR ab("p2y12 antagonist") OR ab(antiplatelet) OR ab(antithrombotic) OR ab(antiaggregation) OR ab(antiaggregant)) AND (MESH.EXACT("Vascular Patency") OR ab(patency) OR ab(maturation) OR ab(immature) OR ab(stenosis) OR ab(thrombosis) OR ab(fail*))	Human, article	151
Embase	'arteriovenous shunt, surgical'/exp OR 'arteriovenous fistula':ab OR 'arteriovenous shunt':ab OR 'hemodialysis access':ab OR 'dialysis access':ab AND 'clopidogrel'/exp OR clopidogrel OR 'p2y12 inhibitor':ab OR 'p2y12 antagonist':ab OR antiplatelet:ab OR antithrombotic:ab OR antiaggregation:ab OR antiaggregant:ab AND "Vascular Patency"/exp OR patency OR maturation OR immature OR stenosis OR thrombosis OR fail*	('randomized controlled trial'/exp OR 'clinical trial'/exp OR 'cohort') AND (humans)/lim	48
Scopus	"arteriovenous fistula" OR "arteriovenous shunt" OR "hemodialysis access" OR "dialysis access" AND clopidogrel OR "P2Y12 inhibitor" OR "P2Y12 antagonist" OR antiplatelet OR antithrombotic OR anti-aggregation OR antiaggregant AND "Vascular Patency" OR patency OR maturation OR immature OR stenosis OR thrombosis OR fail	"Randomized controlled trial" OR " Clinical trial" OR "Cohort"	171
Cochrane	(MeSH descriptor: [Arteriovenous Shunt, Surgical] explode all trees OR "arteriovenous fistula", "arteriovenous shunt", "hemodialysis access", "dialysis access") AND (MeSH descriptor: [Clopidogrel] explode all trees OR clopidogrel, "P2Y12 inhibitor", "antiplatelet drug", "antiplatelet agent", "antiaggregation agent", "antiaggregation drug") AND (MeSH descriptor: [Vascular Patency] explode all trees OR patency OR maturation OR immature OR stenosis OR thrombosis OR failure)	Trials	305

application. Differences between the authors (WA and LU) were discussed until an agreement was reached or consultation with the third author (IR). Publication bias was analyzed by the Rank correlation test and the Egger regression test.

RESULTS

The search on 6 databases generated 496 literatures. 147 duplicate literatures were removed. There were 348 irrelevant articles based on title and abstracts were removed. Assessment for eligibility based on full text literatures, resulted in the exclusion of 9 articles, with 7 being non-RCT study reports, and 2 other articles did not include clopidogrel in the studies. Therefore, 3 articles remained and were reviewed further.. The literature search flow is shown according to the PRISMA flow chart. We did the last search on 13 July 2022.

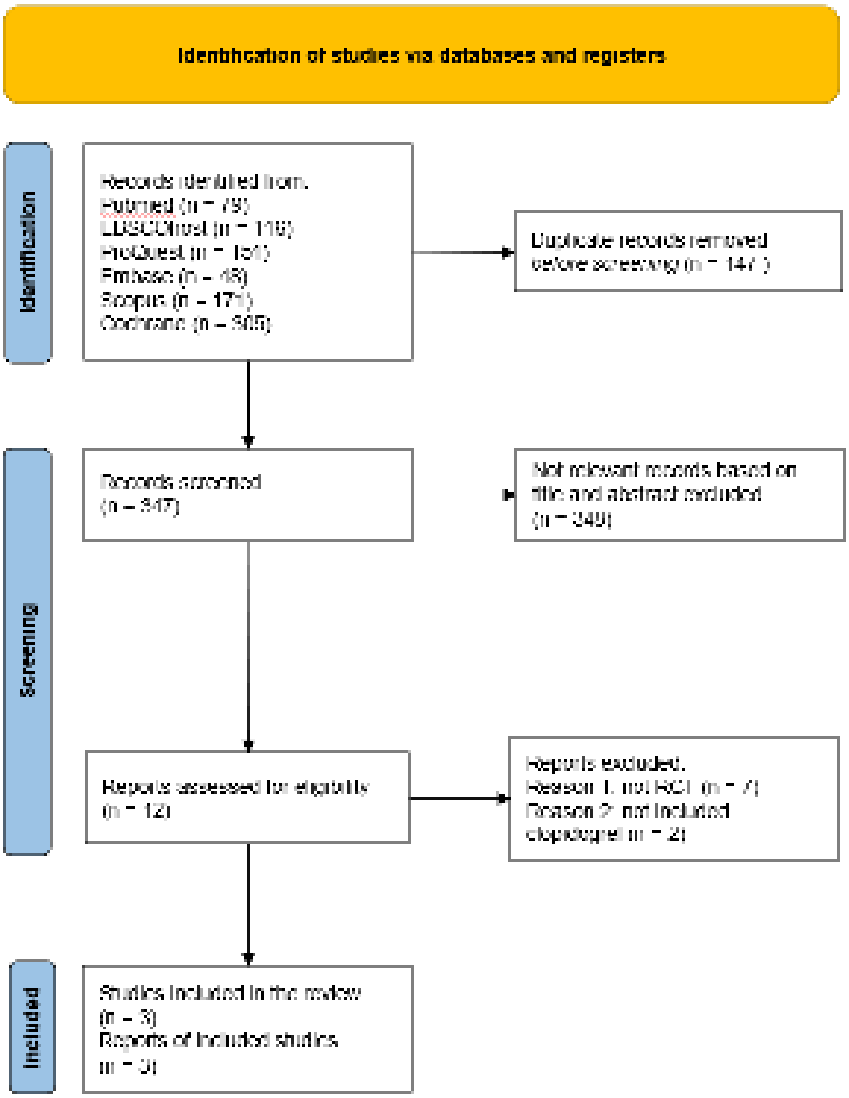

Figure 1. Study flow chart according to PRISMA.

Characteristics of Study

This systematic review involved 3 RCT studies with substantial differences. All participants in the Abacilar study were diabetes mellitus, in contrast to Dember and Ghorbani's study which involved less than 50% of participants with diabetes mellitus. Furthermore, Abacilar administered a combination of clopidogrel 75 mg and prostacyclin 200 mg/day, while Dember gave an initial dose of clopidogrel 300 mg followed by daily dose 75 mg and Ghorbani only gave clopidogrel 75 mg/day. Ghorbani and Abacilar started the intervention 7-10 days before AVF creation, while Dember started 1 day after AVF creation. There were also differences in the duration of intervention and the time for the assessment of AVF primary failure. Dember gave treatment for 6 weeks with an assessment of primary failure at the end of week 6, Ghorbani's treatment lasted for 6 weeks with an assessment at week 8, while Abacilar gave treatment for one year with an assessment at weeks 4 after AVF creation.[4–6]

Based on the three studies involved, the incidence of bleeding in the intervention group was similar to placebo group. The number of major bleeding events between the two groups was the same but the incidence of minor bleeding was slightly higher in the intervention group than in the control group. In Ghorbani's study, there was no significant difference between bleeding time before and after intervention. A similar result was found regarding mortality in both groups based on $p > 0.99$ (Dember) and $p = 0.47$ (Ghorbani).

Table 2. Characteristics of the study included in systematic review.

Study	Number of Participants		Average Age (years)		Male Gender		Diabetes mellitus		Type of Treatment (Duration)	Primary Failure Assessment	Primary Failure		
	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control			Treatment	Control	P-value
Dember et al, 2008[4]	441	436	52,7 (±14.7)	54,5 (±14.4)	63.1%	61.9%	49.2%	47%	Clopidogrel 300 mg the first day, then 75 mg/day (42 days)	Day 42	12.2%	19.5%	0.18 (RR 0.63)
Ghorbani et al, 2009[5]	40	46	44,23 (±3.36)	45,8 (±2.84)	51.6%	51.6%	15.1%	11.8%	Clopidogrel 75 mg/day (42 days)	Day 56	5.2%	21.6%	0.03 (HR 0.72)
Abacilar et al, 2015[6]	50	46	54,23 (±2.6)	55,8 (±2.84)	68%	69.5%	100%	100%	Clopidogrel 75 mg and prostacyclin 200 mg daily (365 days)	Day 28	8%	30.4%	0.001 (HR 0.82)

Table 3. Comparison of bleeding events in the treatment and the control group.

No.	Study	Major Bleeding		Minor Bleeding		Mortality		Bleeding Time Before Treatment		Bleeding Time After Treatment	
		Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
1.	Dember et al, 2008[4]	7 (1.6%)	7 (1.6%)	6 (1.4%)	5 (1.2%)	4 (0.9%)	4 (0.9%)	No data	No data	No data	No data
2.	Ghorbani et al, 2009[5]	0	0	7 (7.4%)	7 (7.5%)	2 (2.1%)	2 (2.1%)	8.1±0.3 minutes	8.4±0.6 minutes	8.5±0.4 minutes	8.6±0.3 minutes
3.	Abacilar et al, 2015[6]	0	0	9 (18%)	6 (13%)	0	0	No data	No data	No data	No data

Risk of Bias

Based on Cochrane's Risk of Bias 2 application, Ghorbani's research had a moderate risk of bias (some concerns) because it did not explain sequence generation process and concealment in detail. Ghorbani only provided a few variables on baseline characteristic that does not show comprehensive equality, and still shows p-value to express difference. Meanwhile, other studies had a low risk of bias.

Publication Bias

Assessment of publication bias of the three articles were done with Rank correlation tests and Egger regression tests. The results for each test were $p = 1.00$ and $p = 0.446$. A p-value < 0.05 in both tests indicates publication bias, and the results of the two tests were $p > 0.05$.[9]

Table 4. Assessment risk of bias based on Cochrane's Risk of Bias 2

Study ID	Experimental	Comparator	Outcome	D1	D2	D3	D4	D5	Overall
Dember	Clopidogrel	Placebo	AVF Primary Failure						
Ghorbani	Clopidogrel	Placebo	AVF Primary Failure						
Abacilar	Clopidogrel and prostacyclin	Placebo	AVF Primary Failure						

D1 : Randomization process
D2 : Deviations from the intended interventions
D3 : Missing outcome data
D4 : Measurement of the outcome
D5 : Selection of the reported result

Low risk
Some concerns
High risk

DISCUSSION

This systematic study involved three randomized controlled trials with a total of 1048 subjects with mean age being below 65 years. Based on the results, patients above 65 years and underwent FAV surgery have a higher risk of vascular access failure than those aged less than 65 years. This is associated with reduced blood flow and a smaller cross-section of blood vessels, especially in patients with comorbidities such as coronary heart and peripheral vascular disease, as well as diabetes mellitus.[10] Lok et al (2005), reported that patients aged 65 years or above are 1.7 times at risk of experiencing primary failure.[11] Most of the subjects in this systematic study were male, this is associated with a tendency for a larger cross-section of blood vessels in males compared to females.[12,13]

The average number of patients with diabetes mellitus in the Ghorbani, Dember, and Abacillar studies was 13.45%, 48.1%, and 100%, respectively.[4-6] According to Lin, diabetes alone does not predispose an individual to primary FAV failure. However, older adults with diabetes might have an increased risk of primary failure.[14] Afsar showed that diabetic patients with HbA1c less than 7 have a risk of primary failure with no significant difference from patients without diabetes, while diabetes mellitus patients with HbA1c more than 7 have a 2.8 times higher risk for primary failure.[15]

Administration of higher initial dose than the daily dose does not have a significant effect on primary FAV failure. The initial dose is usually given to speed up the onset of action and the time to achieve the maximum anti-aggregation effect. A single dose of 75 mg clopidogrel has an onset of 24 hours and reaches its maximum anti-aggregation effect in 4-7 days. Meanwhile, the administration of clopidogrel 300 mg has an onset of action of 2 hours and reaches its maximum anti-aggregation effect after 24 hours.[16] In Dember's study, administration of clopidogrel 300 mg 1 day after surgery followed by 75 mg/day did not provide a significant reduction in the incidence of primary FAV failure compared with the control group.[4] This can be attributed to the inflammatory process that occurred due to the vascular trauma during the FAV creation operation 1 day earlier.

The administration of clopidogrel 75 mg/day 7-10 days before FAV operation such as in the Ghorbani study culminated in a better reduction in the incidence of primary failure than in Dember. This can be attributed to the action of clopidogrel which has reached its maximum anti-aggregation effect at the time of the FAV preparation operation. Therefore, the incidence of thrombosis due to the postoperative inflammatory process is suppressed. Similar to Ghorbani's study, Abacillar who performed the treatment 7-10 days before FAV surgery also achieved significant results in decreasing primary FAV failure in the treatment group compared to the control. However, Abacilar added prostacyclin 200 mg/day, a derivative of arachidonic acid which is a vasodilator and also anti-platelet aggregation. As a vasodilator, prostacyclin can reduce the risk of primary failure by decreasing shear stress, which in turn reduces the risk of inward remodeling (stenosis).[6,17]

Previous reports suggested pleiotropic effect of clopidogrel as a vasodilator. Clopidogrel may also improve endothelial function as well as anti-inflammation caused by the release of NO and decreased levels of proinflammatory cytokines, including IL-1α, IL-2, IL-6, IL-13, TNF-α, and TNF-β thereby reducing vascular remodeling and the risk of vascular stenosis.[18–21]

There was no difference in the incidence of bleeding between the treatment and the control group presumably due to the mean age of the subjects in each study which was less than 65 years. Age over 65 years is a risk factor for bleeding due to the administration of anti-platelet aggregation.[22]

The limitation of this study is that it involved only three RCT studies and the differences in each study. However, the three studies above had a low risk of publication bias based on Rank correlation and Egger regression tests.

CONCLUSION

The administration of clopidogrel can reduce the incidence of primary FAV failure in patients with end-stage renal disease (ESRD). The incidence of bleeding in patients with ESRD who received clopidogrel was not different from

the control group. Further research is needed for application in daily clinical practice, especially to assess various factors (like age, gender and diabetes melitus) that can affect the risk of primary FAV failure and bleeding following clopidogrel administration. For example, clopidogrel daily dose 75 mg since 7-10 days before AVF creation until hemodialysis initiation.

CONFLICT OF INTEREST

The authors declare that this article has no conflict of interest

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Factors Associated with Sarcopenia in Maintenance Hemodialysis Patients: A Cross-Sectional Study

Ria Jauwerissa[1], Maruhum Bonar Marbun[1*], Pringgodigdo Nugroho[1], Ikhwan Rinaldi[2], Suhardjono[1], Hamzah Shatri[3], Purwita Wijaya Laksmi[4], Irsan Hasan[5]

[1] Division of Nephrology and Hypertension, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[2] Division of Hematology and Oncology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[3] Division of Psychosomatic, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[4] Division of Geriatric, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia.
[5] Division of Gastroentero-Hepatology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

Corresponding Author:
Maruhum Bonar H. Marbun, MD. Division of Nephrology and Hypertension, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo Hospital. Jl. Diponegoro no. 71, Jakarta 10430, Indonesia. Email: ria.jauwerissa@gmail.com; mbhmarbun@gmail.com.

ABSTRACT

Background: *Sarcopenia is associated with worse outcomes in maintenance hemodialysis (MHD) patients. Differences in criteria and methods used to diagnose sarcopenia, results in a wide range of prevalence. Factors associated with sarcopenia in MHD have not been well-studied. This study aimed to investigate the prevalence and factors associated with sarcopenia in the MHD population.* ***Methods****: Observational cross-sectional study was done with 96 MHD patients aged ≥18 years old, with dialysis vintage ≥120 days at Cipto Mangunkusumo Hospital March-May 2022. Descriptive, bivariate, and logistic regression analysis were done to find sarcopenia's prevalence and association with Simplify Creatinine Index (SCI), type 2 diabetes (DM), Interleukin-6 (IL-6), nutritional status, physical activity, and phosphate serum level. Asian Working Group for Sarcopenia (AWGS) 2019 criteria used to diagnose sarcopenia, Hand Grip Strength (HGS) to identify muscle strength, Bioimpedance Spectroscopy (BIS) to calculate muscle mass, and 6-meter walk test to evaluate physical performance.* ***Results:*** *The prevalence of sarcopenia was 54.2%. Factors with a significant association in bivariate analysis were phosphate serum level (p=0.008), SCI (p=0.005) and low physical activity (International Physical Activity Questionnaire) (p-0.006). Logistic regression analysis found higher phosphate serum level and high physical activity protective of sarcopenia (OR 0.677;CI95% 0.493-0.93 and OR 0.313;CI95% 0.130-0.755 respectively).* ***Conclusion:*** *The prevalence of sarcopenia in the MHD population was 54.2%. Phosphate serum level, SCI, and physical activity were significantly correlated with sarcopenia. Both high phosphate level and high physical activity were protective against sarcopenia.*

Key words: *Maintenance hemodialysis, AWGS 2019, sarcopenia.*

INTRODUCTION

Sarcopenia is associated with worsened clinical and nutritional outcomes and is an independent predictor of morbidities and mortality in a maintenance hemodialysis patient.[1] The prevalence of sarcopenia in patients with chronic kidney disease (CKD) population ranges between 4%-68% depending on guidelines, tools, and methods used to identify each variables to define sarcopenia. The prevalence in the dialysis population is found at an average of 37%.[2]

Sarcopenia in chronic kidney disease (CKD) is called uremic sarcopenia.[3] Low physical activity and negative protein balance because of prolonged uremic milieu, chronic inflammation, insulin resistance, hormonal imbalance, malnutrition, vitamin D deficiency, and oxidative stress were proposed to play a role in uremic sarcopenia.[4] To date, there has been no studies in Indonesian MHD population using AWGS 2019 criteria.

This study aimed to find the prevalence of sarcopenia using AWGS 2019 criteria and factors associated with sarcopenia in Maintenance Hemodialysis (MHD) populations.

METHODS

Study Design, Setting, Participants, and Sample Size

We conducted a cross-sectional study at Cipto Mangunkusumo Hospital Jakarta from March to May 2022. Sampling was done consecutively, inclusion criteria were patients aged ≥ 18 years with dialysis vintage ≥120 days. We excluded hospitalized patients, as well as subjects who were unable to follow procedure, amputated, or refused to join. The sample size of this study was 96.

Ethics

This study was approved by the Ethical Committee of Faculty of Medicine Universitas Indonesia (Ref. No. KET-175/UN2.F1/ETIK/PPM.00.02/2022).

Variables and Data Sources

Data from medical records were age, dialysis duration, diabetes history, hypertension, Angiotensin Converting Enzyme (ACE) inhibitor/Angiotensin Receptor Blocker (ARB) use, and phosphate binder use. Physical activities were evaluate using the International Physical Activity Questionnaire (IPAQ), while nutritional status was evaluated using the Subjective Global Assessment (SGA). Laboratory examinations taken before the dialysis session for Interleukin-6 (IL-6) level, calcium ion level, haemoglobin, and albumin serum. Simplify Creatinine Index (SCI) assessed with Single Pool Kt/V (SpKt/V). Body composition data for muscle mass calculation was obtained from Body Composition Monitor (Fresenius) and Appendicular Muscle Mass was calculated using the formula by Lin et al.[5] Hand Grip Strength measurement by Jamar hand dynamometer used to identify muscle strength and physical performance was evaluated with 6-meters walk test. We defined sarcopenia based on the criteria suggested by AWGS in 2019.

Statistical Methods

We analyzed the data using SPSS Version 20, involving descriptive analysis to find the prevalence of sarcopenia, bivariate analysis by independent T-test and chi-square to determine the association between phosphate serum level, diabetes, Il-6, physical activity, SCI, and nutritional status with sarcopenia. $P<0.05$ was considered statistically significant. Variables with $P<0.25$ were then analysed with logistic regression (predictive analysis).

RESULTS

The prevalence of this study was 54.2%, with characteristics as shown in **Table 1**.

Table 1. Characteristics of the subject.

Variables	Frequencies (%)	Mean (SD)/ Median (Range)
Sex		
Male	48 (50.0)	
Female	48 (50.0)	
Age (year)		50.82 (14.9)
Dialysis vintage (month)		48 (24-96)
Nutritional status		
SGA A	89 (92.7)	
SGA B	7 (7.3)	
Diabetes Mellitus		
Yes	30 (31.3)	
No	66 (68.8)	
International Physical Activity Questionnaire (IPAQ)		
Light activity	58 (60.4)	
Moderate activity	38 (39.6)	
Hypertension		
Yes	75 (78.1)	
No	21 (21.9)	
Angiotensin-converting Enzyme inhibitor/ Angiotensin Receptor Blocker (ACEi/ARB) use		
Yes	36 (3.5)	
No	60 (62.5)	
Calcium carbonate ($CaCO_3$) use		
Yes	57 (59.4)	
No	39 (40.6)	
Early Referral		
Yes	18 (18.8)	
No	78 (81.3)	
Simplify Creatinine Index (SCI)(mg/kg/day)		23.02 (3.59)
Interleukin-6 (pg/mL)		5.53 (3.93-10.52)
Single Pool (Sp) Kt/V		2.02 (1.76-2.33)
Phosphate serum (mg/dL)		4.08 (1.45)
Creatinine serum (mg/dL)		11.99 (3.77)
Ion calcium (mmol/L)		1.13(1.08-1.21)
Hemoglobin (g/dL)		9.30 (1.38)
Albumin (g/dL)		3.93 (0.38)
Body mass index (kg/m^2)		23.07 (4.91)
Fat mass (kg)		14.15 (9.23-21.30)
Lean tissue mass (kg)		35.35 (30.57-41.80)
Appendicular skeletal muscle mass (ASM) (kg/m^2)		
Male		4.92 (0.84)
Female		3.76 (0.79)
Hand grip strength (kg)		
Male		24 (20-30)
Female		18 (12-20)

Independent T-test analysis showed a significant difference in mean phosphate serum level (p=0.008) and Simplify Creatinine Index (p=0.005). The Chi-Square analysis of physical activity (using IPAQ score) (p=0.006) was significantly associated with sarcopenia (**Table 2**).

Researchers elaborated analysis with a predictive model using variables with p<0.25 and found higher phosphate serum levels (OR 0.677, p=0.016) and higher physical activity (OR 0.313, p=0.01) were protective of and significantly correlated with sarcopenia in MHD population (**Table 3**).

DISCUSSION

Muscle loss is a common finding in CKD patients, especially in the hemodialysis patients. The prevalence of sarcopenia is greatly influenced by the variability of diagnostic criteria and patient characteristics. Sarcopenia in the MHD population's prevalence from various studies ranges from 4-68%.[6,7] The prevalence of sarcopenia in this study population was 54.2%. This study is the first in Indonesia that used AWGS 2019 criteria to diagnose sarcopenia. Researchers use Bio-Impedance Spectroscopy (BIS) to assess Appendicular Skeletal Mass (ASM), Hand Grip Strength (HGS) to assess muscle strength, and 6-meters walk test to evaluate physical performance. The examinations were done before hemodialysis session on the non-AV-shunt arm.

The etiologic factors that contribute to muscle loss in hemodialysis are diverse and can be grouped into factors that contribute to increased protein degradation (reduced energy and protein intake, inflammation, insulin resistance, metabolic acidosis, vitamin D deficiency, and oxidative stress) and factors that related to decreasing protein synthesis (loss of amino acid and protein during dialysis, reduced regenerative stimulus, hormonal derangements, sedentary lifestyle, ageing).[8]

Table 2. Association of SCI, type 2 DM, IL-6, nutritional status, physical activity with sarcopenia.

Variables	Sarcopenia		p
	Yes	No	
Phosphate serum level, mean (SD)	3.73 (SD 1.18)	4.5 (SD 1.63)	0.008
Simplify creatinine index (SCI), mean (SD)	22.09 (SD 3.56)	24.12 (SD 3.35)	0.005
Type 2 DM, n (%)			
Yes	20 (66.7)	10 (33.3)	0.095
No	32 (48.5)	34 (51.5)	
IL6, n (%)			
Normal	33 (56.9)	25 (43.1)	0.507
Above normal level	19 (50.0)	19 (50.0)	
Nutritional status, n (%)			
SGA A	46 (51.7)	43 (48.3)	0.120*
SGA B	6 (85.7)	1 (14.3)	
IPAQ, n (%)			
Light activity	38 (65.5)	20 (34.5)	0.006
Moderate activity	14 (36.8)	24 (63.2)	

Table 3. Multivariate analysis logistic regression.

	Variables	B	SE	Z	P
1	Phosphate serum	0.390	0.162	2.407	0.016
2	Physical Activity	1.162	0.449	2.588	0.010
3	Constant	-3400	0.962	3.534	<0.001

Impaired muscle regeneration also often develops in CKD patients, proven by reduced cell activation and expression of myogenic regulatory factors (a negative regulator of skeletal muscle mass).[9] Furthermore, there is increased catabolism in CKD due to the accumulation of uremic toxins, chronic inflammation, insulin resistance, hormonal imbalance, malnutrition, vitamin D deficiency, oxidative stress, and increased ubiquitination.[4]

Emerging evidence addressed an association between phosphate and muscle function, but only a little attention focused on this issue.[10] High phosphate concentrations are associated with an increased incidence of cardiovascular complications and mortality in CKD patients, hence dietary and pharmacotherapeutic interventions aimed to reduce phosphate serum level.[11] However, a meta-analysis study by Block et al[12] found significant increases in the relative risk of death in lower phosphate serum concentrations (<4.0 mg/dL).

Bivariate analysis in this study showed a significant association between the lower level of phosphate serum with sarcopenia and accordance with the following study by Umakanthan, et al[13], Ren, et al[14], and Cai, et al[15]. The findings suggested that a high protein diet also contained high phosphate, and patient in the uremic milieu usually lost their appetite or even has anorexia. Reduced energy and protein intake will lower phosphate serum levels, causing malnutrition, catabolic protein condition, and sarcopenia. Another mechanism that could explain hypophosphatemia in sarcopenia is that inorganic phosphate also plays a role in cell membrane, energy production, and transduction signal in all body cells.[16] Study by Pesta et al.[17] in animal showed that genetically and diet-induced hypophosphatemic mice has a decreasing muscle ATP synthesis rate.[18]

This study also found a significant correlation between Simplify Creatinine Index (SCI) with sarcopenia (p=0.005). This finding also followed Canaud, et al[19] study which stated that SCI is a reliable and inexpensive marker of muscle metabolism and can be used as a nutritional and skeletal muscle marker in the dialysis population. Yamamoto, et al[20] found that SCI's long term predictive value was comparable with hand grip strength and walking speed.

In this study, physical activity was analysed with IPAQ and had a significant correlation with sarcopenia in this study (p=0.006). Regolisti, et al[21] stated that low physical activity was commonly found in MHD populations and related to muscle disuse which could further cause loss of muscle mass and eventually sarcopenia. Limited physical activity during hemodialysis session and lethargic feelings that are often felt by MHD populations reduce their activity time.

This study did not find a significant correlation between DMt2 and sarcopenia in the MHD population (p=0.095); nevertheless, 66.7% of DMt2 participants in this study were diagnosed with sarcopenia. This finding was following Giglio, et al[1], Hoppe, et al[22], and Visser, et al[23]. This could be explained by the fact that both diabetes and CKD present with chronic inflammation that could disrupt muscle metabolism in the long term.

Nutritional status was assessed with SGA as recommended by KDOQI. The majority of samples in this study (92.7%) have good nutritional status (SGA A). Macedo, et al[24] found that 51.2% of samples in the non-sarcopenia group suffered from malnutrition. Vettoretti, et al[25] also found no significant correlation between sarcopenia and malnutrition and stated that both were different domains of nutrition abnormalities. Similar finding was also found in Ren, et al's study.[14]

The inflammation factor (using IL-6 as a marker) did not meet the significance's requirement in this study (0.507). Many studies found similar results using the same or different markers.[1,14,26,27] This could explained by the fact that inflammation in MHD population are increase in an episodic manner and do not picture the chronic conditions state of the patient.

The logistic regression analysis showed the ideal model to detect sarcopenia, where higher phosphate level and high physical activity were both protective factors to prevent sarcopenia (OR 0.677;CI95% 0.493-0.93 and OR 0.313;CI95% 0.130-0.755 respectively).

This study presented the recent data about the prevalence of sarcopenia in MHD populations

in Indonesia using AWGS 2019 criteria with variables that are proposed to play a role in the pathogenesis of sarcopenia. This study's high prevalence of sarcopenia implies the necessity to address and treat sarcopenia in the clinical setting. Variables used in this study was objective and easily retrieved in hemodialysis centers to diagnose sarcopenia in MHD patient, so the reproducibility of this study was good.

This study was cross sectional, so the causal relationship between variables was not achieved. This study did not include the role of hormones like ghrelin and angiotensin. Bias potentials from this study required considerations were glucocorticoid use and HGS examination in the non-AV shunt arm, which could be the non-dominant arm. Factors that were not significant in logistic regression analysis might reach significance in larger samples study; hence the follow-up study is encouraged.

CONCLUSION

The prevalence of sarcopenia in the MHD population in Cipto Mangunkusumo Hospital was 54.2%. This study's factors significantly associated with sarcopenia were phosphate serum level, SCI, and physical activity. Logistic regression analysis showed that higher phosphate serum level and high physical activity were protective against sarcopenia in the MHD population.

On the basis of these findings, it seems prudent to suggest that early detection of sarcopenia is crucial in MHD populations, and by increasing physical activity and a better control of phosphate serum level could prevent this condition.

AUTHOR'S CONTRIBUTION

RJ designed the study, collected the data, performed data analysis, and drafted the original manuscript. MBM and PN participated in designing the study and revised the manuscript. IR helped in data analysis and revising the manuscript. S, HS, PWL, and IH participated in revised the manuscript. All authors read and approved the final manuscript.

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Depression Symptoms and Inflammation in Chronic Functional Constipation Patients

Hamzah Shatri[1], Edward Faisal[1], Murdani Abdullah[2,3], Ari Fahrial Syam[2], Amanda Pitarini Utari[2], Virly Nanda Muzellina[2], Saskia Aziza Nursyirwan[2], Aly Lamuri[3]*

[1] Division of Psychosomatic and Palliative, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[2] Division of Gastroenterology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[3] Medical Technology Cluster, IMERI, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia.

Corresponding Author:
Amanda Pitarini Utari, MD. Division of Gastroenterology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital. Jl. Diponegoro no. 71 Jakarta 10430, Indonesia. Email: amanda.pu@hotmail.com.

ABSTRACT

Background: *Inflammation in chronic functional constipation (CFC) occurs systemically and has association with depressive symptoms. Biomarkers of inflammation can be assessed by the neutrophil to lymphocyte ratio and platelet to lymphocyte ratio. These inflammation biomarkers are stable, cheap, and widely available. This study aimed to determine the profile and the correlations between depressive symptoms and inflammation in CFC patients.* ***Methods:*** *This cross-sectional study involved subjects aged 18-59 years with chronic functional constipation. We use validated Beck Depression Inventory-II (BDI-II) to assess depressive symptoms. We collected the data regarding complete peripheral blood examination, liver function, kidney function, electrolytes, and neutrophil-lymphocyte ratio (NLR), and platelet-lymphocyte ratio (PLR). Bivariate analysis with Chi-Square test for categorical data and t-test or ANOVA test for numerical data. Multivariate analysis used logistic regression to look at risk factors for depression with $p < 0.05$ as a statistical significant level.* ***Results:*** *A total of 73 subjects with CFC were recruited with a mean age is 40.2 years, mostly women and working as housewives. Proportion of depressive symptoms in CFC patients was 73.0%, including mild depression 16.4%, moderate depression 17.8%, and severe depression 28.8%. The mean NLR in non-depressive subjects was 1.8 (SD 0.7), while in depressive subjects was 1.94 (SD 0.1) ($p>0.05$). The mean NLR in mild depression subjects was 2.2 (SD 1.7), in moderate depression was 2.0 (SD 0.7), and in severe depression was 1.9 (SD 0.5) ($p>0.05$). The mean PLR in non-depressive subjects was 134.3 (SD 0.1), whereas in depressive subjects it was 138.9 (SD 46.0) ($p>0.05$). The mean PLR in mild depression subjects was 142.9 (SD 60.6), in moderate depression was 135.4 (SD 41.2), and in major depression was 139.0 (SD 37.1) ($p>0.05$).* ***Conclusion:*** *This study found that CFC patients were middle-aged, mostly women and working as a housewife. In general, biomarkers of inflammation were found to be higher in depressive subjects than non-depressive subjects, although not statistically significant.*

Keywords**:** *Chronic functional constipation, depressive symptoms, inflammation, lymphocyte to neutrophil ratio, platelet to lymphocyte ratio.*

INTRODUCTION

Chronic functional constipation (CFC) is an often-neglected digestive tract disorder. Low-grade inflammation, cell degeneration, and increased oxidative stress impair functional conditions in CFC.[1,2] In vivo studies demonstrated that inflammation in mice's colon occurred after transplanted with CFC patients feces, as suggested by Gobert et al.[3] Food containing antigen entering the digestive tract will incite the adaptive immune system.[4,5] Further inflammation might play a role in various disorders or diseases involving the gastrointestinal tract and psychological factors, e.g., depressive disorders.[6]

One probable mechanism is dysbiosis and inflammation in CFC, which interrupt serotonin regulation in the brain, and in turn, cause behavioral disorders with depression as a particular impact.[7] The presence of pro-inflammatory cytokines increases serotonin reuptake transporter (SERT) activity in the intestine, causing serotonin decrease as a potential cause of depression.[8,9] Occurring distress can impair intestinal defense, increase bacterial permeability and translocation, also activate the inflammatory system.[6,10] In contrast, psychological distress also activates the immune system in response to inflammation, an increase in cell movement that plays a role in immunity between blood vessels and tissues.[6]

The association between serotonin and serotonin transporters located in the gastrointestinal tract and inflammation has attracted the attention of researchers as to the etiology of depressive symptoms. At the same time, inflammatory markers may increase in response to depression. Simple, inexpensive, and easily reproducible inflammatory parameters may include the neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR), C-reactive protein (CRP) and High sensitivity C-reactive protein (hsCRP).[11,12] Various studies have shown that NLR and PLR are in line with CRP and hsCRP as markers of inflammation.[11–18] It is currently unclear whether local inflammation in CFC can cause systemic inflammation; and whether it is associated with depressive symptoms. For this reason, this study further examines inflammation marked by NLR and PLR.

METHODS

This cross-sectional study involved 73 subjects of chronic functional constipation (CFC) subjects. Diagnosis of CFC was screened using the ROMA IV criteria and Bristol stool chart form (BSCF), validated by Blake et al.[19] Subjects willing to participate signed the informed consent filled out the validated, Indonesian version (in Bahasa), BDI-II questionnaire to evaluate depressive symptoms.[20] This study has been approved by the Ethics Committee of the Faculty of Medicine Universitas Indonesia, and all participants signed informed consent forms.

Blood sample was taken to analyze peripheral blood cell count and inflammation of the neutrophile-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR). Demographic characteristics are age, gender, ethnic group, occupation, the socioeconomic level, the education level, and clinical characteristics, including CFC signs and symptoms. This study considered high socioeconomic status as those with monthly income higher than the minimum wage in Jakarta. Based on the educational attainment, this study grouped those attained at least a highschool diploma as highly educated.

We provided depression symptom data according to BDI-II in the form of proportion, mean, and or median. We performed bivariate analysis with Chi Square test for categorical data and t test or ANOVA test for numerical data. Multivariate analysis relied logistic regression to find out risk factors for depression with $p < 0.05$ as a significant level. Pearson's or Spearman's rho method independently assessed the correlation between NLR and PLR to depressive symptoms. Pearson's or *Spearman's rho* method independently assessed the correlation between NLR and PLR to depressive symptoms.

RESULTS

The mean age of the subjects in this study was 40.2 (SD 11.25) years, and almost all CFC patients were women (90.4%). A larger proportion of subjects were of Betawi ethnic group (64.3%), working as house wife (78.1%), having low socioeconomic status (95.8%) and high education levels (64.5%). The three most commonly reported

constipation-related complaints were straining, defecate <3 times every seven days and hard stool consistency. Laboratory examinations of complete peripheral blood, liver function, kidney function and electrolytes were within normal range. The proportion of depressive symptoms (73.0%) was high in CFC patients, further classified into mild depression (16.4%), moderate depression (17.8%), and severe depression (28.8%). A complete description of the subject profile, see **Table 1**. In **Table 2** and **3** were the results of bivariate and multivariate analysis of factors related to depressive symptoms, respectively. The results of significant level of peripheral blood cell count, NLR and PLR *of CFC based on depression and without depression symptoms* (**Table 4**) and the severity level of depressive symptoms (**Table 5**).

Table 1. Characteristics of the study participants.

Profiles	All (n=73)
Age (Mean) years	**40.2 (11.25)**
Gender, n (%)	
Female	66 (90.4)
Male	7 (9.6)
Race, n (%)	
Betawi	47 (64.3)
Sunda	3 (4.1)
Jawa	11 (15.1)
Sumatra	7 (9.7)
Nusa Tenggara	2 (2.7)
N/A	3 (4.1)
Work, n (%)	
Housewife.	57 (78.1)
Entrepreneur	7 (9.6)
Employees/Labourers	5 (6.8)
Jobless	4 (5.5)
Socioeconomic status, n (%)	
Low	70 (95.8)
High	3 (4.2)
Level of Education, n (%)	
Low	28 (38.4)
High	45 (61.6)
Sign and Symptoms CFC, n (%)	
Straining	67 (89.3)
Hard stool	60 (80.0)
Perceived incomplete evacuation of bowel movements	48 (64.0)
A blocked or full sensation in the lower intestine or anus	38 (50.7)
Doing movements to make defecation easier (requiring tools)	20 (26.7)
Less than 3 bowel movements per week	64 (85.3)
Laboratory Result (Mean (SD)	
Haemoglobin. (Hb) g/dL	12.97 (1.55)
Haematocrit (Ht) %	38.71 (3.81)
Leucocyte 10^3/µl	7.67 (2.07)
Absolute Neutrophil count 10^3/µl	4.42 (1.60)
Absolute Lymphocyte count 10^3/µl	2.49 (0.94)
Platelet 10^3/µl	318.31 (78.41)
NLR	1,90 (0.88)
PLR	137.17 (47.74)
AST (U/L),	20.84 (9.99)
ALT (U/L)	20.99 (18.02)
Bilirubin (mg/dL)	0.50 (0.18)
Creatinine (mg/dL)	0.71 (0.16)
Urea (mg/dL)	20.68 (5.46)
Blood Glucose(mg/dL)	98.11 (51.49)
Potassium (K)	4.18 (0.53)
Sodium (Na)	139.00 (2.17)
Chloride (Cl)	104.66 (2.25)

Depression symptoms	
No symptoms of depression	27 (37.0)
With depression	46 (73.0)
Depression symptoms level	
No symptoms of depression	27 (37.0)
Mild	12 (16.4)
Moderate	13 (17.8)
Severe	21 (28.8)

Note: ALT: Alanine transaminase; AST: Aspartate aminotransferase NLR = Neutrophil lymphocyte ratio; PLR = Platelet lymphocyte ratio.

Table 2. Bivariate analysis of factors related depression symptoms in CFC patients.

Profiles	No depression n (%)	Depression n (%)	P-value
Gender			
Female	23 (34.8%)	43 (65.2%)	p 0.014
Male	6 (85.7%)	1 (14.3%)	
Ethnic group			
Betawi	18 (38.3%)	29 (61.7%)	p 0.775
Non-Betawi	8 (34.8%)	15 (65.2%)	
Work			
Unemployed or housewife	23 (37.7%)	38 (62.3%)	p 0.426
Employee, labourer, or entrepreneur	6 (50.0%)	6 (50.0%)	
Socioeconomic status			
Low	27 (38.0%)	44 (62.0%)	p 0.154
High	2 (100.0%)	0 (0.0%)	
Level of Education			
Low	6 (21.4%)	22 (78.6%)	p 0.012
High	29 (39.7%)	44 (60.3%)	
Smoking			
yes	6 (85.7%)	1 (14.3%)	p 0.014
no	23 (34.8%)	43 (65.2%)	

Table 3. Multivariate analysis of factors related depression symptoms in CFC patients

Profiles		No depression	Depression	p
Level of Education	Low	6 (21.4%)	22 (78.6%)	0.032
	High	29 (39.7%)	44 (60.3%)	
Smoking	yes	6 (85.7%)	1 (14.3%)	0.06
	No	23 (34.8%)	43 (65.2%)	

Table 4. Peripheral blood cell count, NLR, and PLR of CFC based on depression and without depression symptoms.

Profiles	No depression (n =27)	Depression (n=46)
Leukocytes, mean (SD) 10^3/µl	7.3 (2.1)	7.9 (2.0)
Absolute neutrophil count, mean (SD) 10^3/µl	4.1 (1.4)	4.6 (1.6)
Absolute lymphocyte count, mean (SD) 10^3/µl	2.45 (0.7)	2.6 (1.1)
Platelets, mean (SD), 10^3/µl	304.6 (68.2)	326.35 (83.5)
NLR, mean (SD)	1.8 (0.7)	1.94 (0.1)
PLR, mean (SD)	134.3 (43.9)	138.9 (46.0)

Note: NLR = Neutrophil lymphocyte ratio; PLR = Platelet lymphocyte ratio; SD = Standard Deviation. * t-Test p>0.05/ not statistically significant

Table 5. Peripheral blood cell count, NLR and PLR of CFC based on the level of depression symptoms.

Profiles	No Depression (n=27)	Mild Depression (n=11)	Moderate Depression (n=14)	Severe Depression (n=21)
Leukocytes, mean (SD), 103/µl.	7.3 (2.1)	8.1 (2.1)	7.9 (2.4)	7.7 (1.9)
Absolute Neutrophils, mean (SD), 103/µl	4.1 (1.4)	4.9 (2.1)	4.5 (1.8)	4.50 (1.4)
Absolute Lymphocytes, mean (SD), 103/µl	2.4 (0.7)	2.5 (0.6)	2.7 (1.7)	2.6 (0.8)
Platelets, mean (SD), 103/µl	304.6 (68.2)	339.2 (97.6)	311.2 (83.8)	329.7 (78.6)
NLR, mean (SD)	1.8 (0.7)	2.2 (1.7)	2.0 (0.7)	1.9 (0.5)
PLR, mean (SD)	134.3 (43.9)	142.9 (60.6)	135.4 (41.2)	139.0 (37.1)

Note: NLR = Neutrophil lymphocyte ratio; PLR = Platelet lymphocyte ratio; SD = Standard Deviation.

DISCUSSION

Complete laboratory examination of peripheral blood, liver function, kidney function and electrolytes were within normal limits which indicated that all subjects of this study were functional disorders, thus strengthening the diagnosis of functional constipation in Rome IV criteria.[21] This study found that 73 subjects of CFC were middle-aged, primarily women, mostly the ethnic is betawi, working as house wife, with low socioeconomic status, and high education levels. The demographic profile in our study was similar with study conducted by Mokhtar et al.[22] that 240 subjects of CFC highest among female, 72.3%, non-smokers 93.6% and had lower income 89.4%.

Meanwhile, the total number of subjects experiencing depressive symptoms was 73.0%; with 34 % having moderate and severe depressive symptoms and only 12% having mild depressive symptoms. This result was higher than studies that have been done by Mokhtar et al.[22] which found 67.1% had no depressive symptoms, 32.1% experienced mild/borderline depressive symptoms and only two (0.83%) had probable a moderate-severe depressive symptoms. However Mokhtar et al.[22], use Rome III criteria for functional constipation and the Center for Epidemiologic Studies Depression Scale Revised (CESD-R) to asses depression which can make the difference.

There was a bidirectional relationship between depressive symptoms and gastrointestinal disorders. Psychological disorders such as depression will contribute negatively to a person's life and his/her family. Poor understanding of psychological health results in bias, namely that respondents fill out a depression symptom screening questionnaire that does not match the actual situation, but this study shows that screening can find depressive symptoms faster. Psychological disorders such as depression are still a stigma in society, so many do not realize they are experiencing depressive symptoms. This argument is per a qualitative study conducted by Subu et al.[24] in 2017, which suggested that the stigma against patients with psychological disorders in Indonesia is quite apparent, preventing patients from seeking professional help. Subu et al.[24] also found that a person who has a psychological disorder will continue to worsen and find it difficult to access mental health services because of the ongoing stigma. A qualitative study by Holis et al.[25] shows that the wrong coping mechanism occurs due to the stigma against psychological disorders. According to the qualitative study of Meng et al.[26], it was found that dealing with distress depends on one's psychodynamic and adaptive abilities.

Until now, there were no studies that measures inflammatory conditions in CFC systemically. Our findings suggested no systemic involvement due to local gastrointestinal inflammation. Peripheral Blood Cell Count, NLR and PLR of CFC were higher in depression than in normal conditions, but not statistically significant. The finding of this study is similar to that of study conducted by Mazza MG et. al.[27] who found that NLR and PLR were higher in patients with mood disorders compared to healthy controls and were also not statistically significant, which means that

the inflammatory condition that occurs is only local gastrointestinal tract which has no impact systemically. This study also found that NLR and PLR ratio in CFC is higher than in the normal population, especially in CFC with depression.[12]

According to a study by Singh et al.,[28] there were mastocytes and eosinophil cells in the descending colon associated with irritable bowel syndrome. Leukocytes, absolute neutrophils, NRL, and PLR were higher in subjects with depressive symptoms than those without depression. Meanwhile, the lymphocyte levels were almost equal between the two subject conditions. This finding is consistent with the study of Ucar et al.[29] which showed that depressed patients experienced a decreased lymphocyte response to mitogen stimulation and impaired neutrophil activity. This study also follows the study of Ozturk et al.[30] which showed statistically insignificant but apparent trend in inflammatory biomarker levels.

Although NLR and PLR were not significantly correlated with depressive symptoms, there was a higher NLR and PLR in depressed CFC patients compared to patients without depressive symptoms. Further research should be done. This study of inflammation in depressive symptoms was confirmed by Sunbul et al.,[11] who explained that NRL was significantly increased in both major and very severe depressive symptoms. Although subjects with major depression were not large enough in this study, the correlation test may have had less statistical power to assess significance.

This study has a fairly good internal validity, so that bias in the selection can be minimized. Due to the strict inclusion criteria, the number of samples obtained was not large, but still sufficient to represent the research sample to be generalized. The study location may represent urban areas in one research group to be generalized to other urban areas in Indonesia. The significant factors for the incidence of depression in CFC from this study were only low education and not smoking. Therefore, it is necessary to do future studies from the aetiological side of the diagnosis of depressive symptoms. Other contributing factors to depressive symptoms that require further investigation are stresses in daily life, lack of social support, and a history of eating disorders.[31] In depression, serotonin levels decrease because the serotonin transporter mRNA and its regulatory proteins are increased. Inflammatory conditions associated with free serotonin depletion are changes in inflammatory cytokines such as IL-1, IL-6, and TNF-α.[29]

CONCLUSION

This study found that CFC patients were in middle age, mostly women and working as housewives with depressive symptoms. Eventhough not statistically significant, it tends that biomarker of inflammation is higher in depressive symptoms subjects than in non-depressive symptoms subjects, represented by mean of NLR and PLR. Increasing of NLR and PLR values might indicate depression. In the future, researchers are advised to reproduce similar study with a larger sample population, to analyze serum serotonin levels, other inflammatory factors, and to analyze other factors associated with depressive symptoms.

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The Role of New Pulmonary Artery Wedge Pressure Formula to Predict Diastolic Dysfunction in Obstructive Sleep Apnea

Telly Kamelia[1], Lukman Hakim Makmun[2]*

[1] Division of Respirology and Critical Care, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo General Hospital, Jakarta, Indonesia.
[2] Professor Emeritus of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo General Hospital, Jakarta, Indonesia.

Corresponding author:
Telly Kamelia, MD, PhD. Division of Respirology and Critical Care, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo General Hospital. Jl. Diponegoro no. 71, Jakarta 10430, Indonesia. Email: tellykamelia99@gmail.com.

ABSTRACT

Background: *Heart failure (HF) is a common condition with high morbidity and mortality in Obstructive Sleep Apnea (OSA), especially in obese patient. The causes of HF are often abnormal conduction pathways, pump filling and/or heart valves. Right heart catheterization using Swan-Ganz catheter remains the gold standard to determine pulmonary hemodynamics, but it is costly and invasive. Herein, we propose a new formula for non-invasive Pulmonary artery wedge pressure (PAWP) measurement using tissue Doppler echocardiography. The purpose of this research is to explore the correlation between the new formula to calculate PAWP to predict diastolic dysfunction in OSA patients.* ***Methods:*** *A cross-sectional study was conducted in Jakarta, in March until October 2021. Eighty-two subjects were enrolled in the study, consist of 34 females and 48 males. All subjects underwent polysomnography and tissue Doppler echocardiography. Noninvasive measurement of PAWP were obtained from combined assessment of E/e'and left atrial parameters.* ***Results:*** *Based on 82 subjects included, 66 subjects (80.5%) had obstructive sleep apnea, and 16 subjects (19.5%) did not have it. There was a significant difference in PAWP between patients with and without OSA (p value <0.01). Ten subjects OSA (12.1%) had diastolic dysfunction, while all non-OSA subjects had normal diastolic function, with no statistical significance between two groups (p value = 0.20). Diastolic dysfunction significantly associated with PAWP measured using proposed formula (R = 0.240, p value = 0.030).* ***Conclusion:*** *The new formula could be used to indirectly calculate PAWP and predict diastolic dysfunction in OSA. Obstructive sleep apnea is associated with elevated PAWP. The increased risk of diastolic dysfunction in OSA, especially in obesity patient may indicate for the risk of cardiovascular morbidities.*

Keywords**:** *pulmonary artery wedge pressure, obstructive sleep apnea, diastolic dysfunction.*

INTRODUCTION

Heart failure (HF) is a common condition with high morbidity and mortality, which places a significant financial burden on the community due to reduced productivity, repeated hospitalizations and treatment costs. Moreover, 40% to 50% of patients who now present with heart failure with preserved ejection fraction (or HFpEF) are reported to have sleep apnea.[1] Left ventricular diastolic dysfunction, defined by impaired relaxation of the myocardium, is a hallmark of heart failure in patients who present with heart failure reduced ejection fraction (HFrEF) or heart failure preserved ejection fraction (HFpEF). This classification of HF causes is important, when one has to assess

patients with HF for the possibility of sleep apnea and develop a treatment plan as some HF types may be more sensitive to obstructive sleep apnea (OSA).[2]

Diastolic dysfunction is a condition that reflects an impairment of the filling properties of the left ventricle (LV) that has been demonstrated to be a predictor of future development of heart failure. The association between OSA and diastolic dysfunction is not well studied, although OSA is frequent in heart failure patients. Proposed mechanisms that affect left ventricular performance in patients with OSA include several mechanical, neurohumoral, inflammatory, endothelial, and oxidative effects. More research is required to determine basic mechanisms by which OSA exerts its adverse effects on the cardiovascular system. Such investigations could include studies of the impacts of intermittent hypoxia on cardiovascular function at both cellular and molecular levels, and genetic susceptibilities to adverse cardiovascular risks of OSA.[3]

Left ventricular filling can be measured directly by placing a catheter in the left ventricle to obtain the end-diastolic pressure (LVEDP), or indirectly by placing a catheter in the pulmonary artery to measure the pulmonary capillary wedge pressure (PCWP). Both of these invasive techniques involve cardiac catheterization with its attendant risk and expense.[1] Echocardiography is essential to the evaluation of heart failure, and guidelines exist to identify diastolic dysfunction non-invasively. However, up to 50% of patients with HFpEF have normal resting diastolic function parameters. On echocardiographic examination with Doppler, the velocity of V_E and V_A can be determined, where V_A is the rate of filling of the end-diastolic blood phase from LA to LV due to LA contraction as seen with P waves on the ECG. With this V_A velocity component, it can be used to determine the conversion formula from V_A to pulmonary artery wedge pressure (PAWP), so that it is enough with a non-invasive examination to determine PAWP pressure (PC = Pulmonary Capillary) indirectly, and there is no need to perform invasive catheterization again.[2]

Extensive technical improvements in echocardiography have increased its sensitivity for quantifying PAWP and it is now recognized as a safe and available alternative to right heart catheterization. PAWP represents an alternative measure to left ventricular end-diastolic pressure (LVEDP), which is the gold standard for determining left ventricular filling pressure.[4] The mean PAWP that integrates the atrial pressure tracing throughout systole and diastole provides an integrated measure of the hemodynamic burden imposed by the left atrial (LA) operating compliance on the pulmonary circulation.[5] PAWP is a surrogate marker of left atrial pressure (LAP).

Sleep apnea is often found in patients with heart failure. Obstructive sleep apnea patients reveal acute and chronic hemodynamic changes. Nevertheless, the combination of sleep apnea and heart failure is different to the previously mentioned combinations (i.e. OSA with hypertension and CAD) as the majority of the sleep apnea cases are central or mixed in heart failure patients.[3] Nevertheless, OSA can cause heart failure: systolic heart failure as after a myocardial infarction, or diastolic heart failure as is often the case in hypertensive patients. Almost half of heart failure patients have a preserved systolic function, i.e. a left ventricular (LV) ejection fraction (LVEF) >45%. LV hypertrophy (LVH) often seems to be associated with this LV diastolic dysfunction.[6]

Several predisposing factors for OSA include obesity, neck circumference size, age, sex, hormones, and airway anatomic abnormalities. Another study reported that neck circumference (>42.5 cm) was associated with an increase in Apnea-Hypopnea Index (AHI).[7] Obesity can change the volume and anatomical shape; the tongue can be raised thereby reducing the volume of the upper airway. Four large-scale prevalence studies suggest that one in five white adults with an average body mass index (BMI) of 25–28 kg/m^2 has an AHI 5 times per hour. It is reported that one in fifteen of OSA patients has an AHI of 15 or more.[8]

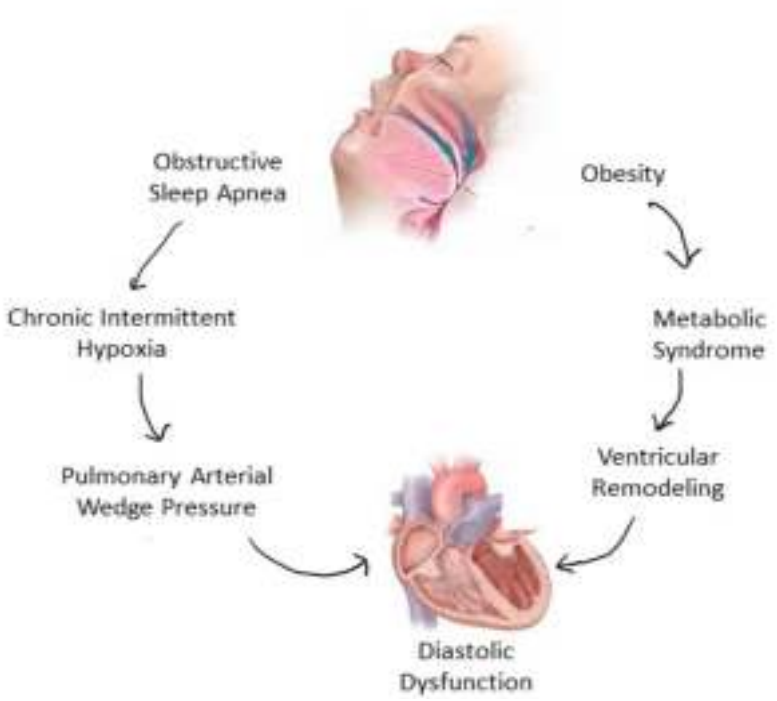

Figure 1. Pathophysiology of diastolic dysfunction in obstructive sleep apnea and obesity[6]

Since the PAWP is a substitute marker of left atrial pressure and chronic airflow obstruction is the most important cause of pulmonary artery remodeling that can lead to diastolic dysfunction, the main purpose of this study was to obtain the pulmonary artery wedge pressure (PAWP) based on echocardiography, which was estimated using a new mathematical model formula based on measurements of left atrial pressure and the influencing factors with parameters related to the OSA incidence. In addition, to build a new PAWP formula using a mathematical model that functions as a non-invasive procedure to predict diastolic dysfunction and compare the new formula with the existing formula.

METHODS

This cross-sectional study was conducted in Jakarta, between March until October 2021. The study was approved by Universitas Indonesia, Faculty of Medicine Ethic Committee (KET-1205/UN2.F1/ETIK/PPM.00.02/2020). This formula has been registered to the Indonesian Copyright Service for predicting diastolic dysfunction in obstructive sleep apnea the new pulmonary artery wedge pressure (PAWP) formula by Lukman H. Makmun (Reg. no. EC00202297046 and Reg. no. EC00202297063).

We included obese subjects aged from 18 to 65 years. In Asia-Pacific countries, the agreed cut-off point for obesity was defined as BMI (kg/m^2) between 25.0 and 29.9. Written informed consent was obtained from all subjects. Exclusion criteria included the following: unstable cardiorespiratory status, defined as the existence of respiratory failure, congestive heart failure, or if they were unable to participate. Patient demographic data were obtained, including age, gender, body mass index, and blood pressure.

Each subject underwent polysomnography (SomnoMedics type 2) according to well-established procedure. The monitoring included recording from surface leads for electroencephalography, bitemporal electro-oculography, submental and leg electromyography, and electrocardiography. Oxygen saturation and respiration was monitored by oronasal airflow and finger-pulse oximeter. Polysomnography recordings were scored for sleep, breathing, and oxygenation. We took the average number episodes of apnea and hypopnea per hour of sleep (apnea/hypopnea index) and the time during sleep spent with an oxygen saturation below 90%. OSA was diagnosed if the apnea-

hypopnea index (AHI) was more than 5 times in 1 hour of sleep.

Echocardiography was performed with an ultrasound system (General Electronics Ultrasound Vivid E95) using 2.5- and 3.5-mHz. Images were stored digitally for off-line analysis using EchoPAC software (General Electronics). Standard M-mode and 2-dimensional views were used. The following measurement were determined, such as peak early (E) and late (A) diastolic mitral annular velocity, the ratio of E and A velocities (E/A), and the deceleration time (DT). e' ≤ 8 was used as an indicator of left ventricular diastolic dysfunction and E/e' was calculated for the prediction of PCWP.[9]

Diastolic dysfunction was classified according to recent guidelines: 1) normal diastolic function (e' (lateral) >10 cm/s, e' (medial) >8 cm/s and LAVI <34 mL/m^2 ; 2) mild diastolic dysfunction (E/A <0.8, e' (lateral) <10 cm/s and e' (medial) <8 cm/s and; 3) diastolic dysfunction with elevated filling pressures (E/e' >13 cm/s or E/e' >9 and LAVI >34 mL/m^2).[9]

During end diastolic phase, left atrium contracts to empty the remaining early and mid-diastolic blood volumes that remain in the left atrium (LA-V_{ed} = LA-Volume end diastolic). There are amounts of blood transferred from the LA into the LV. When the afterload has reached the maximum load, there is no further increase in the dimension length (maximum isometric), so it can be assumed that the magnitude of *S* and the LA- V_{ed} is constant.[10]

The systolic left atrial pressure determined as depicted previously relates to strain in the left atrium during the pulmonary *S* wave. Essentially, diastolic left atrium pressure is the pressure during the pulmonary D wave.[10] Since pulmonary S and D waves have generally a similar duration, we estimated mean left atrial pressure as the average between systolic and diastolic left atrial pressure.[10]

At this end diastolic time, the LA space is also filled with blood volume which will be partially flowed at a smaller speed, namely V_A which is normally smaller than V_E. Because after this phase, there is an isometric contraction phase, where there is no change in the size of the magnitude again, the volume of the heart chambers do not change. Thus, the components for measuring LA pressure can be collected, namely: - geometric LA volume, so that the blood volume in LA can be calculated, so that the amount of blood mass that will press against the LA stereometric wall is a force (F = Force). - LA area dimensions can be calculated using stereometric mathematics. Blood flow or displacement from LA to LV, defined from LA midpoint to LV midpoint or modified from LA basal to mitral valve cross section, the distance or distance (d) can be determined.[11]

In the phase of ejection of blood into the Aorta is also due to LV contraction which is an electrical stimulation of the LV, seen from the ECG picture with QRS complex. Then begins the relaxation phase (diastole), in which LV pressure decreases and muscle tone decreases. Meanwhile the LA is filled with blood volume that has returned from oxygenation in the lungs, the pressure in the LA increases while the pressure in the LV decreases, so that the Mitral valve opens, massive LV filling occurs.[11] While filling the LV, there is a replenishment of blood to the LA. At the end of diastole, LA contraction occurs which is indicated by a P wave on the EKG and a wave on the LA pressure curve, and LV filling is seen which is described as an A wave which is a velocity so it is named VA. The final pressure in the LV is LVEDP (Left ventricular end diastolic pressure). This LVEDP pressure is equivalent to LA pressure and is also equivalent to PAWP (Pulmonary Artery Wedge Pressure).[12]

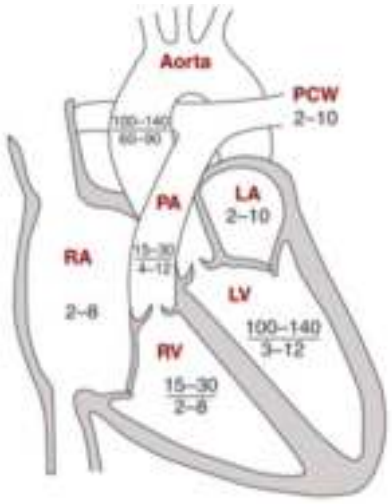

Figure 2. Normal Cardiac Pressure.[5]

Based on that condition, Pulmonary artery wedge pressure (PAWP) can be estimated using new formula that we proposed using calculation of the conversion of the value of V_A to the PAWP value (PC) equivalent to P_{LA} (pressure on the LA area). This pressure stereometrically caused by the suppression of the blood volume contained in the LA in the end diastolic phase. The final new simplified equation formula to calculate PAWP:

$$P_{LA} = 18{,}525 \times V_A^2 \text{ mmHg } (V_A \text{ on m/s})$$

In determining the geometry calculation, because the shape of the heart resembles the shape a tube and also the length of the outer curved line is approximately equal to the height, it will have an error factor value for the final number of the constant 18.525. To determine the value of this error factor, it is actually necessary to compare it with the Swan Ganz catheter examination, which is the gold standard method for determining PAWP. However, it is expensive, invasive, and there is also no medical indication for healthy people.

Amr Abbas et al, using subjects with cardiovascular and pulmonary disease, compared the results of Echo directly, namely TRV (Tricuspidal Regurgitas Velocity) with a right heart catheter and obtained PVR (Pulmonal Vascular Resistant) values.[13] The Amr Abbas Formula would be (sensitivity 77% dan specivicity 81%):

$$PVR = TRV/TVI_{RVOT} \times 10 + 0{,}16$$

Nagueh S et al, performed simultaneous examination of HF patients with Doppler echo and invasive.[10] The researcher used the components: e' and E velocity of the Mitral valve. The Nagueh formula is as follows:

$$PCWP = 1{,}24^* (E/ e') + 1{,}9$$

$$e' = (e'_{lat} + e'_{med}) /2.$$

The mean value of the early diastolic velocity of the mitral annulus is e'. E is the mitral inflow velocity in early diastolic. The Echo mode used to determine e' is TDI (Tissue Doppler Imaging), by measuring the velocity of blood flow in the myocardial tissue at the angles of the mitral annulus septal and lateral during the early diastolic phase. By using the ratio E/e', it is possible to determine the approximate size of the PCWP, the sensitivity and specificity are 66% and 50%. It is necessary to compare the PAWP results according to the new formula with the Nagueh formula as validation.

Statistical analyses were performed with SPSS version 25.0 for Windows. The baseline subject characteristics were expressed as mean and minimum-maximum values or frequency and percentages for categorical data. Normally distributed data were presented as mean and analyzed using Student's t test for comparisons of baseline characteristics and parameters between groups. Non-parametric data were analyzed with Mann-Whitney U test. Fisher's exact test was used for univariate analysis to look for association between various factors. A value of $p < 0.05$ was considered significant. Spearman's correlation analysis was used to assess the possible correlation between OSA and clinical data or echocardiographic variables.

To identify significant independent determinants of resting and dynamic LV diastolic function in OSA patients, their individual association with echocardiographic variables was assessed by multivariate analysis, using a bidirectional stepwise regression.

RESULTS

Of 82 patients in this study, 48 (58%) were male and 34 (41%) female, with mean age was ± 49 (40-51) years. Subjects in the OSA group were 65% male and 34% female with mean age ± 49 (42-52) years than those in the non-OSA group 31% male and 68% female with mean age ± 40 (30-49) years with p value < 0.01. The mean BMI measured in all study subjects was 32.60 ± 4.76 kg/m^2. Subject in the OSA Group had a BMI 32.87 ± 5.24, while those in the non-OSA group 32.42 ± 3.81 but BMI did not significantly different between the 2 groups (p=0.75).

The following variables were included into the analysis: age, gender, systolic and diastolic blood pressure, body mass index (BMI), neck circumference, mid upper arm circumference, waist circumference, fat percentage, muscle mass, visceral fat, Mallampati score, and laboratory parameter such as HbA1c and soluble ST-2 and standard echocardiographic measurements.

From all obese subjects included in this study, there were 66 subjects (80.5%) had obstructive sleep apnea, and 16 subjects (19.5%) did not have obstructive sleep apnea. The mean AHI was 9 to 47 events per hour in OSA group and 5 to 34 events per hour in all subjects. Baseline subject characteristics are presented in **Table 1**.

Fat percentage was found to be higher in the non-OSA group (37.95 (30.68-42.23)) than in the OSA group (30.70 (28.55-38.90)) and were considered significant with p value 0.04. While for muscle mass and visceral fat was found to be higher in the OSA group 28.30 (22.70-29.95) and 19.83 ± 6.33 respectively, although that two variable were not significant with p value 0.07 and 0.32.

There were 10 (15.2%) subjects with OSA have abnormal diastolic dysfunction, while all 16 subjects without OSA have normal diastolic function, without significant correlation among subjects with OSA and no OSA. According to the standard classification, all 10 subjects with OSA are considered to have diastolic dysfunction grade I. However, derived indices of diastolic dysfunction showed significant differences (p value = 0.01), with E/e' as a central parameter, increased with presence of OSA. Left atrial volume as a marker of left atrial size correlated significantly with OSA (p value = 0.01).

Table 1. Subject characteristics.

Variable	All n = 82	No OSA (AHI < 5) n = 16	OSA (AHI ≥ 5) n = 66	p value
Age (years)	49 (40-51)	40 (30-49)	49 (42-52)	< 0.01
Gender, Male	48 (58%)	5 (31%)	43 (65%)	
Gender, Female	34 (41%)	11 (68%)	23 (34%)	
Systolic Blood Pressure (mmHg)	140 (128-147)	132 (128-139)	141 (127-149)	0.05
Diastolic Blood Pressure (mmHg)	90 ± 11	87 ± 9	91 ± 11	0.18
Waist Circumference (cm)	105.03 ± 10.03	102.88 ± 11.47	106.02 ± 10.24	0.28
Mean Upper Arm Circumference (cm)	35.72 ± 3.65	35.41 ± 3.33	36.01 ± 3.96	0.58
Neck Circumference (cm)	40.00 (36.58-43.00)	38.50 (36.25-41.63)	41.00 (36.80-43.00)	0.32
BMI	32.60 ± 4.76	32.42 ± 3.81	32.87 ± 5.24	0.75
Fat Percentage (%)	31.90 (28.65-40.25)	37.95 (30.68-42.23)	30.70 (28.55-38.90)	0.04
Muscle Mass	27.45 (22.03-29.78)	23.00 (21.30-28.78)	28.30 (22.70-29.95)	0.07
Visceral Fat	19.39 ± 6.20	18.06 ± 5.84	19.83 ± 6.33	0.32
Mallampati Score	2 (1-3)	2 (1-2)	2 (1-3)	0.09
HbA1c (%)	5.80 (5.40-6.23)	5.55 (5.40-5.98)	5.80 (5.50-6.30)	0.13
sST2 (ng/mL)	13.12 (10.49-18.43)	12.28 (9.12-16.87)	13.13 (10.72-18.65)	0.39

Table 2. Polysomnographic variables.

Variable	All n = 82	No OSA (AHI < 5) n = 16	OSA (AHI ≥ 5) n = 66	p value
AHI (events/hour)	12 (5-34)	3 (2-3)	20 (9-47)	< 0.01
Lowest SaO2 (%)	81 (75-87)	87 (84-90)	79 (72-85)	< 0.01
ODI (%)	21 (11-42)	8 (6-11)	27 (17-58)	< 0.01
Arousal Index (events/hour)	42 (34-48)	43 (36-48)	41 (33-51)	0.75
Sleep Duration (minute)	300.73 ± 161.43	340.69 ± 116.92	286.77 ± 166.67	0.23

Table 3. Echocardiographic characteristics.

Variable	All n = 82	No OSA (AHI < 5) n = 16	OSA (AHI ≥ 5) n = 66	p value
Ejection Fraction (%)	71.34 ± 6.29	71.44 ± 5.60	71.09 ± 6.61	0.85
Diastolic Dysfunction	10 (12.2%)	0 (0%)	10 (15.2%)	0.20
TAPSE	2.40 (2.18-2.60)	2.50 (2.33-2.98)	2.40 (2.10-2.60)	0.07
E/A Ratio	1.04 (0.51-2.39)	1.28 (0.62-2.39)	0.98 (0.51-1.72)	0.04
E/e'	7.78 (0.47-14.85)	6.41 (0.53-9.42)	8.11 (0.47-14.85)	0.01
S'	13 (11-14)	13 (12-14)	12 (11-14)	0.96
Deceleration Time	193.17 (77-311)	176.50 (119-303)	197.21 (77-311)	0.03
LA Volume (ml/m^2)	23.04 (11.94-34.92)	20.47 (13.45-28.46)	23.67 (11.94-34.92)	0.01
Pulmonary Artery Wedge Pressure (mmHg)	10.14 (8.01-13.07)	8.07 (6.07-9.48)	10.70 (8.07-13.07)	< 0.01

**Using New PAWP Formula*

Of note, this study time measured in both groups also showed difference with p value 0.03. There was a significant difference in non-invasive measurement of PAWP between OSA group and non-OSA group with p value <0.01 and measured higher in the subjects with OSA (10.70 (8.07-13.07)) than in the non-OSA group (8.07 (6.07-9.48)). Echocardiographic measurements are outlined in **Table 3**.

The equation derived was then tested prospectively in obese population for the prediction of PAWP. Further analysis was performed to compare subjects with high PAWP and group with normal PAWP, in OSA group study. Of the 66 subjects with OSA, there were 23 subjects who had higher PAWP.

In subjects with OSA, there were significant differences in systolic blood pressure, HbA1c, and ejection fraction between subject group with abnormal PAWP and normal PAWP. Systolic blood pressure was found to be significantly higher in the OSA group with high PAWP values (146 (139-155)) compared to the OSA group with normal PAWP values (140 (122-145)). HbA1c values were also found to be significantly higher in the OSA group with high PAWP values (6.00 (5.50-7.43)) than in the OSA group with normal PAWP values (5.70 (5.40-6.05).

In addition, the OSA group with high PAWP values had a significantly higher ejection fraction value (74.22 ± 5.54) compared to the OSA group with normal PAWP values (69.42 ± 6.58).

Multiple stepwise logistic regression analysis was performed with variables, including age, fat percentage, AHI, ODI, lowest SaO2, and echocardiographic variables such as E/e', E/A, LA volume, deceleration time, and pulmonary artery wedge pressure calculated. This analysis showed that E/e' and deceleration time was the predictor of diastolic dysfunction.

Table 4. Multivariate analysis to identify predictors of diastolic dysfunction.

Variable	OR (95% CI)	Correlation Matrix (R^2)	p value
Age	0.967 (0.662-1.236)	-0.449	0.69
Fat Percentage (%)	1.000 (0.840-1.191)	-0.352	0.99
AHI	1.003 (0.906-1.112)	0.061	0.94
ODI	1.017 (0.909-1.137)	-0.259	0.77
Lowest SaO2	1.015 (0.904-1.139)	-0.554	0.80
E/e'	1.874 (1.052-3.340)	-0.169	0.03
E/A	0.001 (0.000-1.027)	-0.380	0.05
LA Volume (ml/m^2)	1.149 (0.879-1.502)	0.009	0.30
Deceleration Time	1.032 (1.005-1.059)	-0.393	0.02
Pulmonary Artery Wedge Pressure	0.904 (0.662-1.236)	-0.060	0.52

DISCUSSION

The mean LA pressure is the source pressure for LV filling, determining the LV filling pressure is a key element in the diagnosis and management of patients with suspected decompensated heart failure. Measurement of the pulmonary capillary wedge pressure with the Swan-Ganz catheter has become the gold standard for determining LV filling pressure. This invasive procedure is more expensive and produce complications, especially in critically ill patients. Two randomized clinical studies found no benefit from the use of the Swan-Ganz catheter to manage critically ill patients. Thus, A reliable non-invasive method for determining LV filling pressure is needed.[9]

Although right heart catheterization remains the gold standard for measurement of intracardiac pressures, enthusiasm for placement of Swan–Ganz catheters has dwindled over the past decade on account of complications that include infection, cardiac perforation, and tamponade. In addition, the use of Swan-Ganz Cathters is commonly used in the sick population so that it requires measuring the use of a catheter.[14]

In healthy populations, measurements can be performed noninvasively using Doppler echocardiography in an outpatient setting that is more cost-effective. Doppler echocardiography is generally acknowledged to be a noninvasive alternative to Swan–Ganz catheterization. Hitherto, a number of noninvasive Doppler echo measurements of left ventricular (LV) filling pressure, right atrial pressure, and cardiac output and the changes following load interventions have correlated closely with measurements made by Swan–Ganz catheterization.[13]

Pulmonary vascular resistance (PVR) is a hemodynamic variable that contributes to the management of patients with advanced cardiovascular and pulmonary conditions. Based on the formula issued by Abbas et.al , doppler echocardiography may provide a reliable, non-invasive method to determine PVR using mean pulmonary artery pressure and pulmonary artery wedge pressure (PAWP) or cardiac output. The weakness of this formula is there must be a pulmonary artery systolic pressure (PASP), pulmonary artery diastolic pressure (PADP) and mean pulmonary artery pressure (MPAP) value. However, the formula is directly related to transpulmonary pressure gradient (Δp) and inversely related to transpulmonary flow (Qp).[13]

The term PAWP is used interchangeably with pulmonary capillary wedge pressure and pulmonary artery occlusion pressure in the general literature.[10] Non-invasive measurement of PAWP is useful for the evaluation intravascular fluid volume and pressure status to identify diastolc dysfunction. Nagueh formula can be used for calculation of PAWP from the Doppler derived mitral E/e' ratio. PAWP is usually equal to the left atrial pressure and hence the left ventricular filling pressure. E' (Ea) has been considered as a preload independent index of left ventricular relaxation. Nagueh formula uses mitral E velocity during early diastolic flow corrected for the influence of left ventricular relaxation (E/e' ratio) to estimate the mean PAWP. Nagueh formula: PAWP = 1.24 [E/e'] + 1.9. Ea was taken from the lateral mitral annulus in the pioneering study of Nagueh SF et al. patients had invasive measurement of PAWP and simultaneous Doppler echocardiography.[9]

In addition, the formula proposed by Vladislav et.al also plays a role in determining PAWP using echocardiography. In his research tricuspid regurgitation velocity (TRV), average E/e′ ratio, LV ejection fraction (LVEF), RV fractional area change (RVFAC), IVC diameter, and left atrial volume index (LAVi) were found to be independent predictors of PAWP ratio without any evidence of multicollinearity between variables. The model accurately identified patients with precapillary, isolated postcapillary, and combined PH, with no cases of undetermination and outperforming current echocardiographic algorithms, by using variables routinely acquired in echocardiographic laboratories.[15]

This present study compared PAWP with the value predicted using standard tissue Doppler measurement method validated by Nagueh et al.[9] and it is found that PAWP derived using these 2 methods has fair correlations, suggesting that this method will give similar results to the tissue Doppler method in most examples. Diastolic dysfunction significantly associated with pulmonary artery wedge pressure

measured using proposed formula (R = 0.240, p value = 0.030). These results emphasize the pathophysiologic process of passive pulmonary hypertension; namely, that increased left atrial pressure will necessitate a higher driving pressure across the pulmonary capillary bed.[11]

At present, echocardiography is the only non-invasive technique that allows estimation of pulmonary and LV filling pressures in HF).

Precisely defining the role of OSA in the origin of some cardiovascular complications has been difficult for several reasons. One limitation

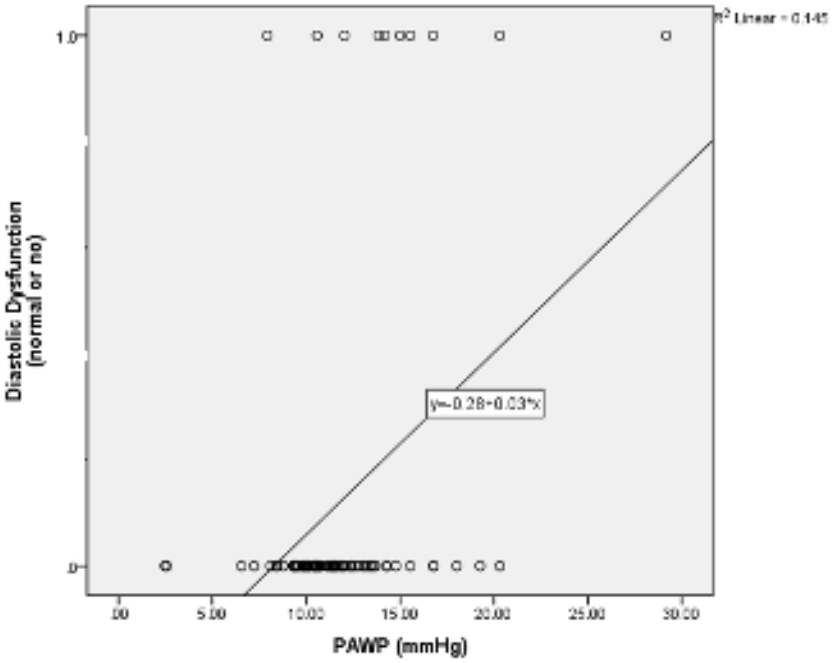

Figure 3. Spearman Correlation between PAWP and Diastolic Dysfunction

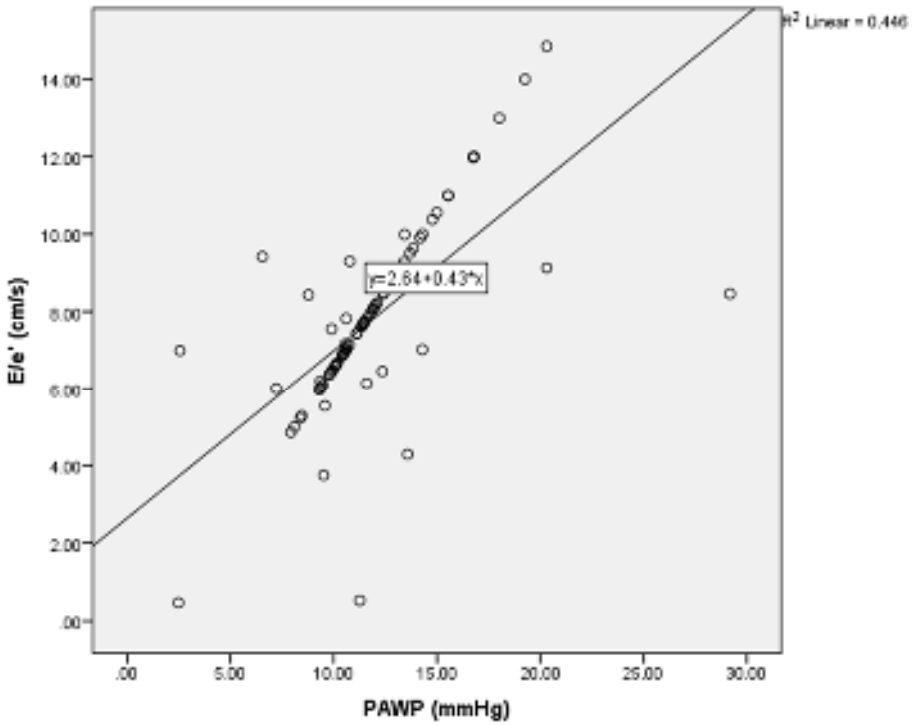

Figure 4. Pearson Correlation between PAWP and E/e'

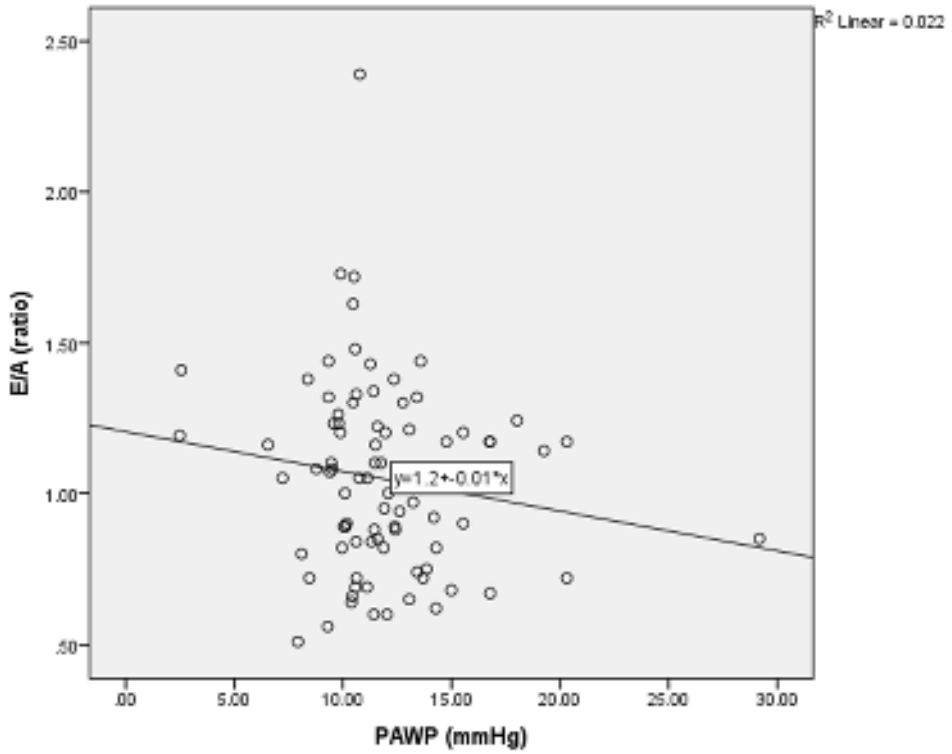

Figure 5. Pearson Correlation between PAWP and E/A

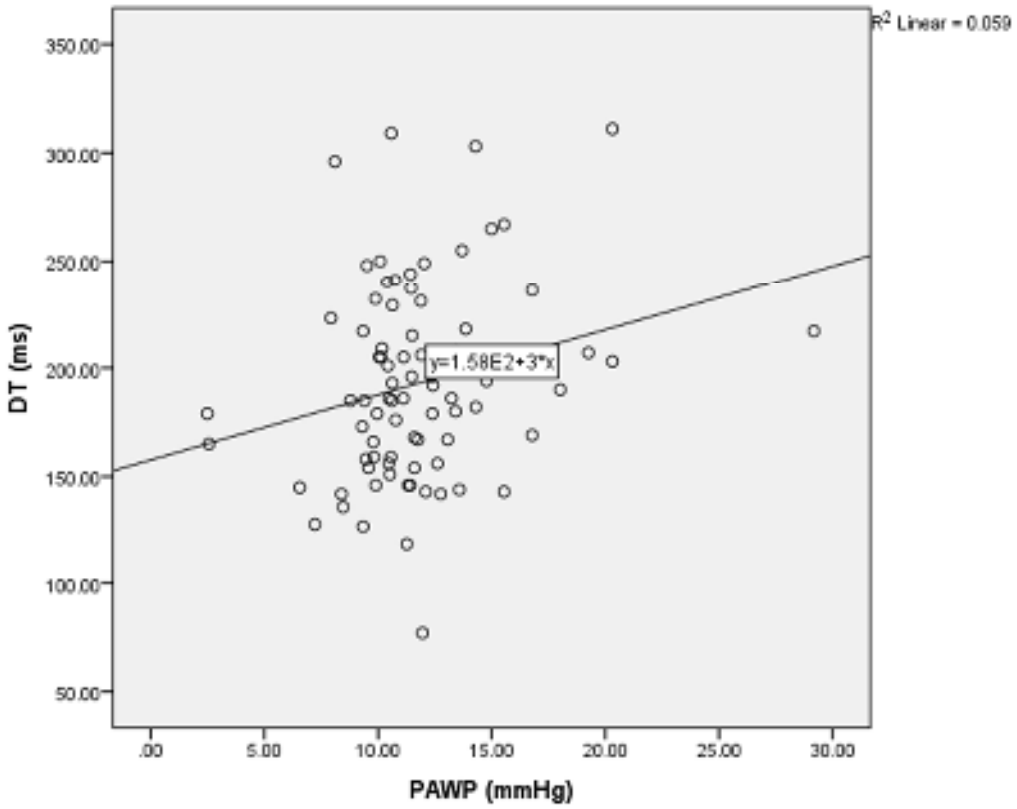

Figure 6. Pearson Correlation between PAWP and DT

Table 5. Comparison between The New PAWP Formula, Nagueh Formula, and Abbas Formula [16]

	The New PAWP Formula	Nagueh et al. Formula	Abbas et al. Formula
Formula	P_{LA} = 18,525 x V_A^2 mmHg (V_A^2 on m/s)	PCWP = 1,24* (E/ e') + 1,9. e' = (e'$_{lat}$ + e'$_{med}$) /2.	PVR= TRV/TVI$_{RVOT}$ x10 + 0,16
Research Population	Obese with Obstructive Sleep Apnea patients	Heart failure patients	Cardiovascular and pulmonary disease patients
Strengths	This formula has more complete parameters and does not require tricuspid regurgitation to be used	This formula does not require tricuspid regurgitation to be used	This formula had been validated by the Swan-Ganz catheter
Weaknesses	This formula had not been validated by the Swan-Ganz catheter	Other echocardiographic and even invasive measurements should be used to supplement the E/e' parameter in some cases.	This formula requires a tricuspid regurgitation condition on patients

has been the various methods by which the diagnosis of pulmonary hypertension is made in studies of subjects with OSA, many by way of Doppler echocardiography, with varying pulmonary artery pressure thresholds.

This study used echocardiographic procedures to assess the impact of OSA on cardiovascular function and structural without symptoms of heart failure. Subjects with OSA jshowed a decreased in E/A ratio, higher LA volume and deceleration time compared with subjects with no OSA. All subjects with OSA manifest diastolic dysfunction, while none of the subjects without OSA have abnormal diastolic function. However, this difference was not statistically significant between the two groups. Various mechanisms might explain the presence of diastolic dysfunction in OSA patients. Patients who experience chronic hypoxemia, which might result in abnormalities of myocardial relaxation because of myocyte hypoxia due to intracellular calcium transport disturbances.[16]

The results of this present study suggest that subjects with OSA have changes in pulmonary hemodynamics. Obstructive sleep apnea can be assimilated to a Müller's maneuver, which is an inspiratory effort against a closed glottis, and the PAWP reflects the changes in intrathoracic pressure; the latter may decrease by as much as 30 mmHg during an OSA, with a subsequent fall of PAWP which decreases to negative values.[17]

These results are in accordance with the research conducted by Solin et al. which analysed sleep-disordered breathing with hemodynamic parameters. From this study, the group with sleep disorders had higher PCWP and pulmonary artery pressure (PAP) values compared to the control group or the group without sleep disorders.[18] Other studies have also shown that PAWP experienced significant changes during the cycle in patients with OSA.

It has been shown that OSA patients might have an increased in left ventricular mass, and alterations of the neurohumoral system might contribute to the elevation of pulmonary artery pressures and PCWP in patients with OSA.

Diastolic dysfunction is a condition with increased resistance to filling of the left ventricle, leading to an inappropriate rise in the diastolic pressure-volume relationship and causing symptoms of pulmonary congestion during exercise. The potential mechanisms leading to changes in cardiac structure and function in patients with OSA have been studied in animal models. Fletcher et al demonstrated ventricular hypertrophy in rats exposed to short bursts of repetitive hypoxia over an extended period and that intermittent severe hypoxia can lead to a sustained rise in BP within 35 days.[4] Diastolic dysfunction leads to elevated left atrial filling pressures which are transmitted to the pulmonary venous system. Long standing elevation in pulmonary venous pressures leads to secondary changes in pulmonary vascular resistance.

CONCLUSION

The pulmonary artery wedge pressure (PAWP) is a surrogate marker of left atrial pressure and chronic airflow obstruction is the most important cause of pulmonary artery remodelling, that

can lead to diastolic dysfunction. This study has proved echocardiography can be replaced invasive cardiac catheterization to assess LV filling with high feasibility and good accuracy to estimate LV filling pressure that leads to diastolic dysfunction.

The new mathematical formula based on echocardiographic variables had a good accuracy because it can estimated indirectly the LA pressure and PAWP which can described the stage of diastolic dysfunction and it could be readily applied in daily clinical practice.

Obstructive sleep apnea is one of the health problems that may lead to cardiovascular complications. This study showed that patients with OSA have a higher risk of cardiovascular remodeling than those without. Obscure cardiac comorbidities may be present in patients with significant OSA. It is highly recommended for patients with OSA to have routine echocardiogram, as development of cardiovascular morbidities is common and proper treatment should not be delayed.

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The Comparison of Monocyte Human Leukocyte Antigen-D-Related (mHLA-DR) Expression Levels Between Corona Virus Disease 2019 (COVID-19) Patients and Healthy Subjects

Musholli Himmatun Nabilah[1,2], Puspa Wardhani[3,4,5], Aryati[3,4], Yetti Hernaningsih[3], Bambang Pujo Semedi[6]

[1]Laboratory Medicine Study Interest, Master Program of Basic Medical Science, Faculty of Medicine Universitas Airlangga, Surabaya, Indonesia.
[2]Department of Medical Laboratory Technology, Health Polytechnique, Ministry of Health Republic Indonesia, Surabaya, Indonesia.
[3]Department of Clinical Pathology, Faculty of Medicine Universitas Airlangga - Dr. Soetomo General Academic Teaching Hospital, Surabaya, Indonesia.
[4]Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia.
[5]Postgraduate School of Universitas Airlangga, Surabaya, Indonesia.
[6]Department of Anesthesiology and Reanimation, Faculty of Medicine Universitas Airlangga - Dr. Soetomo General Academic Teaching Hospital, Surabaya, Indonesia.

Corresponding Author:
Puspa Wardhani, MD. Department of Clinichal Pathology, Faculty of Medicine Universitas Airlangga. Jl. Mayjen. Prof. Dr. Moestopo no. 47, Surabaya 60131, Indonesia. Email: puspa-w-2@fk.unair.ac.id.

ABSTRACT

***Background**: SARS-CoV-2 can trigger a dysfunctional immune response in COVID-19 patients and lead to immunosuppression. HLA-DR molecule expressed on the surface of monocytes, known as mHLA-DR, has been widely used as a reliable marker of immunosuppression. Downregulation of mHLA-DR reflects an immunosuppressed state. This study aimed to compare the expression level of mHLA-DR between COVID-19 patients and healthy subjects concerning immune system dysregulation that can be triggered by SARS-CoV-2 and lead to immunosuppression. **Methods**: This was an analytic observational study with a cross-sectional design that measured the mHLA-DR expression in EDTA blood samples from 34 COVID-19 patients and 15 healthy subjects using the BD FACSLyric™ Flow Cytometry System. The mHLA-DR examination results were expressed in AB/C (antibodies bound per cell) that were quantified using a standard curve constructed with Quantibrite phycoerythrin beads (BD Biosciences). **Results**: Expression of mHLA-DR in COVID-19 patients (n = 34) were 21,201 [2,646-92,384] AB/C, with 40,543.5 [9,797-92,384] AB/C mild cases (n = 22), 21,201 [9,831-31,930] AB/C moderate cases (n = 6), and 7,496 [2,646-13,674] AB/C severe to critical cases (n= 6). Expression of mHLA-DR in healthy subjects (n = 15) was 43,161 [25,147-89,846] AB/C. Based on the Mann-Whitney U test, the mHLA-DR expression in COVID-19 patients significantly differed from the mHLA-DR expression in healthy subjects (p = 0.010). **Conclusion**: The level of mHLA-DR expression in COVID-19 patients was lower and significantly different from healthy subjects. Moreover, immunosuppression could be indicated by the decrease of mHLA-DR expression, which was below the reference range found in severe to critically ill COVID-19 patients.*

***Keywords**: COVID-19, Monocyte Human Leukocyte Antigen-D-Related (mHLA-DR), Immunosuppression.*

INTRODUCTION

Coronavirus disease (COVID-19) is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Clinical manifestations in COVID-19 patients range from asymptomatic, mild, moderate, and severe to critical symptoms.[1] Viral and host factors play a role in SARS-CoV-2 infection. The cytopathic effect of the virus and its ability to defeat immune response determine the severity of infection in COVID-19 patients.[2] Several studies have shown that infection with SARS-CoV-2 could trigger immune dysregulation and immunosuppression that is associated with disease severity in COVID-19 patients.[3-5] Some dysregulation of immune response in COVID-19 patients can be triggered by excessive production of proinflammatory cytokines.[3] Excessive production of these proinflammatory cytokines lead to impaired antigen presentation by inhibiting HLA-DR molecule expression.[6] The HLA-DR molecule is a transmembrane glycoprotein that plays a role in antigen presentation and T-cell activation.[5] The stage of antigen presentation to lymphocytes is critical for activating an adaptive immune response and a sustained immune response to clear pathogens.[7] Zmijewski & Pittet (2020) mentioned that HLA-DR was a class II human leukocyte antigen (HLA) expressed on the surface of antigen-presenting cells (APCs), such as monocytes, differentiated macrophages, dendritic cells, and B lymphocyte cells.[8]

The mHLA-DR is an HLA-DR expressed on the surface of monocytes. Monocytes derived from myeloid bone marrow precursors are considered as the key immune cell that takes part in the infection. Furthermore, it is a first-line cellular response that initiates and promotes an adaptive immune response.[9] The mHLA-DR expressed on $CD14^+$ monocytes plays an important role in synapsing innate immunity with adaptive immunity in infectious diseases.[10]

Downregulation of mHLA-DR expression on $CD14^+$ monocytes represents a state of immunosuppression, also refers to an injury-associated immunosuppression.[9] As in the sepsis case, the expression of HLA-DR in monocytes is expected to be a reliable marker for evaluating immune dysfunction in COVID-19 patients.[11,9] Recent studies confirmed that immune dysregulation and immunosuppression in COVID-19 patients with respiratory failure were associated significantly with downregulation of mHLA-DR.[3]

Monitoring immune dysfunction in COVID-19 patients is still not a complete concern because it is generally not monitored in clinical routines during patient care, especially in Indonesia. Understanding the role of immune cells (mHLA-DR) in the various clinical symptoms of COVID-19 is critical to develop the effective treatment strategies. Accordingly, data regarding the measurement of mHLA-DR expression in COVID-19 in Indonesia needs to be made available. Studies on the measurement of mHLA-DR expression in the COVID-19 population in Indonesia and healthy subjects as a comparison have never been carried out until now. From measurements in healthy subjects, normal values of mHLA-DR expression can be obtained in the population of Indonesia. This study uses the AB/C (antibodies bound per cell) unit of measurement as the result of mHLA-DR expression using the flow cytometry method, in which AB/C describes the quantitative amount of the targeted molecule through its binding to specific fluorescently labeled antibodies.[13] The unit is different from the MFI (*mean fluorescent intensity*) which is often used in the measurement results of flow cytometry method, which can only describe the fluorescence intensity of the targeted cell or molecule. This study aimed to analyze the level of mHLA-DR expression in COVID-19 patients concerning the incidence of immunosuppression by comparing the mHLA-DR expression in COVID-19 patients with healthy subjects.

METHODS

Study Design, Participant, and Data Collection

This cross-sectional study analyzed the differences of mHLA-DR expression in COVID-19 patients and healthy subjects. This study was conducted from June to September 2022 at Dr. Soetomo General Hospital, Surabaya, Indonesia. Inclusion criteria included COVID-19 patients (aged >18 years), confirmed by nucleic acid amplification test (NAAT) from nasopharyngeal swab specimens using real-time PCR (RT-PCR)

and was hospitalized, also healthy subjects (not having an acute illness or comorbidities) aged >18 years who have been confirmed negative through a rapid diagnostic test for SARS-CoV-2 antigen. We excluded COVID-19 patients who receive immunosuppressive drugs, human immunodeficiency virus (HIV) patients or other diseases related to immune system disorders, patients with bacterial sepsis, or critical patients who were not accompanied by COVID-19 pneumonia with ARDS despite showing positive PCR results. The Hematology laboratory examination of the research subjects was evaluated on the blood specimens collected simultaneously for measurement of mHLA-DR expression.

Ethics

This study received approval from the Health Research Ethics Committee of Dr. Soetomo General Academic Teaching Hospital, Surabaya, Indonesia (reference no. 0382/KEPK/III/2022, on March 8, 2022).

Sample Size and Sampling Technique

The method used in collecting samples in this study was consecutive sampling. The sample estimate was calculated using the "compare two means" formula, with $\alpha = 0.05$, $\beta = 0.2$, and ratio groups 1 and 2 (r) = 1. The minimum number of samples required for each group was 13.

The Procedure of mHLA-DR Measurement with Flow Cytometry

The instrument used for measuring mHLA-DR was the BD FACSLyric™ Flow Cytometry. The reagents needed in this measurement were BD Quantibrite™ HLA-DR PE/Monocyte PerCP-Cy5.5 (catalog numb: 340827), BD Anti-Human CD45 FITC (catalog numb: 347463), BD Quantibrite™ Beads PE (catalog numb: 340495), BD FACS Lysing Solution (catalog numb: 349202), and BD FACSFlow™ (catalog numb: 342003). The compensation was done before acquiring the samples using BD CS&T beads (Lot ID: 2031932). The instrument acquisition was set at 10.000 events at high speed.

Sample Preparation

We collected blood samples from COVID-19 patients and healthy subjects with 3 mL volume in EDTA tubes. The blood was stored at 4-8 ^{0}C and immediately processed within 4 hours of withdrawal.[12] Before measuring with flow cytometry, whole blood EDTA (50 µl) was added into a falcon test tube, then stained with the addition of BD Quantibrite HLA-DR PE/Monocyte PerCP-Cy5.5 (20 µl) and Anti-Human CD45 FITC (20 µl). The mixtures were incubated in a dark chamber at room temperature for 25 minutes. The sample was lysed with the addition of BD FACS Lysing Solution (450 µl), homogenized by vortex, and incubated at room temperature in a dark chamber for 10 minutes. Next, Centrifugation at 500 g (0.5 rcf) for 5 minutes after the incubation process was completed before discarding the supernatant. It was then rinsed with 1 mL BD FACSFlow™, homogenized with a vortex and centrifugation again at 500 g (0.5 rcf). After centrifugation, the supernatant was discarded, and BD FACSFlow™ (400 µl) was added, which then was homogenized with a vortex. The sample was ready to run on BDFACSLyric™ Flow Cytometry.

Beads PE Measurement to Calculate AB/C

The number of antibodies bound per cell (AB/C) was quantified by calibration with a standard curve, determined with BD Quantibrite™ Beads PE (Phycoerythrin). One tube of BD Quantibrite™ Beads PE was removed from the foil punch just before they were used. Then it was reconstituted with 0.5 mL BD FACSFlow™, homogenized with vortex, and run on BD FACSLyric™ Flow Cytometry. Each BD Quantibrite™ Beads PE tube contained lyophilized pelletized beads conjugated with four phycoerythrin (PE) grades. Standard curves and linear regression equations were made from Log10 of PE molecules per bead (Low, Med-Low, Med- High, and High) from insert kit (x) against Log10 of PE geo means of 4 populations of PE beads results from running with flow cytometry (y).[13]

mHLA-DR Expression Measurement

The same instrument was used to measure mHLA-DR in the prepared sample. Monocytes were first gated out from other leukocytes expressing CD45 (detected with BD Anti-Human CD45 FITC) based on their CD14 expression (detected with anti-CD14 conjugated with PerCP-Cy5.5 in BD Quantibrite™ HLA-DR

PE/Monocyte PerCP-Cy5.5, anti-CD14 PerCP-Cy5.5 could detect all monocytes [CD14 brightly positive and weakly positive]). The mHLA-DR expression was then measured on their surface (detected by anti-HLA-DR conjugated with PE (phycoerythrin) in BD Quantibrite HLA-DR PE/Monocyte PerCP-Cy5.5) as the median fluorescence intensity (MFI) which was associated with the entire population of monocytes.[12,14,15] A linear regression line equation was used to quantify AB/C (figure 1). Log10 of geo means of the sample measurement results were entered in the linear regression line equation as the "y" value. The equation was solved to find the "x" value as log AB/C, and then the antilog of "x" was determined to get the number of AB/C.[13] The normal values of mHLA-DR expression were >15,000 AB/C.[12]

Statistical Methods

The data obtained in this study were presented in tables and graphs. Data were analyzed by univariate and bivariate analysis using SPSS software (IBM Statistical Package for Social Sciences, version 26.0, Chicago, Illinois). In bivariate analysis, the data obtained were tested for normality with the Shapiro-Wilk test, and then tested for homogeneity with Lavene's test. We used independent T-test to find out whether there was a difference in the data on COVID-19 patients with the healthy group, if the data were normally distributed. In contrast, the Mann- Whitney U was used if the data were not normally distributed. In categorical data, the Chi-Square test was used to see whether there were differences between groups. We considered difference between groups to be statistically significant if p value < 0.05. We presented normally distributed data in the form of mean ± SD, whereas skewed data were described with median and range as a median with the minimum-maximum value (median [min-max]).

RESULTS

Characteristics and Laboratory Data of Research Subject

This study involved 49 subjects consisting of 34 COVID-19 patients and 15 healthy subjects. The characteristics and laboratory data of COVID-19 patients and healthy subjects are presented in **Table 1**.

Table 1. Characteristics and Laboratory Data of Research Subjects.

Characteristics		Research subjects		p-value
		COVID-19	Healthy	
	n	34	15	
Gender n (%)	Male	20 (58.8)	8 (53.34)	0.633[a]
	Female	16 (41.2)	7 (46.66)	
Age (years) n (%)	18-30	2 (5.89)	6 (40)	<0.001*[b]
	31-40	2 (5.89)	3 (20)	
	41-50	4 (11.76)	4 (26.67)	
	51-60	8 (23.53)	2 (13.33)	
	61-70	7 (20.59)	-	
	71-80	5 (14.70)	-	
	>80	6 (17.64)	-	
	Mean ± SD	62.12 ± 18.35	36.67 ± 9.75	
Conditions n (%)	With Comorbidities	25 (73.53)	-	<0.001*[a]
	Diabetes	9 (26.47)	-	
	Hypertension	7 (20.58)	-	
	Kidney disease	9 (26.47)	-	
	Pulmonary disease	5 (14.7)	-	
	Malignancy	6 (17.64)	-	
	Without Comorbidities	9 (26.47)	15 (100%)	
Laboratory Data (Median [min-max])	Total leukocyte counts (10^3/uL)[#]	9.02 [3.39-26.05]	7.89 [5.4-14.1]	0.288[c]
	Monocytes (%)[#]	7.4 [1.3-15.3]	6.1 [4-12]	0.079[c]
	Lymphocytes (%)[#]	12.95 [1.2-65]	28.3 [9-43]	<0.001*[c]
	Neutrophils (%)[#]	71.1 [4.8-89.4]	60 [48-82]	0.008*[c]

Notes: The results were expressed in mean ± SD, median [min-max], or n (%). Data analysis using Chi-square test (a), Independent T-test (b), and Mann-Whitney U test (c). Significant p- value <0.05. Data is significantly different*. Reference values of total leukocytes: 3.37 – 10.0[#]; monocytes: 4.3 – 10.10[#]; lymphocytes: 23.1 – 49.9[#]; and neutrophils: 39.80 – 70.50[#].

The Results of mHLA-DR Expression Measurement with Flow Cytometry

Based on the normality test using the Shapiro-Wilk test, the data obtained from the mHLA-DR expression in the two study groups were not normally distributed. Hence, the data were presented as median [min-max] and *p*-value was obtained using Mann Whitney U. COVID- 19 patients as the subject of this research consisted of 22 people with mild clinical manifestations (64.7%), six people with moderate clinical manifestations (17.65%), and six people with severe- clinical manifestations (17.65%). In this study, the mHLA-DR expression in COVID-19 patients was 21,201 [2,646-92,384] AB/C, mild clinical manifestations were 40,543.5 [9,797-92,384] AB/C, moderate clinical manifestations were 21,201 [9,831-3,930] AB/C, and severe-critical clinical manifestations were 7,496 [2,646-13,674] AB/C. Healthy subjects in this study consisted of 15 people with 43,161 [25,147-89,846] AB/C mHLA-DR expression. Expression of mHLA- DR in COVID-19 patients was lower and significantly different from the healthy subjects (p = 0.010) (**Figure 2**). The gating strategy of FACSLyric™ Flow Cytometry in measuring the mHLA DR expression of COVID-19 patients with mild, moderate, and severe-critical clinical manifestations, also healthy subjects, can be seen in **Figure 3**.

Table 2. The results of Log PE molecule/beads on BD Quantibrite™ Beads PE insert kit (catalog numb: 340495) and PE geometric means of four populations of PE beads runned by flow cytometry.

No	Beads Population	Log PE/beads (x)	Log PE geo means (y)
1	Low	2.675778342	2.681241237
2	Med-Low	3.729083757	3.726156466
3	Med-High	4.377360899	4.362199639
4	High	4.794738931	4.801328234

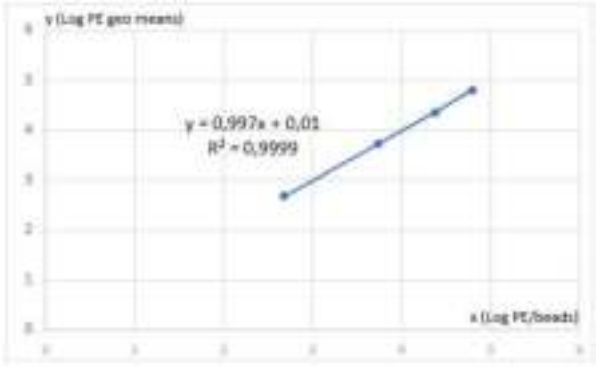

Figure 1. Standard curve and linear regression equation of Log PE/beads against Log PE geo means

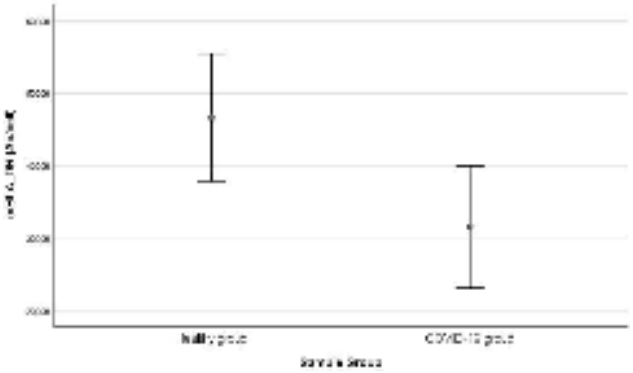

Figure 2. Differences in mHLA-DR expression between the COVID-19 group and thehealthy group

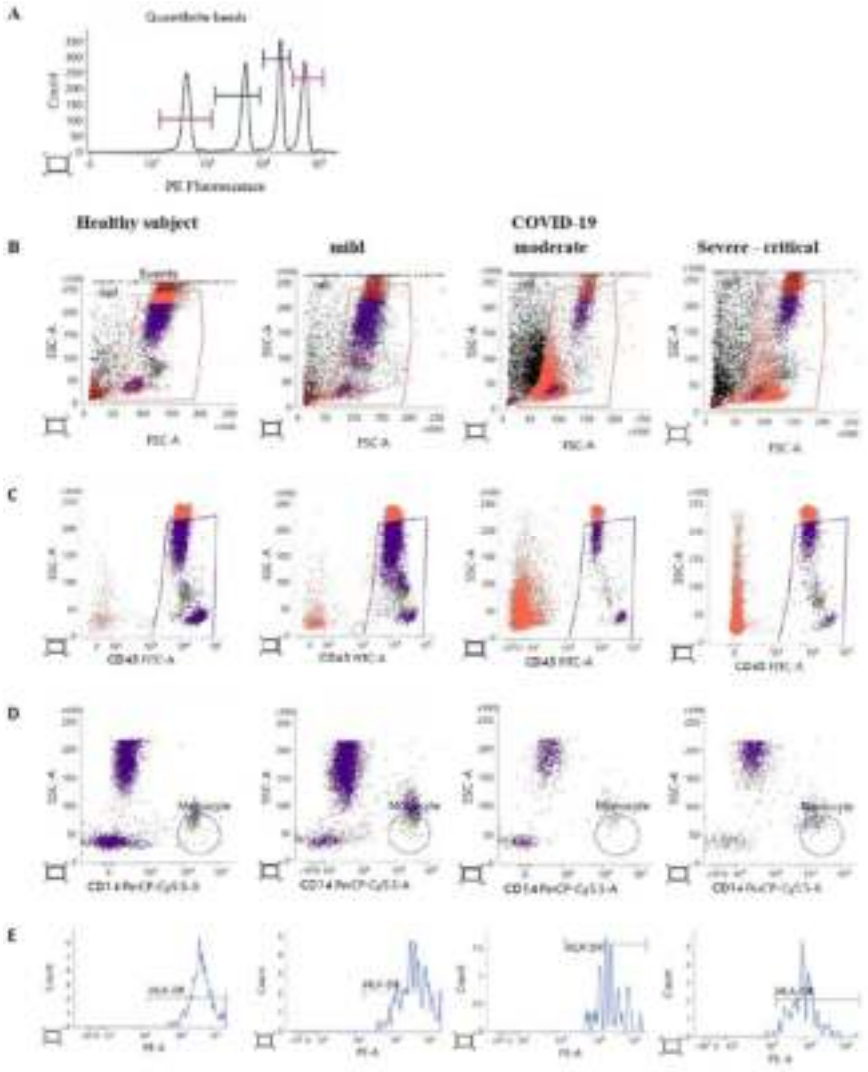

Figure 3. Gating strategy in FACSLyric™ Flow Cytometry on the analysis of mHLA-DR expression of COVID-19 patients with mild, moderate, severe-critical clinical manifestation, and healthy subjects. Beads were gated based on their SSC and FSC characteristics, and PE Fluorescence was plotted (A). Scatter graph of SSC and FSC (B). Patients' leukocytes were gated based on their binding to CD45 FITC-A and the characteristics of SSC and FSC on the scatter graph of SSC to CD45 (C). The patient's monocytes were gated based on their binding to CD14 PerCP-Cy5.5-A and the characteristics of SSC and FSC on the scatter graph of SSC against CD14 (D). The mHLA-DR expression was calculated based on the fluorescence of Anti- HLA-DR PE on monocytes (E). CD45 indicates a cluster of differentiation 45; CD14 indicates a cluster of differentiation 14; PE, phycoerythrin; COVID-19, coronavirus disease 2019; FSC, forward scatter; SSC, side scatter; HLA-DR, human leukocyte antigen-DR; mHLA-DR, monocyte human leukocyte antigen-DR.

DISCUSSION

In this study, the results of measuring the expressions of mHLA-DR in COVID-19 patients and the healthy groups showed a significant difference (p = 0.010), in which the expression of mHLA-DR in COVID-19 patients (21,201 [2,646-92,384] AB/C) were lower than in the healthy groups (43,161 [25,147-89,846] AB/C). This was in line with another study by Bonnet et al. (2021) which stated that the HLA-DR expressed in monocytes was significantly lower in the group of COVID-19 patients, in which the mild case (21,566 AB/C) and severe case (5,926 AB/C) were lower than the healthy subjects (44,544 AB/C)[12]. The expression of mHLA- DR in COVID-19 patients in this study, whose most proportion was the mild case (64.7%), was still at the reference values (>15,000 AB/C). It indicated that the overall mean of immune response in COVID-19 patients was normal and did not lead

to low mHLA-DR expressions in association with immunosuppression. The low expression of mHLA-DR in COVID-19 patients compared to healthy groups could be due to the release of various proinflammatory cytokines, some of which could trigger the low expression of HLA-DR molecules on monocytes through various signal transduction mechanisms.[3,6] Viral load could influence the immune response, including the number of proinflammatory cytokines released.

On the other hand, although the difference could not be seen statistically due to the proportion of the number between groups that did not meet the statistical requirements, the expressions of mHLA-DR in COVID-19 patients with the severe-critical case were lower than in the moderate case. The expressions of mHLA-DR in COVID-19 patients with mild cases were lower than in the healthy groups. Expressions of mHLA-DR in patients with severe-critical clinical manifestation showed the results below the reference values (7,496 [2,646-13,674] AB/C). This was in line with other studies by Spinetti et al. (2020), which stated that the mHLA- DR (AB/C) expression of COVID-19 patients treated in the ICU (severe to the critically ill patients) was significantly lower and below the reference values (9,280 AB/C), compared to COVID-19 patients that were not treated in the ICU (30,900 AB/C).[9] The expression of mHLA-DR which was below the reference values indicated a dysfunctional immune response and led to an immunosuppressed state.[9] SARS-CoV-2 infection, in addition to activating the immune response against the virus, could also cause immune system disorders in severe cases, such as hyperinflammation characterized by excessive release of proinflammatory cytokines resulting in a cytokine storm.[16] One factor that triggered hyperinflammation in COVID-19 patients was the excessive release of the proinflammatory cytokine IL-6.[17] This excessive release of IL-6 could further trigger the low expression of mHLA-DR through signal transduction mechanisms in the STAT3 (signal transducer and activator of transcription 3) signaling pathway.[3,12] Another study conducted by Giamarellos-Bourboulis et al. (2020) reported that high levels of IL-6 were negatively correlated with the levels of mHLA-DR expression in circulating CD14 monocytes.[3] Neutralizing IL-6 via tocilizumab, which could restore HLA-DR expression in monocytes, also supported this hypothesis.[3]

Decreased expression of mHLA-DR in CD14 monocytes indicated a decrease in antigen presentation capacity that caused impaired activation of $CD4^+$ T cells.[5] Decreased expression of HLA-DR led to increased surface expression of negative co-stimulator molecules such asprogrammed death 1 (PD-1), cytotoxic T-lymphocyte antigen 4 (CTLA-4), and B-and T- lymphocyte attenuator (BTLA), and their corresponding ligands, such as PD-1 ligand (PD-L1).[8] Increased surface expression of these negative co-stimulator molecules could disrupt innate and adaptive immune responses, such as impaired activation and induction of apoptosis in CD4 T cells that led to immune system dysregulation. Moreover, the presence or absence of immune system dysregulation in patients with COVID-19 also affected or related to the degree of disease severity.[8] Lymphopenia also occurred along with SARS-CoV-2 infection.[18] Decreased expression of mHLA-DR and lymphopenia are some indications of immunosuppressed status.[19] In this study, the results of lymphocyte measurements in COVID-19 patients showed a lymphopenia state and a significant difference with healthy subjects ($p = 0.002$). Lymphopenia in COVID-19 could be caused by several mechanisms, including increased levels of proinflammatory cytokines, which could cause a reduction in the lymphocyte population as the disease progresses[16]. SARS-CoV-2 could directly infect T lymphocyte cells through the ACE2 receptor, which was also expressed in T lymphocyte cells[16]. A damage to lymphatic organs by SARS-CoV-2 infection and an increase in lactic acid, especially in severe degrees, could also inhibit lymphocyte proliferation.[16]

CONCLUSION

The expression levels of mHLA-DR in COVID-19 patients were lower and showed a significant difference compared to the healthy groups. Although a significant difference could not be seen due to the limitation of subjects in this

study, immunosuppression could be indicated by the decrease in mHLA-DR expression below the reference value in severe to critical COVID-19 patients. The limitation of this study was that the clinical degrees of the COVID-19 patients involved were not proportionally (according to the number of statistics) collected due to limited samples collected. It was because the samples were collected when COVID-19 cases were declining in Indonesia. In future research, to see statistical differences in mHLA-DR expression between clinical manifestation groups in COVID-19 and the relationship between mHLA-DR expression and disease severity in COVID-19, it is suggested to group the COVID-19 samples based on their clinical manifestations according to statistical requirements.

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CONFLICT OF INTEREST

The authors ensure that there is no conflict of interest in this study.

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Sarcopenia in a Multiethnic State: A Cross-Sectional Data Analysis of Multicentre Indonesia Longitudinal Aging Study

Kuntjoro Harimurti[1], Siti Setiati[1], Czeresna Heriawan Soejono[1], IGP Suka Aryana[2], Sri Sunarti[3], Fatichati Budiningsih[4], Roza Mulyana[5], Lazuardhi Dwipa[6], Agus Sudarso[7], Rensa[8], Rahmi Istanti[1], Muhammad Khifzhon Azwar[1], Jessica Marsigit[1]*

[1] Division of Geriatrics Department of Internal Medicine – Clinical Epidemiology and Evidence-Based Medicine Unit, Cipto Mangunkusumo Hospital – Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia.
[2] Department of Internal Medicine, Faculty of Medicine Universitas Udayana, Bali, Indonesia.
[3] Department of Internal Medicine, Faculty of Medicine Universitas Brawijaya, Malang, Indonesia.
[4] Department of Internal Medicine, Faculty of Medicine Universitas Sebelas Maret, Solo, Indonesia.
[5] Department of Internal Medicine, Faculty of Medicine Universitas Andalas, Padang, Indonesia.
[6] Department of Internal Medicine, Faculty of Medicine Universitas Padjajaran, Bandung, Indonesia.
[7] Department of Internal Medicine, Faculty of Medicine Universitas Hasanuddin, Makassar, Indonesia.
[8] School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Jakarta, Indonesia.

**** Corresponding author:***
Kuntjoro Harimurti, MD., PhD. Division of Geriatrics Department of Internal Medicine – Clinical Epidemiology and Evidence-Based Medicine Unit, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital. Jl. Diponegoro no. 71, Jakarta 10430, Indonesia. Email: kuntjoro.harimurti@gmail.com.

ABSTRACT

Background: *Previous regional studies related to sarcopenia in multiethnic Indonesia suggested inconsistent findings. We aimed to find the prevalence of sarcopenia and its associated factors among Indonesian older adults.* ***Methods:*** *In this cross-sectional analysis, we utilised the data of Indonesia Longitudinal Aging Study (INALAS) from community-dwelling outpatients in eight centres. Statistical analyses included descriptive, bivariate, and multivariate analyses. We categorised older adults into sarcopenia group based on the criteria of the SARC-F questionnaire, namely strength, assistance with walking, rising from a chair, climbing stairs, and falls questionnaire.* ***Results:*** *Among 386 older adults, 17.6% were in sarcopenia group. The prevalence of sarcopenia was found to be the lowest in Sundanese group (8.2%). Following appropriate statistical adjustment, sarcopenia was associated with female sex (OR 3.01, 95% CI 1.34-6.73), dependent functional capacity (OR 7.38, 95% CI 3.26-16.70), frailty (OR 11.82, 95% CI 5.41-25.80), and history of fall (OR 5.17 (95% CI 2.36-11.32). Sarcopenia was not significantly associated with age 70 years and older (OR 1.67, 95% CI 0.81-3.45), Sundanese group (OR 0.44, 95% CI 0.15-1.29), and being at high risk for malnutrition or malnourished (OR 2.98, 95% CI 0.68-13.15). All centenarians had no sarcopenia nor frailty, and 80% of them were Sundanese older adults.* ***Conclusion:*** *One in five Indonesian community-dwelling older adults had sarcopenia, associated with female sex, dependent functional capacity, frailty, and history of fall. Albeit statistically nonsignificant, there may still be link between Sundanese, age 70 years and older, as well as being at high risk for malnutrition, and sarcopenia.*

Keywords*: sarcopenia, aging, Indonesia, community-dwelling older adults, Sundanese.*

INTRODUCTION

Sarcopenia is an disease of loss of muscle mass, plus low muscle strength, and/or low physical performance. This geriatric disease is linked to higher risk of fall, disabilities, depression, institutionalisation, hospitalisation, and death.[1–4] Sarcopenia can be diagnosed by using several criteria, including those from Asian Working Group for Sarcopenia (AWGS) consensus in 2019,[5] as well as those from European Working Group on Sarcopenia in Older People (EWGSOP2) consensus revised in 2018.[6] Consensus from both working group suggested the importance of the SARC-F questionnaire for case finding,[5,6] which is a simple questionnaire consisting of strength, assistance with walking, rising from a chair, climbing stairs, and falls. The questionnaire was validated in Indonesian population,[7] and can be used for older adults with various co-morbidities.[8,9]

Asian population appeared to have higher prevalence of sarcopenia compared with other areas in the world. Based on the latest criteria of sarcopenia, sarcopenia was found in 9.6-22.1% male Asians and in 7.7-21.8% female Asians.[10] Indonesia is a country with dietary diversity among various ethnic groups,[11,12] and different epidemiological findings related to sarcopenia among previous study locations.[13,14]

The prevalence of sarcopenia based on AWGS 2019 consensus criteria in Surabaya was as high as 13.9% and 27.9% in male and female population, respectively.[13] On the other hand, the prevalence was only 7.4% and 1.7% among male and female older adults in Jatinangor, West Java, respectively.[14] Although previous local studies have suggested the findings related to sarcopenia,[13–16] national multicentre data related to the prevalence of sarcopenia and its associated factors were lacking. Regional and ethnic differences in the prevalence of sarcopenia also warranted further data analysis.

We aimed to find the current prevalence of sarcopenia and its associated factors among Indonesian community-dwelling older adults in this cross-sectional study, based on the SARC-F questionnaire. We also provided descriptive data of Indonesian centenarians in this study. This cross-sectional data analysis is a part of the national multicentre data analyses of Indonesia Longitudinal Aging Study (INALAS).

METHODS

Study Design and Subjects

In this cross-sectional study, we randomly selected 8 out of 17 older adults healthcare centres in Indonesia, namely Atma Jaya Hospital, Jakarta; Sanglah Hospital, Denpasar, Bali; Dr. Wahidin Sudirohusodo Hospital, Makassar, South Sulawesi; Teja Husada Hospital and Ben Mari Hospital, Malang, East Java; Dr. M. Djamil Hospital Padang, West Sumatera; Dr. Moewardi Hospital, Solo, Central Java; Hasan Sadikin Hospital, Bandung, West Java. This study was conducted in several geriatric service care centres in different islands of Indonesia from March to October 2020. The data analysis was a part of INALAS. We utilised the formula for the sample size of the estimated proportion. The minimum number of subjects to be recruited was 202 subjects.

The inclusion criterion was community-dwelling older adults aged 60 years or above in all selected hospitals who agreed to participate in the study. All subjects or their representing family member(s) signed the written informed consent form. The exclusion criteria were older adults with acute manifestations, such as illness or infections, confused mental state, cerebrovascular and/or cardiovascular events. Subjects were recruited using consecutive sampling method. We excluded subjects with incomplete data related to the SARC-F questionnaire results. Unlike the previous report of analysis of INALAS data related to frailty,[17] the data from Jakarta centre were excluded due to incomplete data related to the SARC-F questionnaire results. The study population of this data analysis and that of previous data analysis were not identical. Ethical approval was obtained from the Faculty of Medicine, Universitas Indonesia.

Data Collection

We obtained secondary data from medical records and primary data from questionnaires. We defined sarcopenia as SARC-F result of 4 or higher.[7] The SARC-F consists of five components,

namely strength, assistance in walking, rise from a chair, climb stairs, and fall. The SARC-F has been adapted and validated in Indonesian population.[7] It has acceptable diagnostic value for sarcopenia,[18] since its performance has been shown to be highly sensitive and specific for detecting sarcopenia based on the criteria from AWGS and from European Working Group for Sarcopenia in Older People (EWGSOP).[7]

The data collected for the cross-sectional study were (a) demographic data (i.e., age, sex, and ethnic groups); (b) functional status based on the Barthel Index of Activity of Daily Living (ADL) questionnaire: totally dependent (score 0–4), severely dependent (score 5–8), moderately dependent (score 9–11), slightly dependent (score 12–19), independent (score 20); (c) frailty was assessed using the FRAIL scale, consisting of fatigue, self-reported resistance (defined as the ability to climb one flight of stairs), self-reported ambulation (defined as the ability to walk one block), number of comorbid illnesses greater than 5, and weight loss of more than 5% in the previous year. One point was given if the patients answered yes to each question. If the total score was 0, the patient was categorised as robust or fit. If the total score was 1 or 2, the patient was categorised as prefrail, and if the total score was 3 or higher, the patient was categorised as frail.[19] (d) nutritional status based on the Mini Nutritional Assessment Short-Form: normal nutritional status (score 12–14), at risk of malnutrition (score 8–11), malnourished (score 0–7); as well as (e) history of fall in the past 12 months. Ethnic groups were categorised into Batak, Balinese, Betawi, Chinese, Minang, Javanese, Makassarese, Sundanese, and others.

Statistical Analysis

The prevalence of sarcopenia was calculated by having the proportion of patients who were categorised as sarcopenia divided by total study subjects. For the statistical analysis, sarcopenia was divided into: (1) no sarcopenia, and (2) sarcopenia. The subjects were categorised based on their sex into male and female. Categories of age group were: (1) < 70 years, and (2) ≥70 years, based on the cut-off point set by the recent epidemiological study of sarcopenia in Malaysia,[20] as well as that of government's profile of Indonesian older adults.[21] Since previous data suggested lower prevalence of sarcopenia in Jatinangor, a Sundanese region, than in other regions,[13–15] we categorised the ethnic groups into Sundanese and non-sundanese. Categories of the functional status were divided into two categories: (1) independent, (2) dependent (for subjects with total, severe, moderate and slight dependency). Frailty status was divided into: (1) non-frail (robust and prefrail), and (2) frail. Categories according to the history of fall were: (1) no history of fall and (2) prior history of fall. Categories according to nutritional status were: (1) normal and (2) at risk of malnutrition or malnourished (for subjects with score less than 12).

We utilised SPSS Version 21 (IBM, Armonk, New York, USA) for bivariate and multivariate analyses. We used binary logistic regression to perform the bivariate analysis to assess the association between sarcopenia and the independent variables. Variables with the result of p-value < 0.25 in bivariate analysis were included in multivariate analysis. Multiple logistic regression was utilised to identify the factors associated with sarcopenia among study variables. P-value < 0.05 was considered statistically significant. We also provided descriptive data of Indonesian centenarians and the prevalence of sarcopenia according to the subject's ethnic group in this study.

RESULTS

We collected data from 386 individuals from different geriatric care centres in Indonesia, see Table 1 for subject characteristics. There was a greater proportion of older adults with independent functional status, normal nutritional status, without history of fall and without frailty. Based on SARC-F results, sarcopenia was found in 17.6% of Indonesian older adults. The prevalence of sarcopenia was 40% in Batak people, whereas it was 35.3% in people of Balinese descent, 27.2% in Betawi group, 26.8% in people of Chinese descent, 21.4% in Minang group, 14.6% in Javanese group, 8.3% in Makassarese group, and 8.2% in Sundanese group.

Bivariate analysis results suggested statistical

Table 1. Subject Characteristics (n=386)

Characteristics	Total (n=386)	Sarcopenia (n=68)	Non-Sarcopenia (n=318)
	N (%)		
Sex			
- Male	162 (42.0)	23 (14.3)	139 (85.7)
- Female	224 (58.0)	45 (20.0)	179 (80.0)
Age			
- 60 – 69 years old	227 (58.8)	28 (12.3)	199 (87.7)
- ≥70 years old	159 (41.2)	40 (25.2)	119 (74.8)
Ethnic group			
- Javanese	137 (35.5)	20 (14.6)	117 (85.4)
- Batak	20 (1.3)	2 (40)	3 (60)
- Sundanese	61 (15.8)	(8.2)	56 (91.8)
- Chinese	71 (18.3)	19 (26.8)	52 (73.2)
- Makassarese	12 (3.1)	1 (8.3)	11 (91.7)
- Betawi	11 (2.8)	3 (27.2)	8 (72.3)
- Balinese	17 (4.4)	(35.3)	11 (64.7)
- Minang	28 (7.3)	(21.4)	22 (78.6)
- Others	44 (11.4)	6 (13.6)	38 (86.4)
Functional status			
- Independent	323 (83.7)	32 (9.9)	291 (90.1)
- Dependent	63 (16.3)	36 (57.1)	27 (42.9)
Frailty status			
- Non-frail	327 (84.7)	29 (8.9)	298 (91.1)
- Frail	59 (15.3)	39 (66.1)	20 (33.9)
Nutritional status			
- Normal	369 (95.6)	58 (15.7)	311 (84.3)
- At risk of malnutrition or malnourished	17 (4.4)	10 (58.8)	7 (41.2)
History of fall			
- No	326 (84.5)	42 (0.2)	284 (99.8)
- Yes	60 (15.5)	26 (43.3)	34 (56.7)

significance of the study variables, except female sex (p>0.05), see Table 2. Based on the p-value of the results, all study variables were included in multivariate analysis. The results of multivariate analysis showed that sarcopenia was associated with female sex (odds ratio [OR] 3.01, 95%

Table 2. Bivariate and multivariate analyses results.

Variables	Crude OR (95% CI)	Adjusted OR (95% CI)
Sex		
- Male	1	1
- Female	1.52 (0.88-2.63)	3.01 (1.34-6.73)*
Age		
- 60 – 69 years old	1	1
- ≥70 years old	2.39 (1.40-4.07)*	1.67 (0.81-3.45)
Ethnic group		
- Non-sundanese	1	1
- Sundanese	0.37 (0.14-0.97)*	0.44 (0.15-1.29)
Functional status		
- Independent	1	1
- Dependent	12.13 (6.53-22.50)**	7.38 (3.26-16.70)**
Frailty status		
- Non-frail	1	1
- Frail	20.04 (10.35-38.78)**	11.82 (5.41-25.80)**
History of fall		
- No	1	1
- Yes	5.17 (2.82-9.47)**	5.17 (2.36-11.32)**
Nutritional status		
- Normal	1	1
- At risk of malnutrition or malnourished	7.66 (2.80-20.94)**	2.98 (0.68-13.15)

*p-value <0.05
**p-value <0.001

confidence interval [CI] 1.34-6.73), dependent functional capacity (OR 7.38, 95% CI 3.26-16.70), frailty (OR 11.82, 95% CI 5.41-25.80), and history of fall (OR 5.17 (95% CI 2.36-11.32). Sarcopenia was not significantly associated with age 70 years and older (OR 1.67, 95% CI 0.81-3.45), Sundanese group (OR 0.44, 95% CI 0.15-1.29), and being at high risk for malnutrition or malnourished (OR 2.98, 95% CI 0.68-13.15).

In our study populations, there were 5 centenarians. All of them were from Hasan Sadikin Hospital cohort, in Bandung. Four out of five were female. Four out of five were Sundanese older adults, whereas the other one was Javanese older adult. All centenatrians had normal ADL, no sarcopenia, and not at risk for malnutrition. Four out of five were robust with no history of fall, whereas the other one were pre-frail with history of fall.

DISCUSSION

This data analysis was the second batch of statistical analyses of INALAS data, following that of frailty.[17] Unlike the previous report of data analysis,[17] the data from Jakarta centre in this study were excluded due to incomplete data related to the SARC-F questionnaire results. On the other hand, the data of several subjects – which were initially excluded in frailty data analysis due to incomplete data related to frailty – were included in the data analysis of this study, making the population involved in both studies not identical. In addition, this study had a larger sample size than required.

The prevalence of SARC-F-based sarcopenia was 17.6% in Indonesian older adults. The prevalence of sarcopenia was found to be the lowest in Sundanese older adults (8.2%). Albeit statistically nonsignificant, Sundanese may be a factor associated with lower risk for sarcopenia. Factors associated with higher risk for sarcopenia were female sex, dependent functional capacity, frailty, and history of fall. One out of five female older adults had sarcopenia, three out of five older adults with dependent functional status had sarcopenia, two out of three frail older adults had sarcopenia, whereas one out of two older adults with history of fall had sarcopenia. The older adults at high risk for malnutrition or malnourished, or those aged 70 years and older, did not have statistically significant higher risk for sarcopenia. All centenerians in our study did not have sarcopenia.

Our study result suggested that nearly 1 in 5 community-dwelling older adults in Indonesia had sarcopenia. This highlights the importance of case finding in primary healthcare centres with the SARC-F, which was already validated in Indonesian population.[7] The removal of programmes for early detection of geriatric problems in healthcare centres may lead to failure or delay in the diagnosis of the problems, including sarcopenia. Case finding, referral, and evidence-based treatment are essential, because sarcopenia is a mortality predictor in community-dwelling older adults.[22]

The result of our study was within the range of the prevalence of sarcopenia in Asia.[23] The prevalence of sarcopenia does not appear to be uniform across different ethnic groups,[24] as also seen in our multiethnic study. The prevalence of sarcopenia was found to be the lowest in Sundanese group (8.2%). This finding supported the result of previous regional study in Jatinangor,[14] a Sundanese region, in which the prevalence of sarcopenia was found to be lower than in other regional studies.[13,15,16]

In this study, the most important factor associated with sarcopenia was frailty (OR 11.82, 95% CI 5.41-25.78). Two out of three frail Indonesian older adults in this study actually had sarcopenia. Sarcopenia and frailty have overlapping clinical consequences and features.[25] Sarcopenia-related components were a part of FRAIL scale screening tool, which were resistance and ambulation. Resistance was assessed by asking subjects if they had any difficulty walking up 10 steps alone without resting and without aids, whereas ambulation can be assessed by asking if the subjects had any difficulty walking several hundred yards alone and without aids. There is a downward spiral of undernutrition, frailty and sarcopenia. Decreased nutrition intake, impaired protein synthesis, acute insult(s), and immobilisation may cause sarcopenia, leading to the decline of the protein reserve of the body. Diminished capacity to meet the extra demand of protein synthesis related to

an injury or a disease will result in increased frailty. Both frailty and sarcopenia were linked with higher risk for mortality. Afterwards, frailty may cause falls, illnesses, hospitalisations, and failure to recover. The consequences may in turn cause or worsen sarcopenia.[26] Without intervention, this vicious cycle may repeat itself.

British Geriatrics Society (BGS), Age UK and Royal College of General Practitioners (RCGP) suggested that the gold standard for the care of people with frailty is comprehesive geriatric assessment (CGA).[27,28] As pathologic conditions in geriatric population may be found concurrently, early detection of geriatric problems is of paramount importance, irrespective of whether the screening tools are incorporated into CGA. Once physician detects dependent functional capacity, sarcopenia, frailty, or history of fall, detection of the others are warranted.

Female sex was associated with 3 times higher risk for sarcopenia than male sex. One out of four female older adults in this study had sarcopenia. Female older adults have less muscle mass and weaker muscle strength compared to their male counterparts.[29] This may be due to physical inactivity that was found to be significantly higher among female than male adults.[30] The lack of estrogen may also reduce muscle mass in female older adults, although recent systematic review and meta-analysis of randomized controlled trials suggested that estrogen supplementation might not increase muscle mass.[31] Interestingly, the decrease in muscle mass and muscle strength of older male adults was significantly quicker than that of older female adults.[29]

ADL disability is increasingly recognised as a public health problem in an aging world.[32] Sarcopenia was associated with dependent functional capacity in this study (OR 7.38, 95% CI 3.26-16.70). Our finding also suggested that among older adults with dependent functional status, three out of five were considered to have sarcopenia. Skeletal muscle mass is crucial for the maintenance of physical function and performing ADL.[33] As human loses a quarter of motor neurons innervating type II muscle fibers over the lifespan,[34] sarcopenia may be the outcome. Sarcopenia may eventually lead to disability and adverse health outcomes.[35]

Nearly 50% of the older adults with past history of fall were considered to have sarcopenia. History of fall was significantly associated with sarcopenia in our study (OR 5.17 (95% CI 2.36-11.32). Our finding was in concordance with the result of systematic review and meta-analysis suggesting that sarcopenic individuals had a significantly higher risk of falls (cross sectional studies: OR 1.60, prospective studies: OR 1.89). Evidence from cross sectional and prospective studies suggested bidirectional causal relationship between falls and sarcopenia.[36] In the aforementioned downward spiral, falls were a part of the vicious cycle requiring detection and prompt treatment.[26]

Although statistically nonsignificant, high risk for malnutrition may be linked with 3 times higher risk for sarcopenia. Previous systematic review and meta-analysis of observational studies suggested poor macronutrients and micronutrients intakes among Indonesian community-dwelling older adults, especially regarding the intake of protein, calcium, vitamin D and vitamin B12.[37]

The associations between age 70 years and older, Sundanese ethnic group and sarcopenia were not statistically significant in multivariate analysis. However, the finding of both factors were significant in bivariate analysis (OR 2.39, 95% CI 1.40-4.07 for age 70 years and older; OR 0.37, 95% CI 0.14-0.97 for Sundanese group), see Table 2. It is interesting to note that age may not be the most important factor associated with sarcopenia in Indonesian cohort. The descriptive data of all centenarians in our study population suggested that all centenarians had normal ADL and no sarcopenia, which may suggest that older adults may be 100 years old without evidence of sarcopenia. Four out of five centenarians were Sundanese older adults. Since several genetic loci related to energy and lipid metabolism may play role in the pathogenesis of age-related sarcopenia,[38] future studies may shed light on the genetic, as well as lifestyle components of each ethnic group that may contribute to longevity, as well as lower prevalence of and lower risk for certain geriatric problems. On the other hand, the results of statistical analysis of the previous

nonsignificant factors may be affected by the sample size of our study, leading to wide 95% confidence intervals. Thus, there may still be link between Sundanese, age 70 years and older, as well as being at high risk for malnutrition, and sarcopenia.

As stated above, the prevalence of sarcopenia varied in different ethnic groups in this study. The prevalence of sarcopenia was found to be the highest in Batak, Balinese and Betawi people. Previous study in older adults suggested no difference between meat, poultry, and fish intake of Betawi and Sundanese people. Compared with Betawi cohort, Balinese older adults had higher meat and poultry intake, and Sumatran older adults had higher fish intake.[12] Sumatran may include several ethnic groups, including Batak and Minang group in this study. We hypothesised that the variety in food intake may not play the key role in causing difference in prevalence of sarcopenia between ethnic groups in Indonesia. Therefore, as mentioned before, future studies may provide data in other lifestyle and genetic components related to sarcopenia in older adults.

To the best of our knowledge, this is the first multicentre cross-sectional data in Indonesia to clarify findings of previous regional epidemiological studies with various results related to sarcopenia. Indonesia is a multiethnic archipelago and our study helped show differences in the prevalence of sarcopenia in different ethnic groups. Sarcopenic state is a potentially reversible condition and we are determined to continue INALAS data collection and analysis in the future for healthcare information, guideline, and policy. However, we acknowledge the limitations of the present study, particularly the sample size. Although the amount of subjects in our study exceeded the minimum sample size required as calculated with statistical formula, the wide confidence intervals of the study results may result from limited sample size. We also categorised the older adults into those with sarcopenia and without sarcopenia relying on history taking (SARC-F questionnaire). In resource limited settings, such as in several healthcare centres in Indonesia, diagnostic tools to assess appendicular skeletal muscle mass are unavailable. Dual-energy X-ray absorptiometry (c) and bioelectrical impedance analysis (BIA) were not widely available. Thus, we were unable to make definitive diagnosis of sarcopenia based on the AWGS guideline released in 2019. However, the SARC-F was a validated tool in Indonesian population, with high sensitivity and specificity for detecting sarcopenia based on the criteria from AWGS and EWGSOP.[7]

CONCLUSION

In conclusion, the prevalence of sarcopenia was 17.6% in Indonesian older adults. The prevalence of sarcopenia was found to be the lowest in Sundanese older adults (8.2%). All centenerians in our study did not have sarcopenia. Factors associated with higher risk for sarcopenia were female sex, dependent functional capacity, frailty, and history of fall. One out of four female older adults had sarcopenia, three out of five older adults with dependent functional status had sarcopenia, two out of three frail older adults had sarcopenia, whereas one out of two older adults with history of fall had sarcopenia. Albeit statistically nonsignificant, there may still be link between Sundanese, age 70 years and older, as well as being at high risk for malnutrition, and sarcopenia.

COMPETING INTERESTS

The authors declare that they have no competing interests

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AUTHORS' CONTRIBUTIONS

SSe, CS, KH, IA, SSu, FB, RM, LD, AS, RR, RI, MA, and JM contributed to development of study concept and design. SSe, CS, KH, IA, SSu, FB, RM, LD, AS, RR, and RI contributed

to acquisition of data. SSe, RI, MA, and JM contributed to analysis and interpretation of data. SSe, CS, KH, RI, MA, and JM contributed to drafting of the manuscript. All authors contributed to the article, read and approved the final manuscript.

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Giant Left Ventricular Pseudoaneurysm in a 79-Year-Old Female Patient: Diagnostic and Management

Nina Solomakhina[1], Alexey Lishuta[1], Ekaterina Gladysheva[1], Anna Dementeva[2]*

[1]Department of Hospital-based Therapy, I.M. Sechenov First Moscow State Medical University (Sechenov University), Sklifosovsky Institute for Clinical Medicine, Moscow, Russian Federation.
[2] Veterans Affairs Hospital 1, Moscow, Russian Federation.

Corresponding Author:
Alexey Lishuta, MD. *Department of Hospital-based Therapy, I.M. Sechenov First Moscow State Medical University (Sechenov University), Sklifosovsky Institute for Clinical Medicine, 18-2 Trubetskaya str., Moscow 119991, Russian Federation. Email:*

ABSTRACT

Left ventricular pseudoaneurysm is a rare but dangerous complication, which occurs in the early post myocardial infarction period. Small pseudoaneurysms are not fatal, while large ones cause death due to sudden rupture and cardiac tamponade if surgery is not performed on time. As left ventricular pseudoaneurysm is uncommon in population, only few case reports were found in the published literature. In this article, we present a case of left ventricular pseudoaneurysm in a 79-year-old female patient after a silent posterolateral myocardial infarction, which increased to gigantic size for 3 months and was diagnosed accidentally by transthoracic echocardiography. Since the patient refused surgical treatment, the difficulties in deciding on the management of the patient based on a review of the literature is described. The main goal of this case is to describe the 6-month survival rate of a 79-year-old female patient with left ventricular pseudoaneurysm after silent posterolateral myocardial infarction despite refusal of surgical treatment and extremely low adherence to drug treatment due to cognitive impairment.

Key words: *left ventricular pseudoaneurysm, echocardiography, diagnosis, prognosis.*

INTRODUCTION

Myocardial ruptures include left ventricular free wall rupture and internal ruptures of interventricular septum and papillary muscles, the latter being the most severe and potentially fatal complications developing in the early post myocardial infarction (MI) period.[1-3] Some left ventricular free wall ruptures may be subacute, in this case the myocardial rupture is small and sometimes closed by partially thrombosed hematoma "sealed" to the pericardium. It means that the thrombus, hematoma, and pericardium cover the rupture, thus forming a false aneurism or pseudoaneurysm of the left ventricle.[2,3] Pathologically, in pseudoaneurysm, there is a small narrow canal (isthmus) which connects the left ventricular cavity with a massive aneurysmal sac containing blood, clots, and only pericardial fibrous elements – no myocardial tissue.[4] Macroscopically, the rupture is linear or arched, with transmural localization, edges are irregular, the site of rupture is jagged.[5]

Left ventricular pseudoaneurysm is a very rare complication of MI[2,3] and develop in less than 0.1% of all patients with MI.[6] Postmortem examination of 303 patients who died of MI revealed no left ventricular pseudoaneurysm-related deaths.[5] It is known, that only small pseudoaneurysms are compatible with life, but with large ones and absence of timely surgical

treatment patients die because of spontaneous rupture and tamponade development.[2,3] As a rule, reports in the existing literature describe small left ventricular pseudoaneurysms. In this article, we report a case of a giant left ventricular pseudoaneurysm in a 79-year-old female patient after posterolateral silent MI, who refused surgical treatment.

CASE ILLUSTRATION

A 79-year-old patient female was urgently admitted to a cardiology unit with breathlessness on mild exertion, increasing in supine position (at night the patient takes the sitting position), swelling of lower extremities (shins and feet), and weakness. History taking was complicated by patient's marked cognitive impairments. The patient lived alone and had no relatives. The medical records showed that she had been suffering from arterial hypertension for a long period of time, over the past 3 months her blood pressure persisted but was not higher than 110/70 mm Hg. The patient had paroxysmal atrial fibrillation for 5 years. Three years ago she suffered an acute cerebrovascular event which triggered progressive memory loss. Two years ago, the patient developed angina pectoris III functional class. Coronary angiography findings revealed circumflex artery stenosis, stenting was performed. Meanwhile echocardiography showed that local left ventricular contractility was normal. Adherence to drug regimen was poor (ramipril, bisoprolol, atorvastatin, clopidogrel, aspirin, warfarin were taken irregularly, dosage instructions were not followed). Blood pressure and heart rate were not monitored.

The patient noticed the deterioration of her condition 3 months prior to admission, when she suddenly felt weakness when she was at home. Then she developed signs of left heart failure (HF) (breathlessness on mild exertion increasing in supine position), and was hospitalized. Echocardiography showed the left ventricular wall with depressed myocardial contractility in posterolateral segment, the rupture of the basal segments with forming pseudoaneurysm with size 6.2x2.5 cm, and volume about 62 ml. Coronary angiography reveals 70% restenosis in the stent of the circumflex artery. Surgical treatment was recommended, but the patient refused it and was discharged with recommendation to add loop diuretic (furosemide, 20 mg and spironolactone, 50 mg on a once-daily basis) to her therapy.

Over the next three months, the patient's condition progressively deteriorated; manifestations of right ventricular heart failure (swollen lower legs and feet) supervened, and she had to be hospitalized. On admission, the patient's condition is of moderate severity (NYHA class III); due to shortness of breath, she is lying with the head of the bed raised. The respiratory rate is 24 per minute. Auscultation revealed weak breath sounds, moist rales over the lower lateral divisions of the lungs on both sides. Heart sounds are muffled; the rhythm is regular; systolic murmur is heard at the apex and at the base of the xiphoid process of the sternum, diastolic murmur at the left lower sternal border (3rd-4th intercostal space). The heart rate is 92 bpm. BP is 100/70 mmHg. The abdomen is soft and non-tender. The liver is enlarged and 3 cm below the costal margin, tender on palpation. Swollen thighs, shins, and feet.

Complete blood count and basic metabolic panel revealed no significant changes. However, NT-proBNP was increased to 2450 pg/mL, serum creatinine was increased to 140 μmol/L. Electrocardiogram (ECG) showed scarring of posterolateral wall. (**Figure 1**)

Chest X-ray showed signs of venous congestion and left-sided hydrothorax (**Figure 2 A,B**).

Two-dimensional Echocardiography findings revealed dyskinesia of the basal posterolateral and mid posterolateral left ventricular segments, hypokinesia of the lower basal and mid segments of the left ventricular, and hypokinesia of its anterolateral basal and mid segments. At the basal level of the posterolateral wall, there is a defect (wall rupture) up to 1.9 cm, communicating with a cavity with size 6.1x9.4 cm, volume about 315 ml. (pseudoaneurysm). The cavity of pseudoaneurysm demonstrates spontaneous echo contrast and mural thrombi (**Figure 3A, B**). Colour Doppler echocardiography showed blood flow pattern "to-and-fro" between two cavities: circulation from the left ventricular through the isthmus to the cavity of pseudoaneurysm and

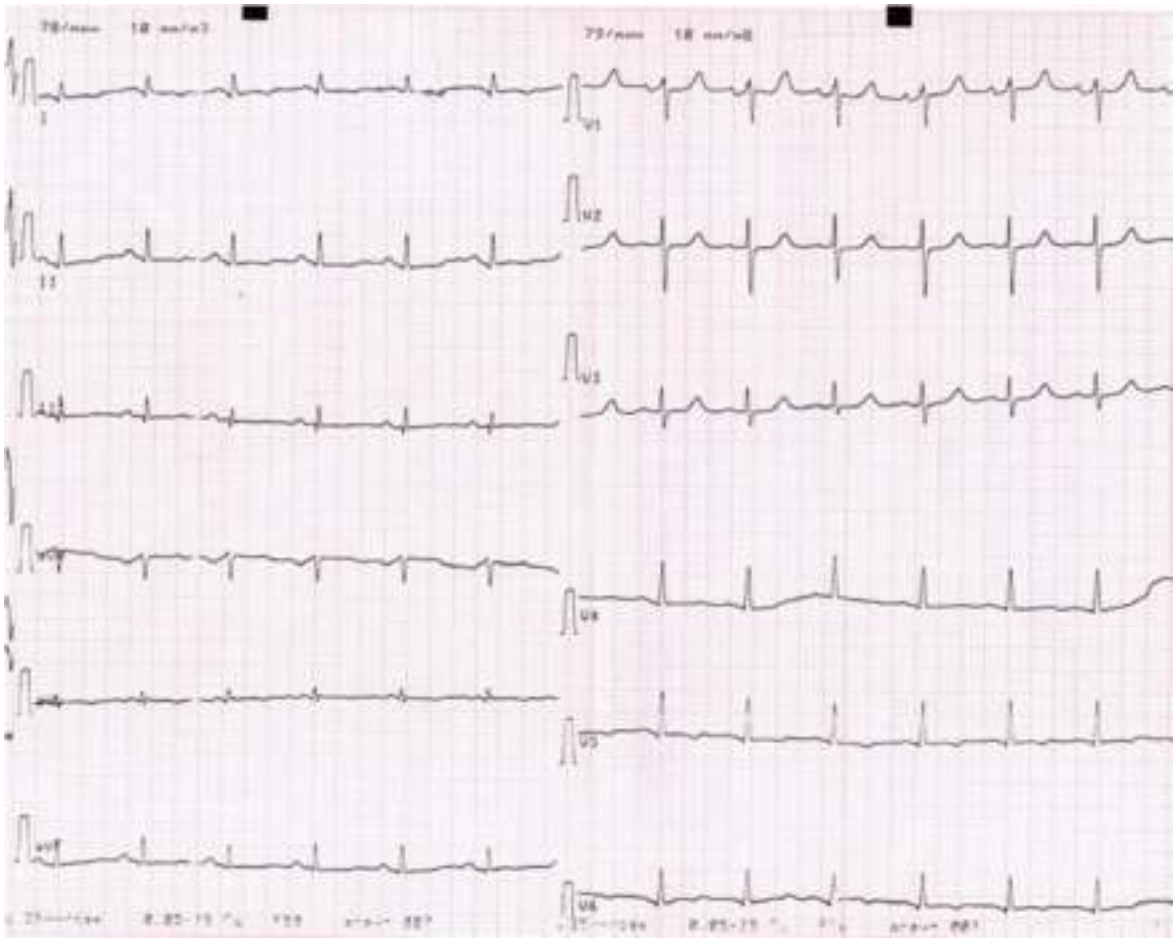

Figure 1. Electrocardiogram Sinus rhythm. qR waves in leads II, III, AVF, AVL, V5, V6, and symmetric inversion of T wave in leads I, aVL, V5 and V6.

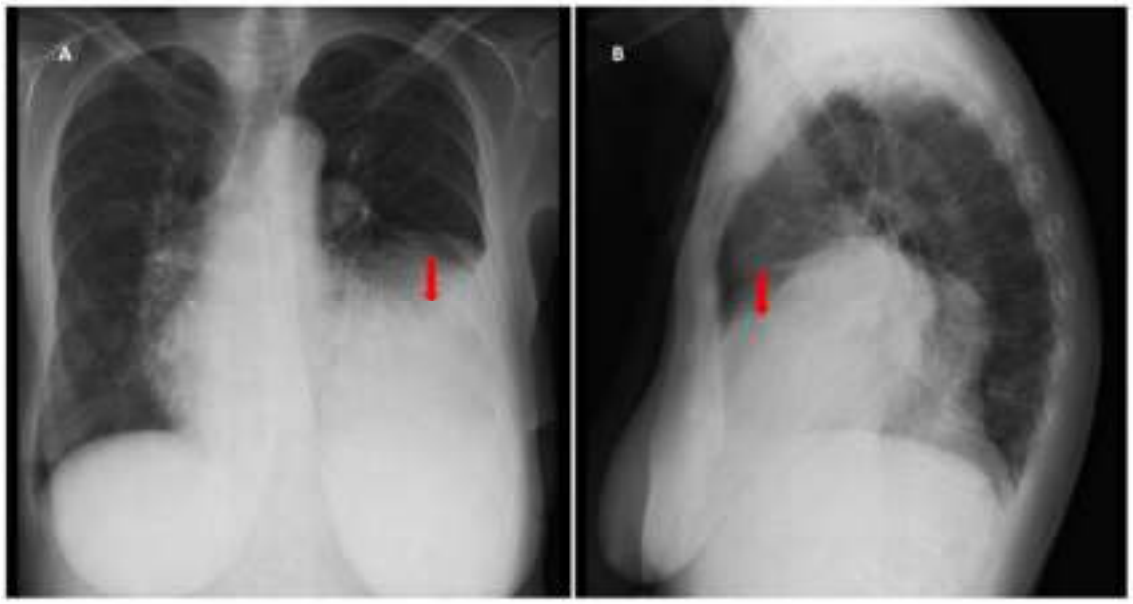

Figure 2. Chest x-ray. A. Frontal view. B. Left lateral view. On the left, there is a homogeneous shadow with an upper indistinct border at the level of rib IV anterior segment (arrow), due to effusion mainly in the anterior sinus.

backflow from the cavity of pseudoaneurysm through the isthmus to the left ventricular cavity (**Figure 4 A, B**). There were also revealed: diastolic dysfunction of the left ventricular of the restrictive type, a decrease in the ejection fraction up to 40%, an increase in pulmonary hypertension up to 55 mm Hg, dilation of the inferior vena cava up to 2.3 cm, which collapsed on inspiration less than 50%. Colour Doppler echocardiography showed moderately severe mitral, aortic and tricuspid regurgitations (**Figure 5A, B**).

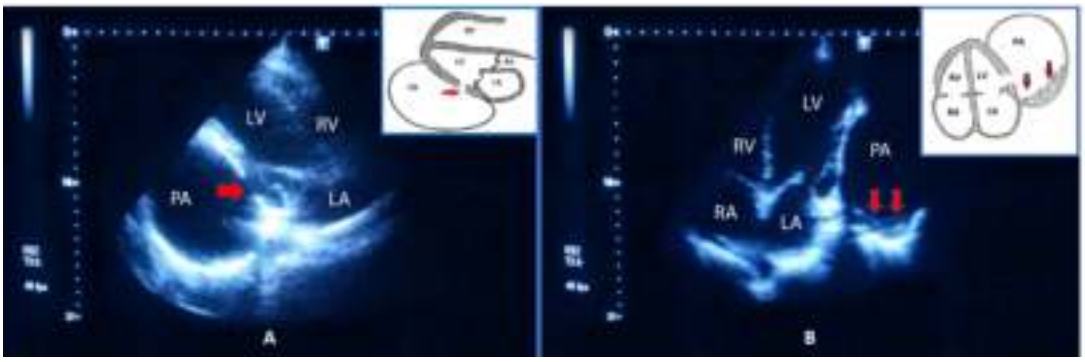

Figure 3. A. Echocardiogram in the parasternal long axis view and scheme. Thinning and wall rupture – a defect in the posterolateral left ventricular wall (arrow) through which the left ventricular communicates with the pseudoaneurysm. B. Echocardiogram in the apical four chamber position and scheme. Mural thrombi in the pseudoaneurysm cavity (arrows).

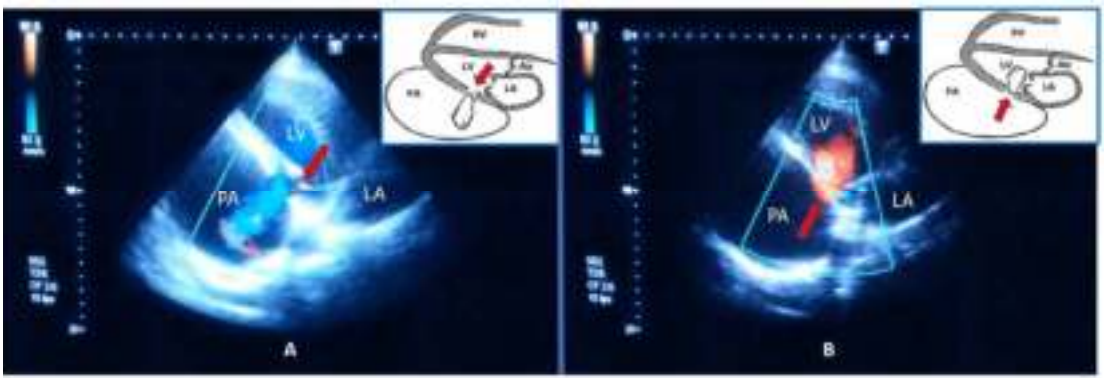

Figure 4. A. Color Doppler echocardiography and scheme. In the projection of the defect, the blue flow indicates cardiac shunt from the left ventricular through the isthmus into the pseudoaneurysm cavity (arrow). B. Color Doppler mapping and scheme. In the projection of the defect, the red flow indicates blood return from the pseudoaneurysm cavity through the isthmus into the left ventricular cavity (arrow).

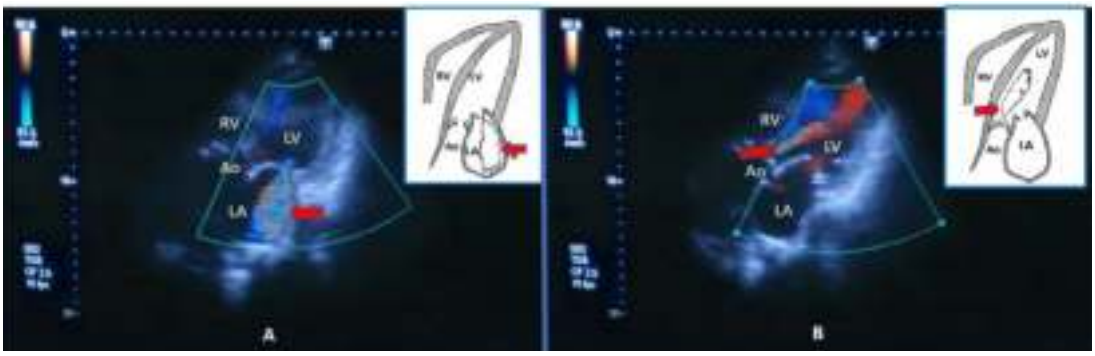

Figure 5. A. Color Doppler echocardiography and scheme. Apical five-chamber position. Turbulent flow in the left atrium during systole reaching the upper wall of the left atrium. The stream of mitral regurgitation is variegated (arrow). B. Colour Doppler echocardiography and scheme. Apical five-chamber position. Turbulent flow in the left ventricular outflow tract and cavity. The stream of aortic regurgitation is colored red (arrow).

Abbreviations: pseudoaneurysm (PA), left ventricle (LV), right ventricle (RV), left atrium (LA), right atrium (RA).

The patient flatly refused to be further examined and operated and was discharged to be followed up by a cardiologist with recommendations to continue drug therapy: ramipril 2.5 mg daily, bisoprolol 5 mg/day, atorvastatin 20 mg QD, rivaroxaban 20 mg, furosemide 20 mg and veroshpiron 50 mg orally on a once-daily basis. However, three months later, the patient died with symptoms of progressive HF up to functional class IY (NYHA) in another hospital.

DISCUSSION

In left ventricular pseudoaneurysm, myocardial rupture develops slowly for several hours or even days[7] and progresses from endocardium to pericardium.[8] Blood continually oozes to the pericardium through a small defect in the left ventricular wall causing local inflammation and "de novo" pericardial adhesions.[8] In addition, myocardial rupture may be contained by existing pericardial adhesions.[2] The above described mechanism clearly explains how the patient's posterolateral silent MI resulted in left ventricular pseudoaneurysm, which prevented rapid cardiac tamponade followed by patient's death.

Due to lack of medical documentation, the date of MI and how long pseudoaneurysm developed are not known. Having studied incomplete medical record of the patient, we suppose that it happened 3 months prior to admission, when being in stable condition, the patient felt worse and developed acute left HF and paroxysmal dyspnea with no previous attacks of angina pectoris, which could prompt her to seek medical attention earlier. The literature describes a case of left ventricular pseudoaneurysm development as a result of transmural MI without preceding angina within 5 months, with HF also being the main clinical manifestation.[9] According to the literature, the time interval between MI and the diagnosis of pseudoaneurysm is from 1 to 11 months,[8] and the median time from MI to diagnosis of pseudoaneurysm is 3.9 months.[10]

We also suppose that the initial rupture of the left ventricular wall was small. However, later due to the continuous blood flow to the cavity of pseudoaneurysm, its volume increased to 315 ml. It significantly exceeded volume of the left ventricular, which cavity, due to the constant flow of blood into the pseudoaneurysm cavity decreased to 80 ml. Decreased blood flow resulted in spontaneous echo contrast and massive thrombus formations on the aneurysmal sac walls which are the major risks for a thromboembolic event.[1,11]

Based on literature, the most common localization of both left ventricular free wall ruptures, and true left ventricular aneurysms is the anterior wall of the left ventricular.[5] This results from frequent atherosclerosis of the left anterior descending artery,[1,8] while 78.6 % of left ventricular pseudoaneurysms[8] develop in posterolateral MI as a result of circumflex artery thrombosis.[7,8,12] An explanation for the greater prevalence of pseudoaneurysms in the posterolateral left ventricular wall, as compared to the anterior wall, is that anterior wall ruptures always have a vivid clinical picture, a fulminant course with the development of tamponade and do not have time to confine themselves to pericardial adhesions.[8]

Furthermore, the patient had the majority of the known risk factors for both, left ventricular rupture – female gender, age over 65, uncontrolled arterial hypertension, history of prior cerebrovascular event, and for pseudoaneurysm – silent posterolateral MI, transmural lesion and the wall thinning at the site of MI.[1,7,13,14] Clinical manifestations following pseudoaneurysm development were the signs of congestive HF, i.e. were not specific and could not alert the clinicians to possible left ventricular pseudoaneurysm. Other authors also pay attention to the non-specificity of clinical manifestations and physical examination data.[8,15,16] Chest X-ray in terms of exclusion or confirmation of pseudoaneurysm, as in this case, is often not informative (a non-informative method).[1,16]

It should be noted that the ECG was not very informative either, since with such a gross damage to the left ventricular wall, instead of qR waves in leads II, III, AVF, AVL, V5, and V6, one should expect the presence of QS or Qrs, which would confirm transmural damage to the myocardium of the posterolateral left ventricular

wall. Other authors also draw attention to the lack of marked ECG changes in the development of left ventricular pseudoaneurysm due to posterolateral MI,[12,17] in contrast to anterior left ventricular wall ruptures, which make the ECG highly informative.[14]

It is obvious that in this case, like in the most cases described in literature, left ventricular pseudoaneurysm was diagnosed accidentally by echocardiography in MI, congestive HF, rhythm disturbances, and thromboembolism.[2,8,9,12] Therefore nowadays, echocardiography as a widely available, noninvasive and informative diagnostic method has become the method of choice for early diagnosing of left ventricular pseudoaneurysm.[1,2,12,13,16,18]

Of course, in diagnostically complex cases, as well as in preparation for surgery may require the additional carrying out of other, but more expensive, imaging techniques: magnetic resonance imaging, multispiral computed tomography or ventriculography.

Since left ventricular pseudoaneurysm is a very rare condition, there is no randomized controlled study, to guide a management strategy. The information on surgically treated and untreated left ventricular pseudoaneurysms is not structured, and based on retrospective analysis of single cases.[13] No large studies comparing the results of surgical and drug treatment of left ventricular pseudoaneurysms have been conducted.

According to the current management strategy, patients with left ventricular pseudoaneurysm require surgical intervention if pseudoaneurysm is large, acute (less than 3 months), and localized in the anterior wall of the left ventricular.[1,8,13] Unlike true aneurysms, in which the wall is intact but fibrous and thin, the wall of pseudoaneurysms contains only pericardium and thrombus[3,4] and no endocardial or myocardial tissue.[1,13] It explains a high risk of rupture and tamponade formation, which accounts for 30-45%.[3] Such ruptures are very unpredictable in terms of onset and development.

However, postoperative mortality is also high (35.7%)[8] because postoperative outcome is influenced by such factors as stage of HF, MI size, patient's overall heath and comorbidities. Moreover, surgical treatment is not always limited to pseudoaneurysm resection and closure of ventricular defect. Some cases require mitral valve replacement or coronary bypass surgery,[8] which makes risks of poor outcome even higher.

Meanwhile, some authors[1,10,11,13] believe that poor prognosis for such patients is more likely to be linked to progressive HF and thromboembolic complications, but not to a possible fatal rupture. Having observed the patients with pseudoaneurysm for four years, T.C. Yeo et al.[10] show that not all pseudoaneurysms have a high risk of rupture. Varvarigos N. et al.[1] suggest that small (up to 3 cm) chronic asymptomatic aneurysms which are not prone to increase may be treated only medically. Moreno R. et al.[11] think that the long-term prognosis for patients with post MI left ventricular pseudoaneurysms is relatively good, with low risk of fatal ruptures, which should be considered while choosing surgical intervention as the main treatment strategy. Díaz-Navarro R. and Nihoyannopoulos P.[18] also it's believed that despite the fact that surgical treatment is the method of choice to avoid the risk of fatal myocardial rupture, the long-term results of medical treatment of patients with left ventricular pseudoaneurysm appear to be relatively favorable. However, when surgery is an absolute indication but the patient's prognosis is quite poor, decision-making process for a clinician is very difficult and even counter-intuitive, especially if a patient refuses surgery. In published literature, there are cases which describe patients who refused surgical intervention in spite of having absolute indications and bad prognosis, medical treatment remained the only option for them.[1,2] It is necessary to note that after diagnosis, such patients were successfully receiving medical treatment for 4[1] and 2 years,[2] their condition being stable. Roa-Castro V.H. et al.[2] suggest that the long survival of such patients is due to dense pericardial adhesions. Varvarigos N. et al.[1] believe medical therapy to be the only optimal treatment for high-risk patients refusing surgery. Hulten E.A. et al.[13] also think that for patients with chronic pseudoaneurysm (more than 3 months) who have high risk for surgical intervention "conservative management may be

prudent".

Taking into account the above information, the opinions of cardiologists and cardiac surgeons on the management of this patient were divided. Those who insisted on surgical treatment argued their position with high risk of pseudoaneurysm rupture and an equally high risk of thromboembolic complications, and those who proposed conservative management of the patient, with extremely high risk of surgical intervention (advanced age, low ejection fraction, presence of severe concomitant pathology: a previous stroke). Nevertheless, the patient was offered surgery which she denied (twice, including previous hospitalization). Next 3 months, as well as 3 previous months, the patient treatment was conservative.

CONCLUSION

The case we report proves that left ventricular pseudoaneurysms have non-specific clinical presentation even if they are giant and have a rough wall defect. They are often diagnosed accidentally by echocardiography after the pseudoaneurysm has already been formed. Therefore, when examining patients with echocardiography, primarily with congestive heart failure, who have had posterolateral MI, it should be recommended to be alert to exclude left ventricular pseudoaneurysm, especially if the patient is a female, older adults and senile, with arterial hypertension and a history of acute cerebrovascular accident.

As the patient with the giant left ventricular pseudoaneurysm, massive thrombi formation, and unsatisfactory compliance to treatment did not develop the two major complications – thromboembolism and wall rupture which could have inevitably led to sudden and rapid death, this case proves that medical treatment can be the method of choice in patients with left ventricular pseudoaneurysm. Although the survival period was limited to only 6 months, it could definitely have increased with the patient's better compliance to treatment. This is especially important for older patients who refuse surgical treatment or for patients with an extremely high risk of surgical treatment, when conservative treatment is forced to become the only method.

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ETHICAL STATEMENT

The research work done for preparing rare the clinical case was approved by the Interuniversity Ethics Committee.

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Cefepime Induced Encephalopathy in a Non-dialysis Dependent Chronic Kidney Disease Patient: A Case Report

*Giritharan Muniandy[1], Lydia Kamaruzaman[2], Tan Hui Jan[3], Rozita Mohd[2], Theepa Neesam Mariamutu[2], Fong Voon Ken[2], Pau Kiew Beng[4], Ruslinda Mustafar[2]**

[1] Department of Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia.
[2] Nephrology Unit, Department of Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia.
[3] Neurology Unit, Department of Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia.
[4] Department of Pharmacy, Hospital Canselor Tuanku Muhriz, Universiti Kebangsaan Malaysia.

**** Corresponding author:***
Ruslinda Mustafar. Nephrology Unit, Department of Medicine, National University of Malaysia Medical Centre. Jalan Yaacob Latif, Bandar Tun Razak, Cheras, 56000, Kuala Lumpur, Malaysia. Email: ruslinda.m@gmail.com.

ABSTRACT

Cefepime is a frequently used fourth-generation cephalosporin antibiotic for a wide variety of infections. Toxic levels of this drug can cause neurological complications. The most common neurological adverse event of cefepime is headache and lightheadedness. Here, we presented a case of cefepime induced encephalopathy in a 57-year-old female patient with acute on chronic kidney disease. With an accurate diagnosis that requires a high index of clinical suspicion, prompt management was instituted. She had full resolution of symptoms following discontinuation of the medication and also emergent dialysis.

Keywords*: Cefepime, encephalopathy, chronic kidney disease.*

INTRODUCTION

Cefepime has a broad antibacterial spectrum covering aerobic for gram-positive and gram-negative bacteria including Pseudomonas.[1] Cefepime was recommended for a wide array of infections such as hospital-acquired pneumonia, febrile neutropenic sepsis as well as soft tissue and intra-abdominal infections.[2]

The neurotoxic effects of Cefepime was first reported in 1999 and the most common being headache and lightheadedness.[3,4] These symptoms are often associated with decreased cefepime clearance in the setting of reduced glomerular filtration rate and increased central nervous system penetration secondary to blood-brain barrier dysfunction.[5] These adverse events are mostly seen in patients who are on renal replacement therapy. It is rarely seen in patients not requiring dialysis. In 2002, the Food and Drug Administration (FDA) adjusted the labelling to account for increased risk of seizures, encephalopathy and myoclonus, especially in the setting of renal impairment.[6]

CASE ILLUSTRATION

A 57-year-old female with stage IIIb chronic kidney disease (CKD) secondary to diabetes mellitus (baseline creatinine of 137 µmol/L), hypertension and dyslipidemia presented with a one-week history of fever, reduced oral intake, nausea lower abdominal pain and acute urinary retention. On examination, she was alert with blood pressure of 138/90 mmHg, pulse rate 98 beats per minute and febrile at 38°C.

Abdominal examination revealed a distended bladder. Therefore emergent catheterization was done. Her blood glucose was 8mmol/L, and urinalysis had leukocyte of 3+. Her renal function test showed raised creatinine level to 246 µmol/L with urea of 11.7 mmol/L, she had no leukocytosis but raised serum C-reactive protein of 5.4mg/dl. She was diagnosed and treated for urinary tract infection and emprically started on intravenous ceftriaxone 2gm once daily.

Ultrasound of urinary tracts showed grossly distended urinary bladder with bilateral hydronephrosis and hydroureter.

On day 3, her urine and blood cultures both grew *Enterobacter species* Beta-Lactamase Group 1, sensitive to cefepime only. Her antibiotic was escalated to intravenous (IV) Cefepime 500mg twice daily. Her renal function was at 267 µmol/L, with an estimated glomerular filtration rate (eGFR) of 16mls/min/1.73m^2. After three days of IV cefepime, she was noted to be disoriented and had incoherent speech. Her conscious level was fluctuating with Glasgow coma scale ranging from 13/15 to 14/15. Capillary plasma glucose was 8mmol/L, other electrolyte parameters and CT brain were all normal. Her neurological manifestations coincide with the initiation of IV Cefepime; thus, the possibility of Cefepime induced encephalopathy (CIE) was entertained.

Electroencephalogram (EEG) showed mild to moderate cerebral disturbance by virtue of excessive theta activity with triphasic waves in keeping with metabolic encephalopathy. The EEG images are as below (**Figure 1**). Therefore, based on clinical manifestations and EEG findings, she was diagnosed with CIE.

Cefepime was withheld, and she underwent two sessions of hemodialysis (HD) lasting 4 hours each which saw a tremendous improvement in her clinical condition with normal orientation and speech. The antibiotic was switched to IV Meropenem for another 5 days after stopping the Cefepime. Her repeated blood and urine cultures were negative and she was allowed to be discharged home. As expected, her renal functions also returned to the baseline (**Figure 2**).

DISCUSSION

Cefepime is a widely used antibiotic in the settings of sepsis and acutely ill. It is a 4th generation cephalosporin with a broad antibacterial spectrum covering aerobic gram-positive and gram-negative bacteria including pseudomonas.[1] It is mainly excreted via the kidneys with 85% unchanged in the urine and the body metabolizes the remainder to N-methylpyrrolidine, a 7- epimer isomer.[1] Approximately 10% of serum cefepime is able to pass through the blood-brain barrier; however in the setting of decreased eGFR, this can increase up to 45%.[5] Treatment with a cephalosporin may also induce endotoxin release which generates cytokines liberation such as tumor necrosis factor- α (TNF- α). TNF-α seems to mediate septic encephalopathy.[7] Alternatively, in an animal study, cephalosporin's may decrease γ-aminobutyric acid (GABA) release from nerve

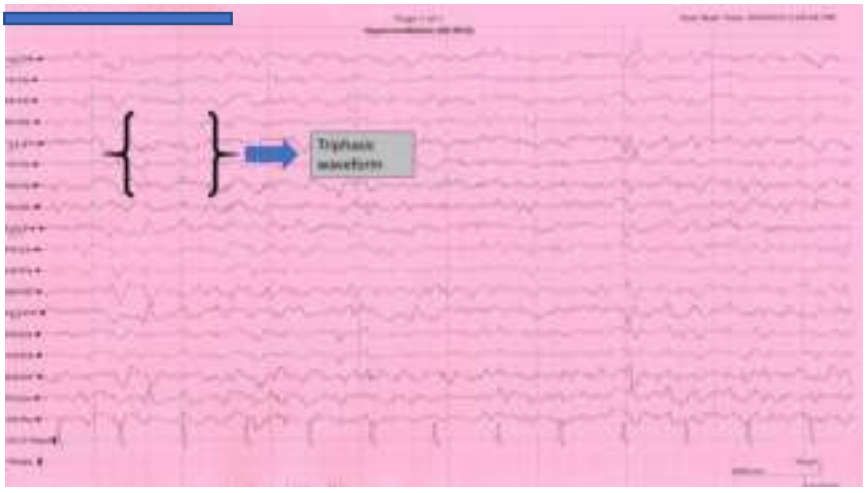

Figure 1. EEG showing encephalopathic disturbances with triphasic waveform.

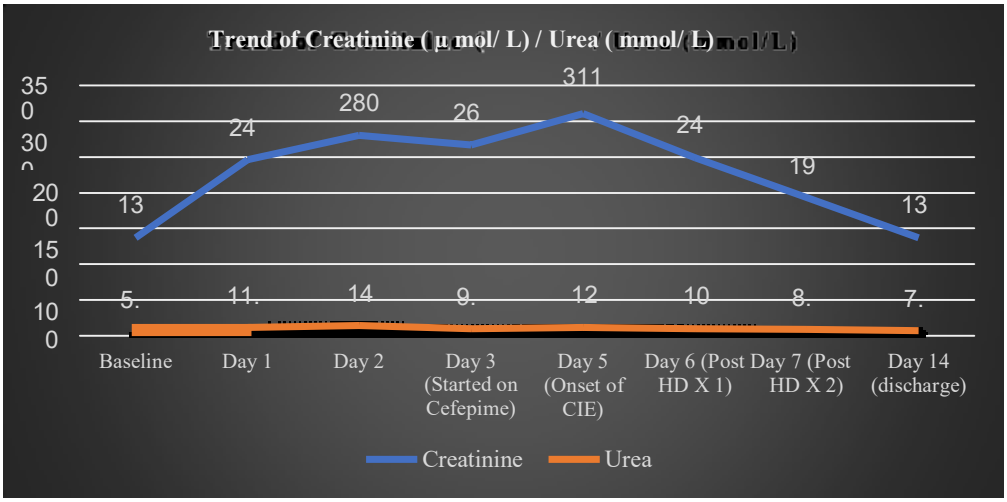

Figure 2. Trend of serum creatinine and urea.

terminals, increase excitatory amino acid release, and exert a competitive antagonism towards GABA.[8] In the settings of acute on chronic kidney disease, the clearance of cefepime is delayed, and in our patient, the blood brain barrier is further compromised due to the underlying bacteremia that can further potentiate risk of encephalopathy.

In patients with end-stage renal disease (ESRD), there are multiple evidence and reports of non-convulsive status-epilepticus and metabolic encephalopathy associated with cefepime.[3] The EEG in these patients shows paroxysmal activity or sharp triphasic waves which are pathognomonic of CIE.[9-11] Cefepime was also found to have significant adverse effects despite renal dose adjustments.[12] Two meta-analysis studies measured all-cause mortality. The first study was in 2006, where it evaluated antibiotic treatment for neutropenic fever with 33 trials showing higher all- cause mortality after 30 days with cefepime when compared to other β-lactams.[13] A second study by Rugate et al. in 2013 evaluated 100 patients from the Intensive Care Unit setting for neurotoxicity and found 15 patients likely to have CIE, and 7 patients were found to be definite. Four patients out of these 7 had their cefepime dose adjusted for their renal function but still experienced adverse effects.[12] The diagnosis of CIE in these studies was made via EEG and also based on clinical features. A case series by the U.S. Food and Drug Administration (FDA) in 2012 also showed the varying occurrence of CIE in patients from different age group and renal status.[14]

Measurement of serum cefepime to predict the neurotoxicity has also been studied. Some early reports suggested neurotoxicity can be expected when the serum level exceeds 22mg/L and the level needed for harm ranges from 2.1 to 18.5 mg/L.[15] Some recent prospective studies in 2017 have suggested neurotoxicity associated with cefepime at concentrations exceeding 35mg/L.[16] The facility to measure serum cefepime level is not readily available in our setting; therefore, we need to have a high index of suspicion based on the clinical manifestations and EEG findings. Apart from the toxic threshold of cefepime concentration, other factors that cause alteration in the blood-brain barrier such as inflammatory conditions, severe sepsis, toxins, metabolic disorder; together with an underlying renal dysfunction has the likelihood to contribute to its neurotoxic potential. In patients with normal renal function, cefepime is eliminated in more than 80% of cases by urine, with a half-life of 2-2.5 hours. In a patient with renal failure and creatinine clearance <10ml/min, the half-life of cefepime is 5 times higher from 2.3 to 13.5 hours and sometimes even up to 22 hours.[17] Cefepime is dialyzable and up to 70% of a given dose can be removed during a 3-hour hemodialysis session.[18]

Haemodialysis therapy has been found to give a good prognosis in patients with CIE. Chatellier et al. reported a series of five cases, all treated with urgent haemodialysis, with full recovery in four cases. Delay in diagnosis in the fifth case could be the reason for the patient's

Table 1. Clinical characteristics and recommended treatment for CIE.

Risk Factors	Signs and symptoms	EEG Characteristics	Treatment
Renal dysfunction Critical illness Altered BBB Older age Drug overdose	Altered mental status Reduced consciousness Confusion Myoclonus Aphasia Seizures	Triphasic waves - multifocal sharp waves - Non-convulsive SE - Generalised slowing - Myoclonic SE	Drug's discontinuation Haemodialysis Benzodiazepine*

*EEG: Electroencephalography, BBB: Blood-brain barrier, SE: Status epilepticus. *for EEG abnormalities/seizure activity associated with toxicity*

death.[18] Haemodialysis seems to be favourable because of its rapid action to clear the drug from circulation. A good understanding of the clinical course and manifestations of CIE may facilitate earlier identification and prompt treatment.[19] However, symptoms may be delayed with a median onset of 4 days after the initiation of the drug. (**Table 1**)

Even though neurotoxicity with cefepime is most commonly occurs when inappropriate doses are administered to patients with renal dysfunction, there are new and emerging evidence that indicate neurotoxicity can happen in patients receiving proper dose adjustments and also in those with normal renal function.[20] The use of alternative antibiotics should be considered for those patients who are at risk of neurotoxicity, at the same time recognizing the potentials emergence of antibiotic resistance. If substituting the antibiotics is not a choice, close clinical monitoring is warranted. Symptoms of CIE can be delayed and progressive, but clinical improvement usually is seen following drug cessation, treatment of epileptiform activity and also drug removal via hemodialysis. In this case, we illustrated the development of CIE in a patient with acute on CKD. However, despite adequate dose adjustment, our patient developed clinical features of CIE after three days of therapy. With a prompt diagnosis of CIE, immediate withdrawal of the drug and urgent hemodialysis showed good clinical outcome for this lady.

CONCLUSION

Recognising cefepime as a source of neurotoxicity can be a challenge given the clinical picture is often clouded by accompanying causes of encephalopathy such as metabolic disturbances, infection, uraemia, and hepatic encephalopathy. For this reason, a heightened index of suspicion is required when treating patients with or without renal impairments, even when cefepime dose is appropriately adjusted.

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Pituitary Macroadenoma in a Girl with Male Karyotype: A Rare Case Study

Fatinah Shahab[1,2], Tania Tedjo Minuljo[3], Calvin Layuk Allo[3], Dody Priambada[4], Mahayu Dewi Ariani[5], Sultana MH Faradz[5*]

[1]Master in Biomedical Science, Faculty of Medicine, Diponegoro University, Semarang, Indonesia.
[2]Faculty of Medicine, Wahid Hasyim University, Semarang, Indonesia.
[3]Division of Endocrine Metabolic, Departement of Internal Medicine, Faculty of Medicine, Diponegoro University - Dr. Kariadi General Hospital, Semarang, Indonesia.
[4]Departement of Neurosurgery, Faculty of Medicine, Diponegoro University - Dr. Kariadi General Hospital, Semarang, Indonesia.
[5]Division of Human Genetics, Center for Biomedical Research, Faculty of Medicine, Diponegoro University, Semarang, Indonesia.

**** Corresponding Author:***
Prof. Sultana MH Faradz, MD, Ph.D. Center for Biomedical Research (CEBIOR). Faculty of Medicine, Diponegoro University. Jl. Prof Sudarto SH No.1, Tembalang, Semarang 50275, Indonesia. Email: sultanafaradz@gmail.com.

ABSTRACT

Macroadenoma is a tumor that typically develops in the epithelial cells of the pituitary gland. Patients suffering from the condition are often asymptomatic with complaints that are caused by hormonal imbalance. Therefore, chromosome analysis needs to be done to females aged >16 years presenting with amenorrhea. Karyotype 46,XY is a disorder of sex development (DSD) that is caused by the complex process of gene interactions, androgen synthesis, and hormone regulation.

The patient initially came to the hospital for a scheduled transsphenoidal surgery due to pituitary macroadenoma, and later complained of primary amenorrhea and atypical external genital. Furthermore, physical examination of genitalia revealed mild clitoromegaly without obvious introitus vagina. Laboratory testing showed elevated prolactin and testosterone level, while ultrasonography imaging revealed the absence of the uterus and ovaries. The brain magnetic resonance imaging (MRI) demonstrated a pituitary adenoma, and cytogenetic analysis showed 46,XY karyotype. Subsequently, hyperprolactinemia, imaging, and histopathology examination were used to confirm pituitary macroadenoma in the patient. It was assumed that the undermasculinized genitalia was caused by hormonal disorders including the deficiency of androgen action or 5-alpha-reductase enzyme. 46,XY DSD has many different symptoms, hence, clinicians need to be aware of potential multifactorial aetiologies. Imaging of internal genitalia, hormonal and chromosomal analysis should be carried out to assess patients with unknown causes of the disorder. Molecular analysis needs to be carried to exclude the possible gene mutation.

Keywords: *Disorder of sex development (DSD), primary amenorrhea, pituitary macroadenoma.*

INTRODUCTION

Disorder of sex development (DSD) is a condition that is characterized by atypical development of chromosomal and gonadal/ anatomical sex organs,[1-6] and it occurs in the ratio of 1: 2500-5000 live births.[6,7] Furthermore, it appears in various forms at different ages,[8,9] and is classified into three main categories, namely 46,XYDSD; 46,XX; and sex chromosome DSD.[1,7,10] Phenotypes of patients with 46,XY DSD range from female external genitalia to atypical male phenotype with testicular regression.[4,5,11,12] The victims generally seek medical attention, which is often delayed until puberty or a later time due to the lack of breast development and/or primary amenorrhea.[13] Meanwhile, the underlying cause of the disorder can be attributed to gene mutations complex process, androgen synthesis disorders as well as hormone regulation. Further tests, such as hormonal, imaging, and cytogenetic analysis, which complement physical examination are necessary to establish a diagnosis of the condition.[2,7,14] The genetic aetiology of most cases of the disorder is heterogeneous, hence, it remains debatable whether every patient with 46,XY DSD needs to experience parallel sequencing of a wide range of genes.[15]

A complete family history, including pedigree and history of consanguinity, is important and need to be carefully reviewed in cases of DSD.[2,7] Furthermore, physical examination of secondary sex characteristics by Tanner staging and a detailed assessment of external genitalia anatomy by Quigley staging are the first steps for its diagnosis. Laboratory evaluation for FSH and LH are needed to observe the pituitary function. Loss of LH and FSH causes amenorrhea, which is characterized by hypogonadotropic hypogonadism. Prolactin and testosterone level tests are also needed to confirm the diagnosis of the disease. Meanwhile, elevated prolactin level is a symptom of pituitary tumor.[2] Laboratory testing for FSH, LH and prolactin levels help to determine the endocrine system's role in the pathogenesis of primary amenorrhea symptoms. Ultrasonography of the pelvic region is used to confirm the presence or absence of female reproductive organs as well as to locate the gonads.[16]

Pituitary adenoma is the third most common brain neoplasm, and it accounts for approximately 15% of all primary brain tumors.[17,18] Furthermore, the increased tumor size produces many symptoms, such as headache, visual defect, olfactory dysfunctions, and various hypopituitarism manifestations. Headache and visual defects are the most common symptoms that occur in 40-70% of patients. Brain MRI (Magnetic Resonance Imaging), ophthalmologic monitoring, and hormone tests are needed to evaluate a pituitary tumor. However, brain MRI is the most sensitive tool to assess the pituitary gland.[18]

Primary amenorrhea has several causes, and chromosomal abnormalities are the most common cause, which account for 40% of cases.[16] Therefore, cytogenetic analysis needs to be performed to evaluate chromosome aberration. This is a case study of a patient who was diagnosed with primary amenorrhea and atypical external genitalia after experiencing transsphenoidal surgery to treat pituitary macroadenoma.

CASE ILLUSTRATION

A 43 years old woman [II.9] was referred to Dr. Kariadi province referral hospital for evaluation of primary amenorrhea. The patient's weight and height were 50 Kg and 157 cm, respectively, with a body mass index of 20,28 kg/ m^2. Furthermore, there was no family history of the condition, as shown in **Figure 1**. The patient also experienced visual disturbances on the right eye since the previous year, which slowly got worse. Visual acuity test using Snellen Chart was on the right eye showed a value of 2/60 and left eye >3/60. Patient also did not complain of headache.

On physical examination, the patient had no female breast development, as seen in Tanner stage 1, while pubic and axilla hair growth was normal, as seen in Tanner stage 5. Inspection of the external genitalia showed normal labia majora and minora. However, mild clitoromegaly and a small perineal opening without obvious introitus vagina were detected. There was also a 2 cm palpable mass that is similar to testes in the left side of labia majora, as shown in **Figure**

2. Based on an interview with the patient and family, the genital ambiguity occurred right from birth.

Laboratory testing revealed an elevated prolactin level of 686.83 ng/mL as well as an increased testosterone level of 85.03 ng/ml. Furthermore, the patient had a normal FSH, LH, and thyroid profile with values of 16.6 mIU/ml, 4.01 mIU/ml, and 15,52 ρmol/L, respectively. **Table 1** shows the result of the hormonal assay.

The pelvis ultrasound imaging showed the complete absence of internal genital organs, namely uterus and ovaries. The initial brain MRI demonstrated a pituitary macroadenoma, which extends to the right parasellar (AP 2.71 cm x CC 2.81 cm x LL 2.72 cm), thereby causing an encasement on the right internal carotid artery and compression in the intracranial part of the right optic nerve and optic chiasm. However, there was no bleeding or sign of elevated intracranial pressure, as shown in **Figure 3**.

Transsphenoidal surgery was performed, which revealed that the tumor was a pituitary macroadenoma. The histopathological microscopic examination of the excised tumor also showed that the hypercellular mass contains medium cells that formed a solid structure bonded by connective tissue and blood vessels. Furthermore, the pseudorossette structure contains polarized cells with elongated cell processes as well as a round or oval nucleus, mild pleomorphic, and stippled chromatin with sparsely granulated cytoplasm. The mitotic structures were difficult to locate, and there were also tumor areas with fibrosis as well as inflammation of lymphocytes, histiocytes, macrophage, cellular organization, prolonged bleeding, and cystic degeneration. However, there were no visible areas of necrosis and no sign of malignancy on the preparation. The histopathological features were consistent with pituitary macroadenoma accompanied by chronic degeneration and inflammation.

Table 1. Hormonal profile of the patient with primary amenorrhea

Hormone	Result	References range
Testosterone in ng/ml	85.03	10.83-56.94
FSH in mIU/ml	16.6	Follicular phase : 3.03-8.08 Mid-cycle peak : 2.55-16.69 Luteal phase : 1.38-5.47 post menopause females without HRT : 26.72-133.41
LH in mIU/ml	4.01	Follicular phase :1.80-11.78 Mid-cycle peak : 7.59-89.08 Luteal phase : 0.58-14.00 Post menopause females without HRT*: 5.16-61.99
Prolactin in ngmL	686.83	5.18-26.53
TSHs in μIU/mL	3.41	0.51-4.94
free T4 in ρmol/L	15.52	10.6-19.4

*HRT: hormone replacement therapy

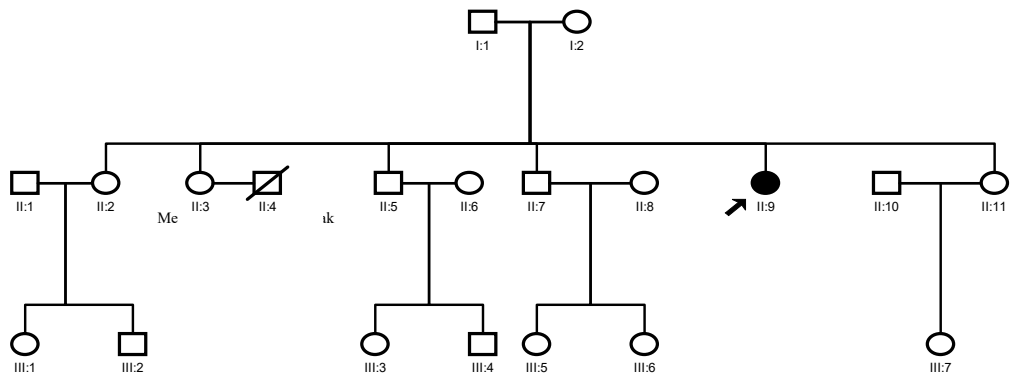

Figure 1. Pedigree of the family showing no family history of the same condition. Circles represent females and squares represent male gender. A slash through the symbol indicates that the family member is deceased. Open symbols are unaffected, while the filled circle denoted by an arrow represents the affected patient [II.9].

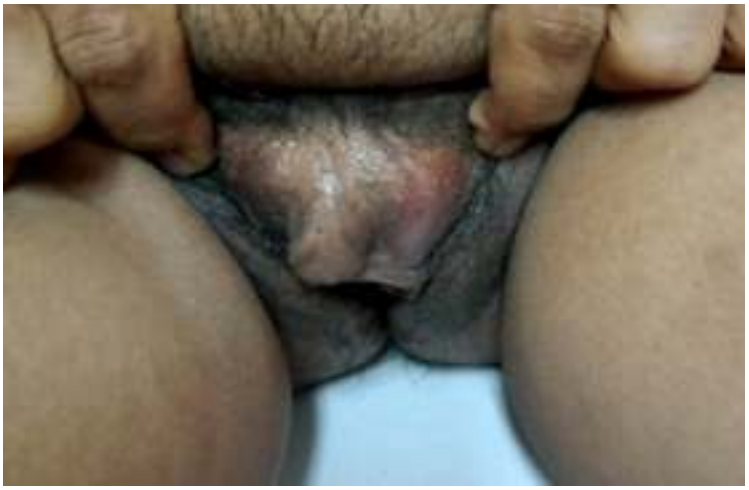

Figure 2. Physical examination of the external genitalia showed normal labia majora and minora. Mild clitoromegaly was detected with a small vaginal-introital opening without obvious bulging of the hymen. A 2 cm palpable mass that is similar to testes was found in the left side of labia majora, while the pubic hair had normal growth, as seen in Tanner stage 5.

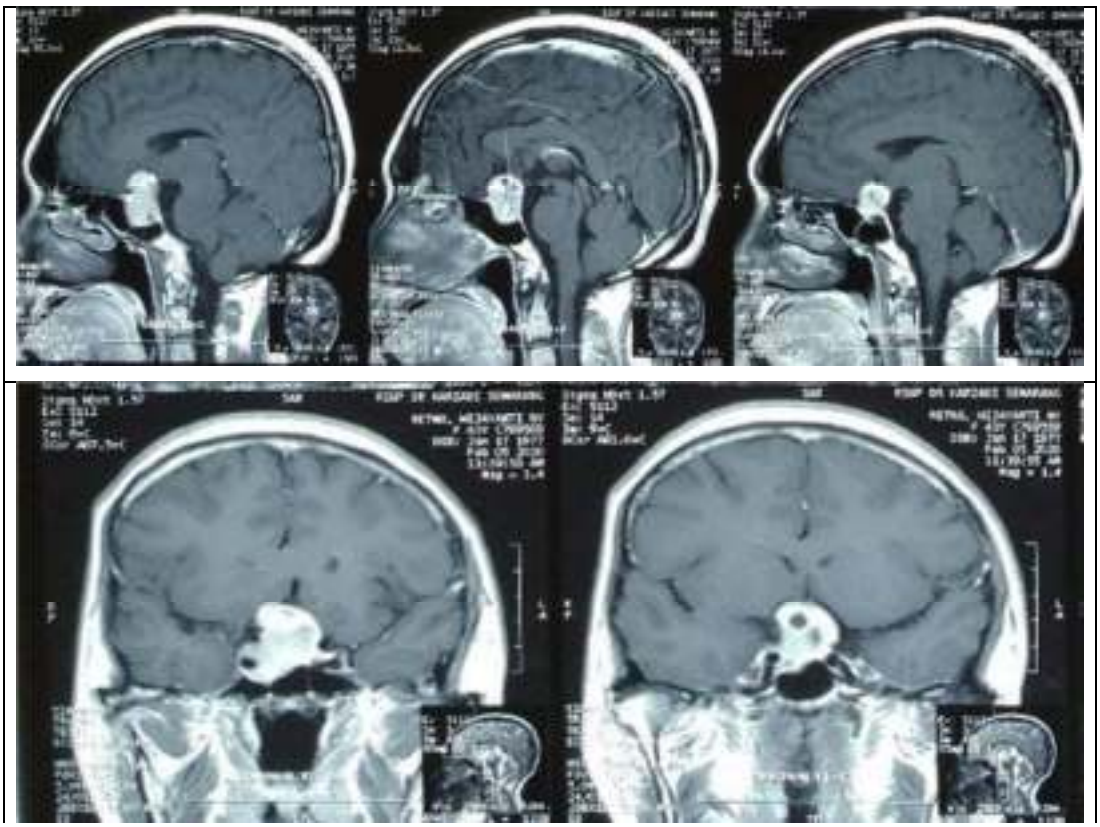

Figure 3. Brain MRI on the first admission. Preoperative MRI revealed a pituitary adenoma, which extends to the right parasella (AP 2.71 cm x CC 2.81 cm x LL 2.72 cm) causing an encasement of the right internal carotid artery as well as compression in the intracranial part of the right optic nerve and optic chiasm. There was no bleeding or sign of elevated intracranial pressure.

Cytogenetic examination was carried out, which indicated a male karyotype 46,XY without structural and numerical abnormalities. Meanwhile, during hospitalization, the patient was monitored daily and Diabetes Insipidus (DI) occurred 3 days after the surgery with polyuria >3 L a day. The DI was transient and recovered in the first postoperative week.

We followed up the patient for three months after the surgery and there was no recurrence of tumor, as shown on the MRI.

Ophthalmological examination showed right-sided visual impairment and bitemporal visual field defect. The neurological examination was normal as well as the muscle power of extremities for both sides (grade 5/5).

DISCUSSION

Aetiology of patients with 46,XY DSD undermasculinized male is associated with enzyme disturbances that occur during testosterone synthesis as well as androgen insensitivity syndrome, deficit of 5-alpha-reductase enzyme, gonadal dysgenesis, and ovotesticular DSD. Meanwhile, some of the 5α-reductase deficiencies are often misdiagnosed as androgen insensitivity syndrome because they have a similar clinical phenotype, while others escape recognition completely.[19,20]

Diagnosis of the disorder is established by assessing the patient's family history, physical examination, hormonal analysis, imaging, and cytogenetic analysis.[2,21] In this case, the diagnosis was made based on the clinical manifestations and available laboratory investigations.

The symptoms of the patient include primary amenorrhea, phenotypic female external genitalia, lack of breast development, and mild clitoromegaly. Meanwhile, mild clitoromegaly was found at birth but the primary amenorrhea was diagnosed late. 7% labial and/or clitoral anomalies were also observed in the patient.[22] There was a small mass in the left side of the labia majora, which was similar to the shape of a testis, while on the right side, there was no palpable mass. Consequently, it was assumed that the right testis was located in the inguinal or intra-abdominal region. Male gonads are palpable in the majority of 46,XY DSD cases depending on the location of the external genitalia.[11] Some of the clues used for the diagnosis of DSD in older patients include unrecognized genital ambiguity, delayed or incomplete puberty, and primary amenorrhea.[16,22,23]

Ultrasonography imaging is an effective diagnostic tool used to identify the presence/absence of uterus and ovaries in 46,XY DSD cases. Furthermore, females with only primary amenorrhea caused by pituitary dysfunction often have a normal uterus. Detailed study on the patient revealed that there was no sign of uterus and ovaries, which indicates the absence of female internal reproductive organs. LH, FSH, and TSH levels were then evaluated to assess the pituitary function, and the result showed that the patient had normal pituitary hormone levels.

The 5-Alpha-Reductase Deficiency (5-ARD) is a rare autosomal recessive symptom of 46,XY DSD caused by mutations in steroid 5α-reductase 2 (SRD5A2).[24] The deficiency was suspected in this case because of the characteristic phenotype and increased testosterone level. Furthermore, testosterone cannot be converted to dihydrotestosterone (DHT) at the external genitalia target cells of a patient with the condition. DHT is required for normal masculinization of the external genitalia, and the patient had an elevated testosterone level of 85.03 ng/ml. Meanwhile, the testosterone level ranged from 35 to 84 in other studies. Diagnosis of mutation in 5α-reductase enzyme can be made by DNA analysis, but it was not performed in this case. The diagnosis of 5α-reductase deficiency was assumed to be based on the patient's clinical presentation and hormonal assay, which revealed an elevated testosterone level. Furthermore, the 3 generation pedigree shows that there was no consanguinity and no other member was affected, hence, the ARD was bearable. Gonadal dysgenesis is characterized by a low testosterone level,[19] but this current case was different.

Complete Androgen Insensitivity Syndrome (CAIS) cases often have a history of primary amenorrhea,[2] and female patients usually have a normal-looking clitoris, vaginal introitus, labia minora, and labia majora. Depending on the severity of androgen resistance, the clinical features also vary with unilateral or bilateral gonads that can be located anywhere along the path of embryonic testicular descent. However, patients with CAIS can be distinguished by adequate breast development and X-linked pattern of inheritance.[7,12,14] Breast development and pubertal growth spurt are normal because testosterone was aromatized to estrogen in the circulation.[2] CAIS causes the production of

testosterone, but androgen action is deficient because of mutations in the androgen receptor (AR) gene. Therefore, molecular sequencing of AR gene is needed to identify mutations in 90-95% cases.[2,15,22,25]

DSD was suspected in the patient due to the presence of clitoromegaly, tanner stage 1 no breast development, and palpated gonad in the left side of labia majora, hence, clinicians are advised to perform a cytogenetic examination to determine the karyotype and gender assignment when needed. CAIS and gonadal dysgenesis are characterized by the presence of female external genitalia.[5] Meanwhile, the absence of virilization results in female–typical genitalia was strictly linked to the androgen action and AR function. Both adrenal and ovarian androgens facilitate the growth of axillary and pubic hair in girls. Therefore, any type of alteration along the androgen pathway can lead to impaired virilization. Patients with the syndrome normally develop testes due to the presence of the SRY region and they also produce testosterone whose action is not effective because of the AR gene mutation. Therefore, they lack male genitalia, except for testes. The hormonal profile of CAIS patients is characterized by high LH and normal FSH levels, while testosterone results are typically within the normal male range but increase relatively to the female range.[25]

There was an elevated prolactin level in the patient due to prolactin-secreting pituitary macroadenoma. Meanwhile, the adenoma accounts for 85% of tumors in the pituitary gland,[26] and it affects approximately 20% of the general population.[17] In this present case, brain tumor is a pituitary adenoma that was caused by hyperprolactinemia. A pituitary macroadenoma was revealed after transsphenoidal surgery, and there was a positive relationship between hyperprolactinemia and visual changes. Several etiopathogenetic hypotheses have been proposed to explain brain tumors and primary amenorrhea. One of the pathological causes of hyperprolactinemia is pituitary adenoma.[27]

At the time of diagnosis, the patient complained of visual disturbance, which was in the form of decreased visual acuity in the right eye due to a pituitary macroadenoma that compressed the intracranial part of the right optic nerve and optic chiasm. Furthermore, compression of the visual pathways causes a disturbance in the visual functions, such as a slow progressive visual loss.[17]

The patient developed DI with polyuria >3 L a day, which was transient 72 hours after surgery, and recovered over the next couple of days. Meanwhile, DI, which can either be transient or permanent is a common complication that occurs after neurosurgery of the pituitary gland, specifically with the transsphenoidal approach.[28,29] Most cases of the disease were transient and the patient recovered within 2 weeks of post-operative.[28] It is usually caused by mild-reversible injury to the pituitary stalk.[29]

After 3 months follow-up, MRI showed that there was no recurrence of the tumor. Meanwhile, the recurrence rate in patients with pituitary adenoma 4 years after surgery was 22%.[17] The recovery of visual function usually correlate with time, but there was a slow improvement of the right eye in this case.[17]

The clinical management of 46, XY DSD patients includes prophylactic gonadectomy, which needs to be carried out due to an increased risk of gonadal malignancy.[11,21] Furthermore, patients with abdominal gonads have a high risk of germ-cell tumors development[30] with an incidence rate of approximately 25-33%.[23]

Another appropriate management of XY female with the disorder includes hormonal therapy and psychological counseling.[14] Optimal care of the condition also requires a multidisciplinary approach.[4,5,23] Psychological counseling for the patient is important, specifically when they experience gender dysphoria after being diagnosed with DSD.[8,12,23]

The presence of female external genitalia and mild clitoromegaly since birth as well as visual disturbance that was observed lately in a single patient is a rare combination, and it indicates the possibility of a dual diagnosis. Therefore, further examination is needed to determine whether the atypical genitalia and pituitary macroadenoma contributed to the disease condition. This rare coexistence of 46,XY DSD with pituitary macroadenoma was assumed to be a coincidence, hence, a case study was carried out. However,

there were some limitations because molecular observation was not carried out.

CONCLUSION

Based on the results, 46, XY DSD has many different symptoms, and this case highlighted the atypical genital and primary amenorrhea, which were caused by various factors, hence, a multidisciplinary approach was carried out. Furthermore, chromosomal analysis is important to assess the genetic factor and sex assignment of patients with the disorder. Hormonal problems in the central pituitary gland can also be considered in some cases.

Although the precise mechanism was not determined with advanced molecular analysis of 46,XY DSD in the patient, a dual diagnosis is still impossible. This case study is expected to provide valuable insight on the approach that can be used to manage the disorder.

FUNDING SOURCE

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Paraganglioma in The Urinary Bladder: A Pitfall in Histopathologic Diagnosis

Jamie Lim Co, Maria Lourdes Luna Goco, Jeffrey Santos So*

Department of Pathology, St. Luke's Medical Center – Global City, Taguig City, Philippines.

**** Corresponding Author:***
Jamie Lim Co, MD. Department of Pathology, St. Luke's Medical Center – Global City. 5th Avenue, Taguig, 1634, Metro Manila, Philippines. Email: bluecojamie8293@gmail.com.

ABSTRACT

Paraganglioma of the urinary bladder is a rare neuroendocrine tumor which originates from the chromaffin tissue of the sympathetic nervous system. It only accounts for about 0.05% of all vesical tumors. Bladder paraganglioma may also present with non-specific symptoms which could easily lead to misdiagnosis. In this report, emphasis on the histomorphology and immunohistochemical profile of the tumor is stressed as the morphological findings could overlap with relatively more common urothelial neoplasms. Distinction from other tumors is of utter importance because of different therapeutic options. Here, we present a case of a 52 year-old, filipino, male, previously diagnosed with colonic tubulovillous adenoma, presenting with dysuria and hematuria who, after undergoing CT Stonogram revealed an incidental finding of a lobulated mass measuring 5.7 cm located at the anteroinferior portion of the urinary bladder wall.

Keywords: *Paraganglioma, pitfall, urinary bladder, diagnosis.*

INTRODUCTION

Paraganglioma of the bladder, also called an extra-adrenal pheochromocytoma, originates from the chromaffin tissues of the sympathetic nervous system located in the detrusor muscle of the urinary bladder.[1-3] The first case was reported by Zimmerman in 1953.[2] Of the 10% of the extra-adrenal paragangliomas', 10% are localized within the bladder which accounts for only 0.05 % of all bladder tumors.[1]

Being a rare entity, it has a propensity to be misdiagnosed because of 1) its frequent involvement of the muscularis propia[1,4-6] 2) its non-specific symptoms which also occur in other tumors[3-6] 3) its morphology and immunohistochemistry that can mimic other tumors[5,7], and 4) its rare occurrence in the bladder.[1,3-6]

CASE ILLUSTRATION

A 52-year-old, filipino man, presented with a 7-month history of intermittent dysuria and hematuria. He had no other irritative lower urinary tract symptoms. The patient had no history of headache, palpitations, and dizziness. His medical history consisted of a history of hypertension and colonic tubulovillous adenoma diagnosed last 2018. There was no significant family history of similar disease. Physical examination was unremarkable and vital signs were stable. A non-contrast axial 256-multislice CT stonogram was done which revealed an intravesical, slightly irregular to lobulated mildly hyperdense mass approximately measuring 4.7x4.4x5.7 cm. This is located at the anteroinferior portion of the urinary bladder wall, near the neck. Cystoscopy revealed a large, irregularly shaped mass located at the

left anterolateral wall of the bladder and adjacent to the bladder neck. Transurethral resection of the bladder tumor was subsequently done. His intra- and post-operative course remained uneventful. The specimen was then submitted to the Histopathology Department. Description of the gross specimen consisted of several cream-tan to brown-tan, irregular soft tissue fragments with an aggregate measurement of 2.0 x 2.0 x 0.7 cm. The entire specimen was submitted for processing.

Microscopic evaluation showed nests of oval to polygonal cells underlying an unremarkable urothelium. These cells are arranged in nested, zellballen pattern, separated by prominent fibrovascular stroma. These cells also have abundant granular, clear to amphophilic cytoplasm, uniform round to oval nuclei with regular nuclear contour. Mitosis was inconspicuous and no necrosis was seen. **(Figure 1 and Figure 2)**.

Histological differential diagnosis at this time were Urothelial Carcinoma, Colorectal Carcinoma, Malignant Melanoma, and Paraganglioma.

Immunohistochemistry showed tumor cells to be positive for Chromogranin, Synaptophysin, and GATA-3 **(Figure 3 to Figure 5)**.

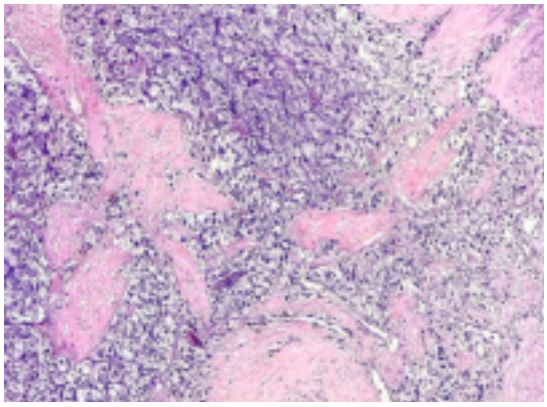

Figure 1. Low magnification of the urinary bladder paraganglioma.

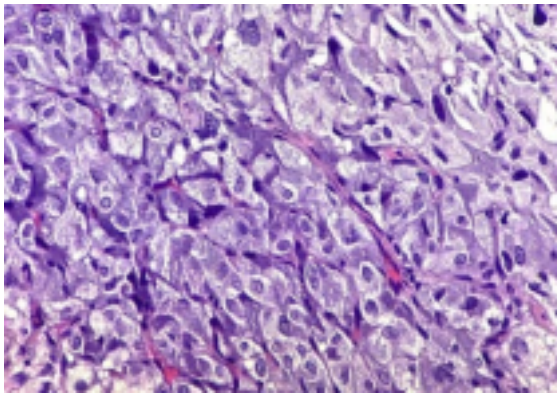

Figure 2. High magnification of the urinary bladder paraganglioma.

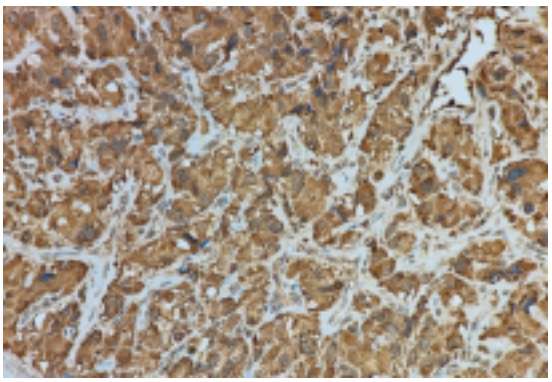

Figure 3. High magnification of positive cytoplasmic staining for Chromogranin.

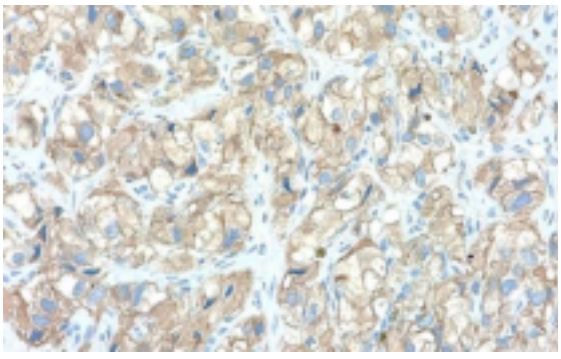

Figure 4. High magnification of positive cytoplasmic staining for Synaptophysin.

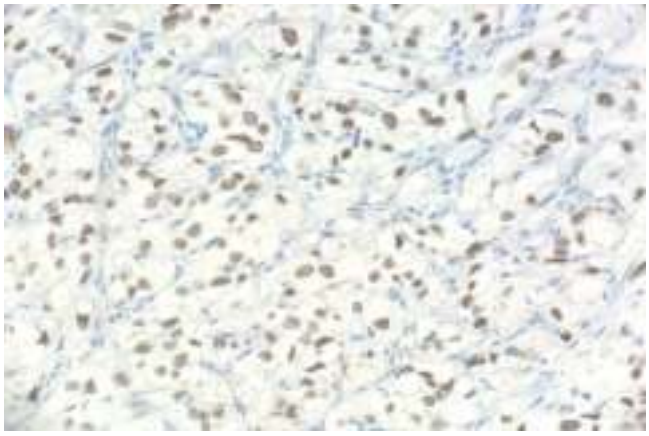

Figure 5. High magnification of positive nuclear staining for GATA-3.

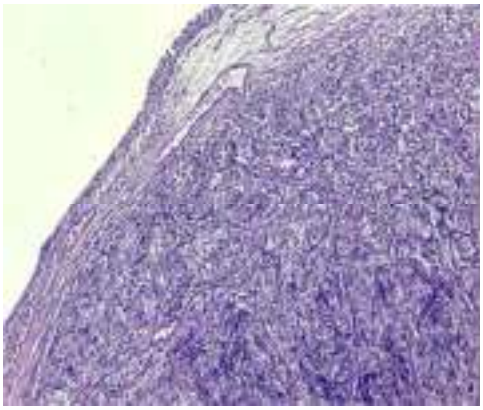

Figure 6. Low magnification of the Bladder Paraganglioma focally extending to the urothelium.

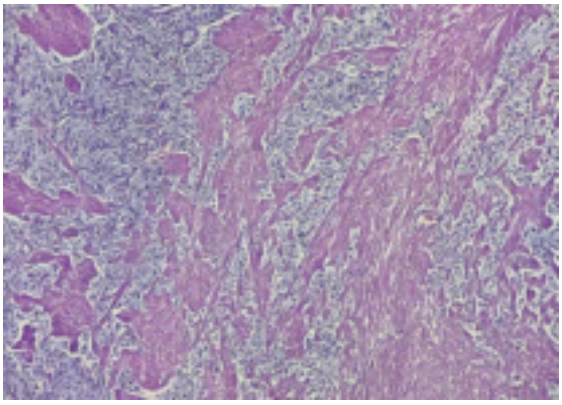

Figure 7. Low magnification of the Bladder Paraganglioma located within the muscularis propria.

The tumor cells are negative for CK, CK20, CDX2, Melan-A, Beta-catenin, and SATB2**.** Given the morphology of the tumor and the immunohistochemical profile, a diagnosis of Paraganglioma was rendered.

Two months after the initial procedure, the patient was admitted for a partial cystectomy. Consent and clearance were secured and on the 2nd hospital day, the patient underwent the planned procedure. Intraoperative finding revealed a 4.0 x 4.0 cm pedunculated tumor extruding from the left posterolateral bladder diverticulum. Specimen was then submitted to the Pathology Department for histopathologic processing. The patient's postoperative course was uneventful and he was then later discharged on the 6th hospital day.

Description of the gross specimen consisted of a tan brown to orange tan, pedunculated, firm tissue measuring 6.0 x 5.8 x 3.5 cm. Cut sections show a fairly circumscribed, pink tan, soft to solid mass measuring 5.5x3x2.7 cm. Representative sections were taken.

Microscopic evaluation showed cells showing the same histomorphology as those seen in the previous biopsy specimen. These tumor cells extend from the urothelium and eventually involve the muscularis propria (detrusor muscle). **(Figure 6 and Figure 7)**

The case was signed out as Paraganglioma with the Tumor involving the Muscularis Propria (detrusor muscle) and focally extending to the Urothelium.

DISCUSSION

About 98% of the paragangliomas are located in the abdomen, 90% of these are in the adrenal

medulla and 10% of them in extra-adrenal sites.[3,8]

Paragangliomas can occur in patients of any age, with a mean age at 43.3 years old.[1] Although more common in females[1,9], there are studies that show that paraganglioma can also occur in males. As shown by four studies where males are affected with ages ranging from 39 to 78-years-old, two of which are Japanese.[4,10-12] In a study done in Japan last 2020, it was found out that both males and females are equally affected. Out of 162 patients diagnosed with the tumor, 50% consisted of males.[11] Molecular studies of said tumor show frequent losses at 1p, 3q, and 22q and germline mutations in SDHA and SDHB genes.[1]

Paraganglioma occurs in any part of the bladder wall but has a predilection for the detrusor muscle with the most common locations being the dome and trigone of the bladder.[1] The tumor can be functional (catecholamine secreting) or non-functional.[1,13] Clinical symptoms are related to catecholamine release. These include hypertension, headache, blurred vision, intermittent gross hematuria, and hypertensive crisis during micturition.[1,4] Only a minority presented with catecholamine-associated symptoms accounting to about only 13% of the total cases.[4,5] While most of the cases presented with nonspecific symptoms such as painless hematuria or no symptoms at all.[4,5,15] Laboratory findings show elevated urine and serum catecholamine levels.[13]

Imaging findings are important for the diagnosis as it assesses the shape, size, and location of the tumor[14] which shows the bladder paraganglioma as a well-defined, solid, ovoid vascular mass located within the urinary bladder wall.[13] According to P. Humphrey, et al (2016), the tumor usually consists of a well circumscribed, exophytic lesion with an average size of 3.9 cm but tumor size can increase to up to of 9 cm.

On histomorphology, the majority of cases consist of the cells arranged in nests, called Zellballen pattern, while the remaining 20% are arranged in a diffuse pattern. They are separated by vascular network or fibrous septa.[1,15] These cells are large and polygonal with abundant clear, granular, amphophilic or acidophilic cytoplasm and uniform round to ovoid nuclei. Mitosis, focal hemorrhage, and necrosis are rare.[1,4,16] The tumor cells can be seen located within the muscle bundles of the muscularis propria (detrusor muscle) with absence of desmoplasia.[1,4,5] Paragangliomas can be confirmed with positive immunostaining for neuroendocrine markers such as chromogranin and synaptophysin[1,4,9], with a majority also staining for GATA3[1,7], and S100 in sustentacular cells.[1] They are negative for epithelial markers.[1,4,9]

Because of the frequent involvement of the muscularis propria and positive staining for GATA-3, a misdiagnosis of Urothelial Carcinoma can be given.[1,4,5,7] Records of fifteen patients diagnosed with paraganglioma of the urinary bladder were reviewed back in 2004. Twelve of which were transurethral resection specimens while the remaining three were partial cystectomies. Tumors showing nested, zellballen pattern consisted of 12 out of the 15 cases while diffuse growth pattern were seen in the remaining 3 cases. Ten cases showed the tumor nests located between the muscularis propria. Eleven of these cases were misdiagnosed as urothelial carcinoma and the remaining four were diagnosed as bladder tumor.[5] In our case, the senior author's previous experience as well as his paper on paragangliomas, helped in including is a differential diagnosis. As well as not readily diagnosing this as a urothelial neoplasm.

Urothelial Carcinoma is the most common malignant neoplasm of the urinary tract with higher pathologic staging indicating the presence of muscularis propria invasion.[1]Majority (67-90%) of the tumor cells express GATA3 and cytokeratin markers.[1] On histomorphology, urothelial carcinoma may exhibit a nesting pattern which could mimic the nesting, zellballen pattern of the Paraganglioma. However, cytologic atypia of urothelial carcinoma is usually more pronounced and there are no vascular networks surrounding the tumor cells. The tumor nests invading the muscularis propria should also exhibit a desmoplastic response.[5]

Malignant Melanoma is a neoplasm of melanocytic origin.[1] As of 2021, there are only a total of 40 cases of Malignant Melanoma of the bladder[17] reported whereas compared to Paraganglioma of the urinary bladder, wherein

there were already a total of 69 reported cases just from the year 2010 to 2021 alone.[18] Extracutaneous melanoma is rare and consists of only 4-5% of all diagnosed melanoma with primary malignant melanoma only accounting for less than 0.2% of all melanomas.[19,20] Classic microscopic feature consists of large epithelioid or spindle cells containing melanin pigments. Melanoma cells show positivity for S100, HMB45, and Melan A.[1] Based from the rarity of the cases as well as the histomorphology and immunohistochemical profile of the index case, we favor a diagnosis of a Paraganglioma rather than a Malignant Melanoma.

For metastatic tumors of the bladder, majority originates from the colorectum[1] with the genitourinary tract infiltrated by 3-10% of the total cases of advanced colorectal tumors.[21-24] Tumors that originate from this location expresses CK20, SATB2, and CDX2.[1,9,25] Due to the presence of APC or CTNNB1 mutation, the tumor also exhibits nuclear expression of β-Catenin.[9] Histomorphology and immunohistochemical findings of the index case does not support these findings. However, due to the patient's aforementioned history of colonic tubulovillous adenoma, a colorectal lesion was still ruled out via immunohistochemistry.

Surgical resection is the common treatment modality for bladder paraganglioma.[10,15,18] This includes: (1) Transurethral resection of the bladder tumor (TURBT) (2) Partial Cystectomy and (3) Total Cystectomy.[15,18] Transurethral resection of the bladder tumor (TURBT) is the treatment of choice for non-functional, non-invasive lesion measuring less than 3 cm in size while partial cystectomy is done when the tumors extend into the deep layers of the detrusor muscles. The last option consists of a total cystectomy if the lesion is too large that preservation of the bladder could not be considered or if there is metastasis to the lymph nodes. Long term follow up with annual measurement of catecholamine is recommended because of the increase chance of recurrence and metastasis.[10,15,18]

CONCLUSION

Although this tumor is rare, having its own histologic and immunohistochemical features should prompt the Pathologists to include it in their differential diagnosis. However, misidentification of the tumor still occurs because of its rare entity, as well as its histological features mimicking other more common urothelial neoplasms. Identification and accurate diagnosis necessitate complete knowledge of the morphological, histochemical, and molecular features of this tumor in order to better differentiate it from other neoplasms.

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We have no conflicts of interest to disclose. All authors declare that they have no conflicts of interest.

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Plasmodium Ovale Malaria: Endemic Areas in Indonesia

Umar Zein[1,2], Hadiki Habib[1], Hadyanto Lim[3]*

[1]Dr. Umar Zein Tropical Diseases and Infectious Clinic, Medan, Indonesia.
[2]Department of Internal Medicine, Faculty of Medicine, North Sumatra Islamic University, Medan, Indonesia.
[3]Division of Cardiovascular Medicine, Department of Pharmacology, Faculty of Medicine, Methodist University of Indonesia, Medan, Indonesia.

Corresponding Author:
Prof. Umar Zein, MD., PhD. Dr. Umar Zein Tropical Diseases and Infectious Clinic. Jl. Jermal 3 no. 12 Medan 20227, Indonesia. Email: hadikihabib@gmail.com.

ABSTRACT

Plasmodium ovale consists of two subspecies – P. ovale wallikeri and P. ovale curtisi. Increased reports of imported malaria ovale in non-endemic regions and mixed infection of P. ovale with other Plasmodium species suggest that P. ovale might be under-detected during routine surveillance. Areas endemic with P. ovale have mostly been reported in African and Western Pacific countries. A recent case report in Indonesia indicated that regions with P. ovale endemicity are not only distributed in Lesser Sunda and Papua, but also in North Sumatra.

Keywords: *Plasmodium ovale, Indonesia, Endemic Area, Malaria, Molecular Tests.*

INTRODUCTION

Malaria is caused by *Plasmodium* parasites. *Plasmodium* can infect humans and animals, such as mammals. Many factors contribute to this infectious disease in humans, such as demography, environment, population mobility, and economic and sociocultural reasons.[1] Malaria is a preventable and treatable infectious disease, and intensive prevention efforts in various endemic areas have reduced the burden of this disease. Endemicity and vector distribution are based on environment, climate, and season among the five *Plasmodium* species, which vary in their distribution. Ovale malaria was seldom reported except in Sub-Saharan Africa and on some islands of the western Pacific.[2] In 2015, 106 countries were reported as sources of malaria transmission. Between 2010 and 2015, the incidence of malaria in the at-risk population (rate of new cases) was 21%. In the same period, the global mortality rate for the at-risk population was 29% in all age groups and 35% in children under 5 years.[3,4] The WHO Global Technical Strategy (GTS) launched in 2015, which aimed to eradicate 90% of the global burden of malaria in 2030, would likely be unmet.[5] Therefore, programs directed at combating malaria in endemic areas should be strengthened, specifically considering the ongoing Covid-19 pandemic.

Although *P. falciparum* is generally considered to cause severe disease and death, a recent meta-analysis reported that *P. ovale* can lead to severe illness with jaundice, anemia, and respiratory failure.[6,7] Thus, it is important to recognize the severity of *P. ovale* infection in order to prevent rare complications. Diagnosis relies on molecular examination using polymerase chain reaction (PCR). Published case reports have shown that all *Plasmodium* species can cause severe malaria.[8-11]

Malaria in Indonesia is still a public health problem. Malaria endemic areas in Indonesia cover several provinces, including the province of North Sumatra. Five species of plasmodium in humans were found with different species in

each endemic area, and the most common was *P. falciparum* species. So far, ovale malaria endemic areas in Indonesia have only been reported in two provinces, namely Papua and East Nusa Tenggara.[6] The limited reports of ovale malaria endemic areas in Indonesia are thought to be influenced by the diagnostic methods used in the field. For malaria blood surveys in the field, a rapid diagnostic test (RDT) is always used, because it is easy, cheap, fast and does not require special skills such as microscopic examination. RDT examination can only differentiate diagnostic *P. falciparum* and non-*P. falciparum* infections (*P. vivax, P. malariae, P. ovale,* and *P. knowlesi* cannot be distinguished). To ascertain the morphology of the five plasmodium species, the gold standard is microscopic examination of thick and thin blood with good staining and the microscopic skill and experience of the examiner. Other possible misdiagnostic factors for determining *P. ovale* species on microscopic examination are the presence of mixed infection, low parasite density, and the subspecies of *P. ovale*, namely *P. ovale wallikeri* and *P. ovale curtisi* which can only be distinguished by RT-PCR examination by sequencing.

The discovery of one case of ovale malaria in Gerunggang Village, Langkat Regency, North Sumatra Province which was reported in 2017, is the basis for making this review. Previously the case was diagnosed as mixed-infection with *P. falciparum* and *P. vivax*. It was not previously thought that *P. ovale* might be found, because malaria endemic areas in Langkat Regency have been reported only *P. falciparum* and *P. vivax*. After re-observation of the patient's blood smear, and confirmed by the parasitologist, it was confirmed that the morphology found was typical for *P. ovale*. Unfortunately, due to limited laboratory facilities, we could not proceed with RT-PCR and sequencing to determine the subspecies of *P. ovale* found.[12]

REPORTS OF *PLASMODIUM OVALE* MALARIA WORLDWIDE

P. ovale was the fourth known cause of malaria before the discovery of *Plasmodium knowlesi* in Sarawak, Malaysia in 2004.[13,14] In 1969, Lysenko and Beljaev conducted a geographical analysis of published cases of ovale malaria across the Western Pacific countries, including India, Nigeria, Philippines, Southern China, Iraq, Pakistan, New Guinea, Solomon Island, Bulgaria, Columbia, Venezuela, Macedonia, S. Epirus, Iran, USSR (Armenia, Georgia, Bashkiria), Palestine, Egypt, South America, Duke of York Island, and Indonesia.[15] Sporadic spread and alteration trends in the prevalence of the four *Plasmodium* species were reported, except *for P. falciparum*. Several countries across the African continent, including Ethiopia, Uganda, Equatorial Guinea, and Kenya have recorded cases with *P. ovale curtisi* and *P. ovale wallikeri*.[16]

An imported case of infection by two species (*P. ovale* and *P. falciparum*) in an Indonesian patient working in Cameroon was reported in north Sumatera.[17] Prakash et al. reported a case of ovale malaria from Assa District, India, which was initially diagnosed as vivax malaria.[18] In Southern Bangladesh, Fuchrer et al. reported the first cases of *P. ovale* infection with a percentage of 1.6% in 189 patients using the species-specific nested PCR technique, targeting the small subunit ribosomal RNA (SSU tRNA) gene from 379 patient samples.[19] Singh et al. in 2010 reported regarding 256 patients with *P. falciparum* malaria who were hospitalized in central India and diagnosed microscopically; three cases (1.2%) of *P. ovale* malaria were detected for the first time using species-specific nested PCR with 18s rRNA.[20] Cao et al. identified 98 cases of ovale malaria out of 1,268 malaria cases from Jiangsu Province, China, from 2011 to 2014, most of which were imported from Sub-Saharan Africa.[21] Mitchel et al. reported that *P. ovale* was widely distributed in the Democratic Republic of the Congo, especially *P. ovale curtisi* and *P. ovale wallikeri* in 2013.[22]

Lim et al. reported the first imported malaria case, which was initially diagnosed as *P. vivax* malaria microscopically.[23] Likewise, a case report from Gujarat, India in 2006, showed that the parasite seemed to be *P. vivax* in a thick smear stained with Leishman stain. However, it was revealed as *P. ovale* in a thin smear using standard microscopic and morphological

evaluation.[2] Misidentification of *P. ovale* can occur microscopically when the parasite density is low and other types of *Plasmodium* infection occur concurrently. The morphology of *P. ovale* is similar to *that of P. vivax*, which can lead to an error in the estimation of the current prevalence of ovale malaria and endemic areas by species. Because *P. ovale* possesses a hypnozoite stage in liver cells similar to that found in *P. vivax*, a relapsing course of infection can ensue. Furthermore, both *P. ovale* species, *P. ovale curtisi* and *P. ovale wallikeri,* are sympatric but distinct species, based on the analysis of the MSP-1 (merozoite surface protein-1) sequence in Thailand.[24] Diversity in PocMSP-1 and PowMSP-1 by the MSP-1 sequences from the isolate sample resulted in a low level of sequence, suggesting that *P. ovale curtisi* and *P. ovale wallikeri* originate from a persistently low prevalence. *P. ovale* infections cannot be diagnosed by SSU rRNA-based PCR if coinfection with other *Plasmodium* species is present at a very low parasite density. Hence, the burden of *P. ovale* infection could be underestimated.

An imported case of ovale malaria was reported in 2011[25] in Brazil, which was later confirmed by standard microscopy and PCR. Based on the patient's travel history, it was concluded that the parasite was in the latent hypnozoite form for a minimum of 2 years. It was difficult to ascertain the relationship between the time of exposure to the parasite and the onset of symptoms because of the relatively long incubation period of *P. ovale.* Asymptomatic *P. ovale* infection is usually found in areas endemic with ovale malaria. Additionally, a report [26] in Senegal showed that there was no risk of fever when the parasitemia was 80–799 parasites/mL of blood. The risk of fever increased 11-fold in mixed infections or 93-fold for *P. ovale* when the parasite count was 800–8000 parasites/mL of blood.

Multiple infections of *Plasmodium* species often occur in certain endemic areas because of the presence of several species in the same area. Microscopic findings of multiple infections of *P. falciparum* and *P. malariae* in two children were reported in Central African Republic.[27] Three species were found (*P. falciparum*, *P. malariae*, and *P. ovale*) in real-time PCR examination of the first blood sample after treatment follow-up. *P. ovale* infection was still found in one child after re-examination on day 28, suggesting a delayed appearance of ovale malaria in this mixed infection. The proportion of imported cases due to *P. ovale* and the difference between *P. ovale curtisi* and *P. ovale wallikeri* are important. A descriptive study to analyze the prevalence, proportion, distribution, and origin of *P. ovale curtisi* and *P. ovale wallikeri* in Henan Province was collected from 2010 to 2017 by Zhou et al.[28], and their findings showed that the proportion of imported cases of *P. ovale* was larger than that of *P. vivax.* The latency period of *P. ovale curtisi* was significantly longer than that of *P. ovale wallikeri* in these two subspecies imported into China. Nolder et al. [29] reported the results of PCR examination of *P. ovale curtisi* and *P. ovale wallikeri* infections in blood samples of a British traveler who had malaria. The suspected asymptomatic period between the time of diagnosis was determined, and the time of the patient entering the UK was compared between the two groups. Showed that there are epidemiologically significant differences between the two cases of ovale malaria, suggesting that targeted treatment for *P. falciparum* may not be sufficient to reduce the malaria burden caused by *P. ovale.*

MALARIA MAP IN INDONESIA

One of the Millennium Development Goals (MDGs) was to eradicate malaria in 2015. This commitment was strengthened by the Sustainable Development Goals (SDGs). In the SDGs, the malaria control program is in the third objective, namely, ensuring the health and welfare of all people, with the specific aim of ending the malaria epidemic and neglected infectious diseases by the end of the year 2030. The level of morbidity due to malaria in an area is determined by the annual parasite index (API), which is the number of malaria cases per 1,000 population in a certain country or territorial area in a year. The API in Indonesia has declined since 2011, indicating the success of the malaria prevention program conducted by the central, regional,

community, and related partners in Indonesia.[4]

To date, there has been no mapping of endemic areas for *P. ovale* malaria in Indonesia. The existing mapping considers all malaria cases without specifying the *Plasmodium* species. The existence of *P. ovale* malaria in Indonesia from Belu (East Nusa Tenggara) was reported by Gundelfinger et al., in 1975.[6] Baird et al. reported 34 cases of ovale malaria infection found in 15,806 peripheral slide smear samples examined from 1973 to 1989 from various islands in Indonesia. Of the 514 samples, 25 were obtained from Owi, Irian Jaya (Papua). Other *P. ovale* infection cases originated in two areas in East Flores. However, there were no cases of *P. ovale* malaria in samples from Sumatra, Kalimantan, Sulawesi, and Java.[30] Reports on parasite surveys in Indonesia were recorded between 1900 and 2008 at 2,366 locations with an uneven distribution of locations; 63% of the surveys were conducted in Eastern Indonesia, namely, Maluku, East Nusa Tenggara, and Papua. Of the 16 survey locations, *P. ovale* was only found in East Nusa Tenggara Province and Papua Province, with a prevalence of 0.003% and 0.02%, respectively.[6]

However, a 2017 case report revealed non-imported *P. ovale* mixed with *P. falciparum* infection in North Sumatra Province, which was confirmed by microscopic examination.[12] The endemicity of ovale malaria in three provinces in Indonesia is shown in Figure 1. The distribution of *P. ovale* in people in Indonesia is presented in **Table 1**.

Figure 1. Three Provinces of Endemic Area Ovale Malaria in Indonesia. *Plasmodium ovale* infection has been reported in three provinces, including North Sumatra, East Nusa Tenggara, and Papua

Table 1. The Distribution of Plasmodium ovale in Indonesia.

Province	Year of sample	No. site	No. exams	No. *Pf* (%)	No. *Pv* (%)	No. *Pm* (%)	No. *Po* (%)
North Sumatra	2015	1	75	2 (0.02)	2 (0.02)	-	**1 (1.33)**
East Nusa Tenggara	1975–2009	609	383,950	23,502 (6.1)	19,401 (5.1)	157 (0.04)	11 (0.003)
Papua	1929–2009	694	193,043	19,848 (10.3)	9343 (4.8)	1395 (0.7)	40 (0.02)
Indonesia	1900–2021	2366	1,062,259	61,415 (5.8)	52,336 (4.9)	2299 (0.2)	52 **(1.36)**

Pf, *Plasmodium falciparum*; *Pv*, *Plasmodium vivax*; *Pm*, *Plasmodium malariae*; *Po, Plasmodium ovale*. Modified from Elyazar et al.[6]

CONCLUSION

The mapping of malaria-endemic areas in Indonesia needs to be reviewed because of the detection of various *Plasmodium* species that have not been previously reported. The report of cases of *P. ovale* malaria by the current author and colleagues in North Sumatra needs to be followed up by the government and related sectors to conduct a widespread blood survey with microscopic examination, followed by nested PCR confirmation. This should yield a new map of malaria-endemic areas in Indonesia in general, and in North Sumatra in particular. The endemicity map may provide a rational basis for future malaria management strategies.

AUTHORS' CONTRIBUTIONS

All authors have helped draft the manuscript. All authors have read and approved the final manuscript.

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The authors declare that they have no competing interests.

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Primary Central Nervous System Lymphoma in an Immunocompetent Young Adult Patient: A Rare Case

Aisyah Wirdah[1], Norman Djamaludin[2], Mediarty Syahrir[2], Yenny Dian Andayani[2], Mita Andriani[1], Yunni Diansari[3]

[1]Department of Internal Medicine, Faculty of Medicine Universitas Sriwijaya - Mohammad Hoesin General Hospital, Palembang, Indonesia.
[2]Medical Hematology Oncology Division, Department of Internal Medicine, Faculty of Medicine, Universitas Sriwijaya - Mohammad Hoesin General Hospital, Palembang, Indonesia.
[3]Division of Neuro Oncology, Department of Neurology, Faculty of Medicine Universitas Sriwijaya - Mohammad Hoesin General Hospital, Palembang, Indonesia.

Corresponding Author:
Aisyah Wirdah, MD. Department of Internal Medicine, Faculty of Medicine Universitas Sriwijaya - Mohammad Hoesin General Hospital. Jl. Dr. Muhammad Ali, Sekip Jaya, Kota Palembang, Sumatera Selatan 30114, Indonesia. Email: aisyahwirdah297@gmail.com.

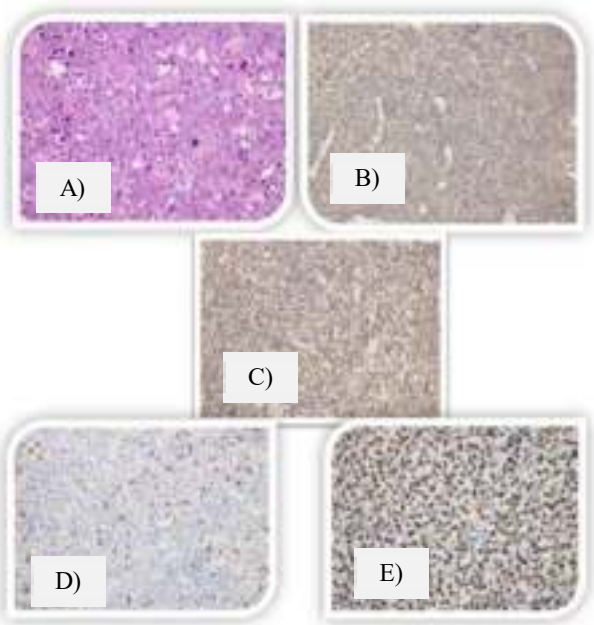

Figure 1. *Pathological Anatomy. A) Hematoxylin & Eosin A PCNSL Biopsy Basal Ganglia. B). CD 20 positive. C). CD 45 positive. D). CD 3 negative. E). KI 67+90-95% high grade.*

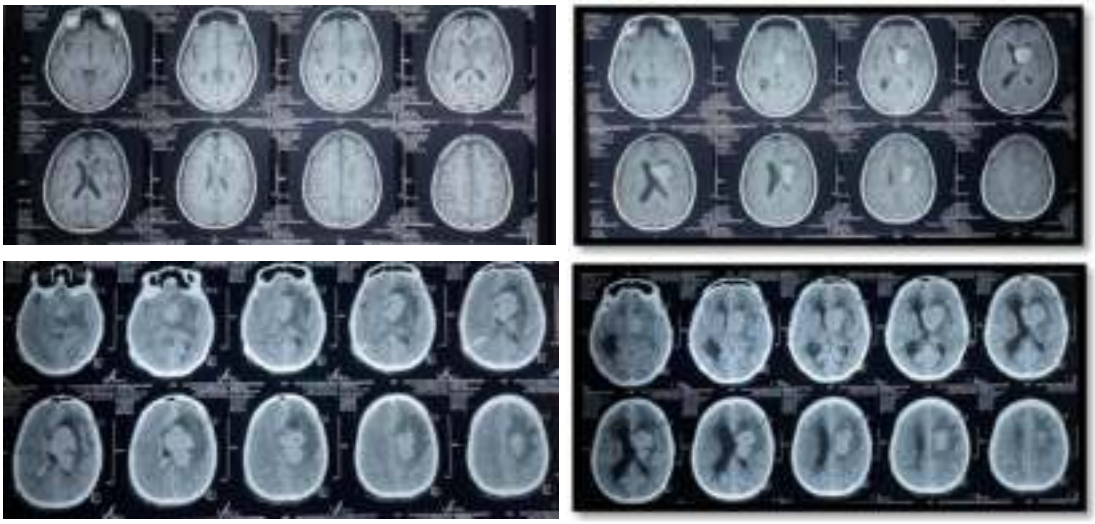

Figure 2. Brain MRI Patients Before Chemotherapy.

Figure 3. Brain MRI Patients After 2 Cycle Chemotherapy.

Primary CNS Lymphoma (PCNSL) is a rare form aggressive extra nodal non-Hodgkin Lymphoma (NHL) that comprising 1-2% of the primary brain tumors that develops in the brain, spinal cord, eye or leptomeningeal area without evidence of systemic involvement.[1] The overall incidence of PCNSL with immunocompetent patients is only 0.47/100,000 year in PCNSL. Approximately 10-20% of patients had ocular involvement and around one third had multifocal neurological disease. Overall long-term survival rate was only 20-40%, this is because the management of PCNSL is limited by ability of the drug due to cross the blood brain barrier (BBB). We present a B-cell central nervous system lymphoma in an immunocompetent patient who responded to chemotherapy.[2]

A 35-year-old man presented to our hospital with sudden change in mental status since 4 hours before admission. He was experiencing headache and blurred of vision within 3 months and have episode seizure. On Examination, Glasgow Coma Scale (GCS) E2M1V aphasia. The patient was found to have right hemiparesis with bilateral papilloedema. Visual acuity of both eyes was no light perception (NLP). The other physical exam was normal. Laboratory tests Hb 10.7 g/dl, LDH 446 U/L, and D-dimer 3.21 ug/ml. Rubella IgG 76.9, CMV Ig G 245.6 and, HSV IgG and IgM negative, HIV test non-reactive, Toxoplasma IgG and Toxoplasma IgM negative, HbsAg and HCV test negative. Brain MRI and MRI Stereoscopy: Lobulated mass size 7.08 cm x 4.75 cm at the left caudate nucleus and left lateral periventricular area. Choline/NAA ratio: 5-9, Choline/Creatine ratio 6-11 suggestive of malignancy, possibly lymphoma. Whole spine MRI showed bulging intervertebral disc at the level of C4-C5. Chest and abdomen CT-Scan result was normal. Bone survey result was normal, Electroencephalogram (EEG) suggested epileptiform discharges of left temporal area. Cerebrospinal Fluid: Showed gliosis and suspected malignancy. The patient underwent

ventriculoperitoneal shunt (VP shunt) due to obstructive hydrocephalus and craniotomy. Pathology biopsy and immunohistochemistry (IHC) of basal ganglia revealed a Diffuse Large B Cell Lymphoma (NHL) Non-Germinal Center, CD 20 +, Ki 67 95% (High Grade), CD 45 +, CD 3 -, BCL6 +, Mum 1+. For this patient, we started induction therapy with RMP Regimens (Rituximab 375 mg/m^2, day 1, 15 and 29, High Dose Methotrexate (HDMTX) 3000 mg/m^2 day 2, 16 and 30, and Procarbazine 60 mg/m^2 day 3-12) Due to the unavailability of procarbazine in town, we decided to change it with Dacarbazine 375 mg/m^2 days 3, 17 and 31), Dexamethasone 5 mg/6 hours, and low dose whole brain radiotherapy (WBRT).

PCNSL is a rare form aggressive extra nodal NHL, especially in Immunocompetent patient. In this particular case of patients High Dose Methotrexate Chemotherapy has achieved high respond especially for this patient that showed GCS E4M5V6 and recovery neurological deficit after 2 cycle chemotherapy.

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TIMP2 and IGFBP-7 as Biomarkers For The Diagnosis of Acute Kidney Injury (AKI) in Post-operative Patients: An Evidence-based Case Report

Igor Ian Wiguna, Gde N. I. Bhaskara, Mutiara R. A. Putri, Priscilla Ardianto, Widi Atmoko

Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

Corresponding Author:
Igor Ian Wiguna, MD. Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital. Jl. Salemba Raya no. 6, Jakarta 10430, Indonesia. Email: igorwiguna@yahoo.com.

ABSTRACT

Background: *Acute kidney injury (AKI) is defined as a sudden reduction in kidney function which commonly occurs as a complication of major surgeries. It is traditionally diagnosed using serum creatinine elevation. AKI has relatively slow kinetics that makes it unable to diagnose at an earlier more reversible stage. Furthermore, previous research has shown that TIMP-2 and IGFBP7 were urinary biomarkers to use as a diagnostic tool for AKI. We aimed to compare the accuracy of TIMP2 and IGFBP-7 to the gold standard (serum creatinine) in diagnosing AKI in postoperative patients.* ***Methods:*** *A thorough search was performed using a search strategy on EMBASE, PubMed, and Medline (Ovid) using keywords according to the objective. The collected articles were critically appraised using CEEBM critical appraisal tool.* ***Results:*** *5 studies that fulfilled the inclusion criteria were selected and evaluated. They all stated that the use of TIMP2 and IGFBP7 could not detect AKI better than the gold standard as shown in the sensitivity and specificity of the biomarkers. Furthermore, the examination of AKI using both biomarkers showed a sensitivity of 60-100% and specificity of 58-91%.* ***Conclusion:*** *TIMP2 and IGFBP7 are promising diagnostic tools for AKI. However, due to the wide variation in results amongst the different studies, further research is required to ensure the credibility of this result.*

Keywords: *Postoperative AKI, Diagnosis, TIMP2, IGFBP7, Serum Creatinine Level*

INTRODUCTION

Acute kidney injury (AKI) is defined as a sudden reduction in kidney function. It is a common complication of major surgeries resulting in a significant increase in mortality and morbidity.[1] The diagnosis of AKI has remained unchanged over the years and is based on the acute rise of SCr and/or a decline in urine output over time. SCr elevation, however, has proven to be an imperfect gold standard due to its relatively slow rise[2], disabling it from detecting AKI early on, during a potentially reversible stage.[3] This prompts the search for potential biomarkers with comparable accuracy to SCr that are able to detect AKI at an earlier stage. An example is the urinary cell-cycle arrest markers, namely TIMP-2 and IGFBP7, whose levels were found to increase following a kidney injury.[4] Both TIMP-2 and IGFBP7 are urinary cell-cycle arrest markers that are secreted by the tubule cells of the kidney. They are capable of inducing G1 cell cycle arrest and differential in secretion localization can predict kidney damage. Research stated that the used of both the markers simultaneously added the predictive value in diagnosing AKI. Nephrocheck is the brand used to diagnose both these markers simultaneously.[4] The clinical and economic

benefits of these biomarkers in diagnosing AKI have been previously evaluated in the US hospital system settings by Berdugo MA, et al. Currently, they have not been evaluated in the Indonesian hospital setting. Furthermore, studies investigating the potential use of TIMP-2 and IGFBP7 in the diagnosis of AKI compared to SCr were collected and analyzed. They were also evaluated to know whether these biomarkers have enough potential to be implemented in Indonesia.

CASE ILLUSTRATION

A 63-year-old male underwent cardio-pulmonary bypass without abnormalities in a tertiary hospital in Indonesia. The patient was then transferred to the general ward and monitored. Laboratory tests (blood and urine) were performed, and the results did not indicate any abnormalities (serum creatinine level 1.1 mg/dL and he was hemodynamically stable) and thus he was treated in the general ward. Suspicion was made after his surgery and Nephrocheck (TIMP2*IGFBP7) was used and found that there were increasing amounts of the biomarkers. He was then admitted to intensive care unit (ICU) due to suspected AKI for early treatment. In ICU, his blood pressure kept increasing from 140/90 to 180/110 mmHg and his SCr increased to 4.7 mg/dL during 3 days after the surgery. He was then diagnosed with Acute Kidney Injury (AKI) and severe hypertension related to the surgery.

CLINICAL QUESTION

Do TIMP-2 and IGFBP7 (Nephrocheck) provide a good accuracy in diagnosing AKI as a postoperative complication, considering its capability of early detection?

METHODS

Search Strategy

A thorough search was performed in three databases, namely EMBASE, Medline(Ovid), and Pubmed, utilizing MesH terms and keywords (**Table 2**). This made it possible to obtain studies investigating the accuracy of TIMP2 and IGFBP7 compared to SCr in diagnosing postoperative AKI. Furthermore, these searches were performed on October 29, 2021 as shown in **Figure 1**.

Eligibility Criteria

Studies were screened based on the following criteria, namely to investigate AKI, which is defined by either an increase in SCr by 0.3 mg/dl (or 26.5 lmol/l) within 48 hours or by 1.5 times baseline, which is presumed to have occurred within the prior 7 days or Urine volume < 0.5 ml/kg/h for 6 hours. Based on KDIGO[5], this involved patients in a postoperative setting older than 18 years old, whereby TIMP2 and IGFBP7 alongside SCr were measured. Scr was used as the compactor because we believe that SCr is still the gold standard in diagnosing AKI. However, we are trying to investigate the diagnosis accuracy of TIMP2 and IGFBP7 in diagnosing AKI in early stages. Studies that investigated pregnant and pediatric patients, and also did not provide full texts or utilize other languages other than English and Bahasa Indonesia, were excluded.

Study Selection

This was performed simultaneously by about four people carried out this process which involved passing the studies through independent screening. The titles, abstracts, and the ones that passed this phase were screened

Tabel 1. PICO Framework.

Patient/Problem (P)	Intervention (I)	Comparison (C)	Outcome (O)
Patients who underwent surgery suspected of AKI as a postoperative complication	Combination of TIMP-2 and IGFBP7 (Nephrocheck)	Serum Creatinine	Diagnosis of postoperative AKI
Type of Clinical Question	Diagnosis		
Study Design	Evidence Based Case Report		

Table 2. Search Terms Per Database.

Database	Search strategy	Hits	Selected articles
PubMed	(((TIMP2[Title/Abstract]) OR (TIMP-2[Title/Abstract])) AND ((IGFBP-7[Title/Abstract]) OR (IGFBP7[Title/Abstract])) OR (Nephrocheck[Title/Abstract])) AND ((acute kidney injur*[Title/Abstract]) OR (AKI[Title/Abstract]) OR (acute renal injur*[Title/Abstract])) AND ((diagnos*[Title/Abstract]) OR (detect*[Title/Abstract])) AND ((creatinine) OR (scr) OR (gold standard)) AND (sensitiv*) AND (specific*) AND ((surger*) OR (postoperative) OR (post-operative) OR (post-op))	2	
EMBASE	('timp2 OR 'timp 2') AND ('igfbp 7' OR igfbp7) OR nephrocheck) AND (acute AND kidney AND injur* OR aki OR (acute AND renal AND injur*)) AND (diagnos* OR detect*) AND (creatinine OR scr OR (gold AND standard)) AND sensitiv* AND specific* AND (surger* OR (post AND operative) OR 'post operative' OR 'postoperative complication'/exp OR 'postoperative complication')	21	5
MEDLINE	('timp2 OR 'timp 2') AND ('igfbp 7' OR igfbp7) OR nephrocheck) AND (acute AND kidney AND injur* OR aki OR (acute AND renal AND injur*)) AND (diagnos* OR detect*) AND (creatinine OR scr OR (gold AND standard)) AND sensitiv* AND specific* AND (surger* OR (post AND operative) OR 'post operative' OR 'postoperative complication'/exp OR 'postoperative complication')	24	

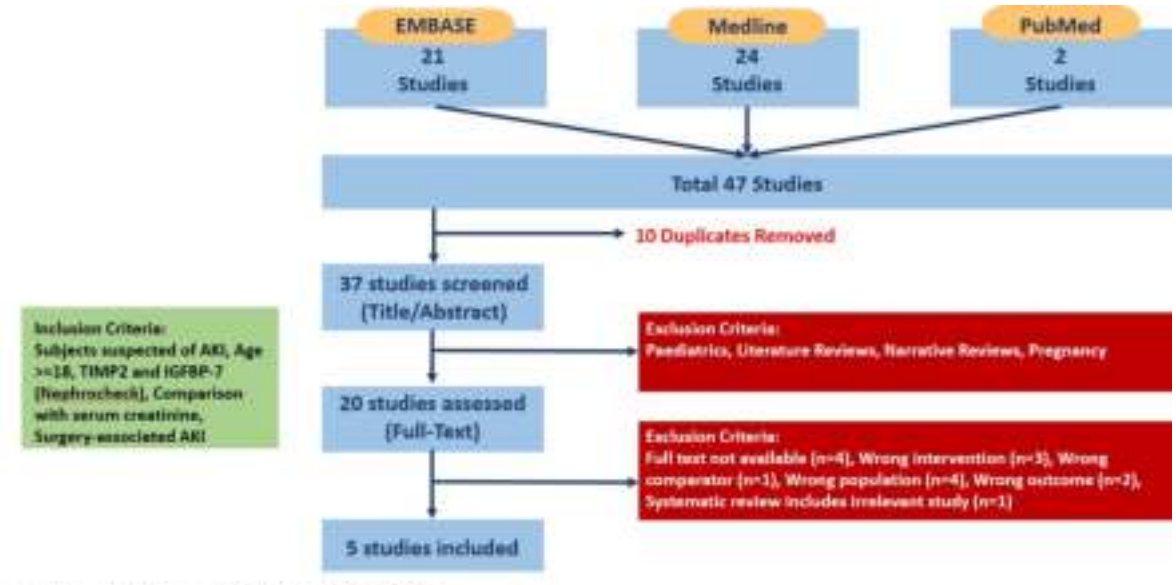

Figure 1. Study Selection Flow Chart.

thoroughly in each of their full texts against the eligibility criteria. A discussion between the two conflicting reviewers was also held in order to reach a consensus in cases of conflicting decisions during the study selection. However, in a situation whereby a consensus was not reached, a third reviewer would provide a final decision. Covidence© was used to perform this selection in order to ensure the accuracy of the selected study.

Critical Appraisal

Critical appraisal was performed using Oxford CEBM diagnostics critical appraisal sheet.

RESULTS

Search Results

Based on the search from the three databases, 47 studies were retrieved consisting of 10 duplicates (**Figure 1**). The remaining had their title and abstract, and later the full text screened against the eligibility criteria. In the full-text screening phase, 15 studies were excluded due to the following reasons namely, 4 studies did not have a full-text version, 3 assessed different biomarkers in combination with Nephrocheck, one study did not include gold standard comparison (SCr), and 4 studies were excluded as it assessed pediatric population. Furthermore, one study used Nephrocheck as a predictor of renal recovery instead of a diagnosis of AKI, another measured the incidence of AKI instead of the accuracy of the diagnostic tools and one systematic review included irrelevant studies. The full-text screening phase resulted in 5 studies which were later critically appraised.

Critical Appraisal

This was performed on the selected studies and the result are summarized in **Table 3**. It was concluded that they were valid and important for the patients. However, about three studies[6–8] did not perform blinding. Based on the applicability test, it was inferred that some were not applicable because the diagnostic test was not available in Indonesia. In addition, 4 out of the 5 studies showed a low percentage of post-test probability. The ones that were selected aimed to determine the level of TIMP2, IGFBP7, and SCr in specific time points and compare the concentration between the biomarkers and SCr as summarized in **Table 4**.

24 hour TIMP2 and IGFBP7 level in detecting AKI

All studies, except for Meersch et al, measured the urinary biomarkers after 24 hours timepoint. There were different cutoff values of

Table 3. Summary of Critical Appraisal Results Performed on the Five Remaining Studies.

			Validity			Importance			Applicability				
Study	Sample Size	Study Design	Representative Patient Spectrum	Compared to Gold Standard	Independent/ Blind Comparison	Sensitivity	Specificity	Likelihood Ratio	Diagnostic test is available, affordable, accurate	Clinical Estimate of Prevalence	Affect Patient Management	Consequences help patients	LOE*
Meersch, et al (2014)	50	Cohort	✓	✓	✓/-	72%	96%	1.74*		✓		✓	2b
Dusse, et al (2016)	40	Cohort	✓	✓	✓/✓	100%	91%	11.11*	-	✓	✓	✓	2b
Oezkur, et al. (2017)	146	Cohort	✓	✓	✓/-	60%	69%	1.94*		✓		✓	2b
Mayer, et al. (2017)	110	Cohort	✓	✓	✓/-	77.8%	64.1%	2.17*	-	✓	-	✓	2b
Pilarczyk, et al. (2015)	80	Cohort	✓	✓	✓/✓	89%	81%	4.68*	-	✓	-	✓	2b

Table 4. Summary of Findings in the Selected Studies.

Author	Patient group	Outcome	Key results	Comments
Dusse, et al.	40 patients with	[TIMP2]*[IGFB	In KDIGO AKI 0/1:	In patients underwent TAVI, [TIMP
(2016), BMC	severe symptomatic	P7] urine	- No significant rise of [TIMP2]*[IGFBP7] urine concentration at	2]*[IGFBP7] concentrations within
Anesthesiology	aortic stenosis who underwent transapical or transaortic	concentration compared to serum creatinine	all times - Serum creatinine and eGFR remained stable at all times;	24 h after surgery is associated with the onset of AKI within the next 72 h.
Cohort study (2b)	Transcatheter Aortic Valve Implantation (TAVI) were included	(sCr)	In KDIGO AKI 2/3: - [TIMP2]*[IGFBP7] increased significantly on 1st postoperative day (POD) (4.62 ± 3.14 (ng/ml)2/1000) - sCr elevated on POD 2 with a maximum of 1.64 ± 0.99 mg/dl [TIMP2]*[IGFBP7] elevated as early as 24h after TAVI	[TIMP 2]*[IGFBP7] urine concentrations show an excellent diagnostic accuracy for the prediction of severe AKI requiring RRT. .
		Sensitivity and specificity of [TIMP2]*[IGFB P7] (Diagnostic accuracy)	Diagnostic accuracy for [TIMP2]*[IGFBP7] 4h after intervention was better than using serum creatinine concentration with sensitivity of 75% and specificity of 55.6% (AUC 0.646). One day after TAVI, [TIMP2]*[IGFBP7] showed sensitivity of 100% and specificity for KDIGO 2 / 3 (AUC 0.971). Within 24 hours after TAVI, [TIMP2]*[IGFBP7] showed sensitivity of 87.5% and specificity of 82.8% (AUC 0.869) compared to serum creatinine concentration.	
Oezkur, et al. (2017), Kidney Blood Press Res **Cohort study (2b)**	148 patients undergoing elective cardiac surgery	Value of [TIMP-2*IGFBP7] compared to serum creatinine (sCr) 24 hours post surgery	24h post surgery measurement of [TIMP2]*[IGFBP7] had significant result.(OR 2.11, p=0.06, sensitivity 60%, specificity 69%). PPV= 57.1%, NPV= 89.9%	It was concluded that early detection of elevated [TIMP-2*IGFBP7] at ICU admission was more likely to predict postoperative AKI compared to subsequent measurements
Meersch et al (2014), PLOS one Cohort Study (2b	50 patients undergoing cardiac surgery	Biochemical value and performance of [TIMP-2*IGFBP7] for early diagnosis of AKI	The first 24 h after surgery urine [TIMP-2]*[IGFBP7] using cutoff 0.3 yielded sensitivity of 73% and specificity 58% (AUC: 0.90, CI:0.79–1.00) PPV=0.66 NPV=0.67, when compared to serum creatinine Maximum [TIMP2]*[IGFBP7] value was achieved at 24h 24 patients who did not develop AKI showed no statistically significant increase in [TIMP2]*[IGFBP7	Urinary [TIMP-2]*[IGFBP7] serves as a sensitive and specific biomarker to diagnose AKI early after cardiac surgery and to predict renal recovery.

Pilarczyk, et al. (2015), Ann Intensive Care	60 patients (>18 y.o.) undergoing CABG	Post-operative course of [TIMP-2]*[IGFBP7] and serum creatinine	In patients with AKI 0/1: - No significant rise of urinary [TIMP-2]*[IGFBP7] was observed at all times - sCr remains stable at all times	Urinary [TIMP-2]*[IGFBP7] (G2 cell cycle arrest iomarkers) allow earlier diagnosis of AKI compared to creatinine-based definition of AKI
Cohort study (2b)		Diagnosis of AKI with [TIMP-2]*[IGFBP7] on the 1st postoperative day	In patients with AKI 2/3: - [TIMP-2]*{IGFBP7] increased significantly 4 hrs post surgery; reached maximum level on 3rd day - sCr elevation observed at POD 2 - POD 4, reached maximum at POD 3 & 4 Accuracy of [TIMP-2]*[IGFBP7] at AKI diagnosis: - sensitivity 0.80, specificity 0.81 (cut-off value 0.89); CI 0.696-1.0, $p = 0.04$	
Mayer et al (2017), Journal of Cardiothoracic and vascular anesthesia	110 patients (>18 y.o.) underwent cardiac surgery	Urine levels of [TIMP-2]*[IGFBP7] at an early time point after surgery for prediction of AKI	1 hour after the start of CPB, the levels of TIMP-2*IGFBP7 were measured to predict postoperative AKI. Sensitivity and specificity were found to be 0.778 and 0.641, respectively. Positive and negative predictive values were also calculated (0.156 and 0.972, respectively).	Urine levels of TIMP-2 IGFBP7 are able to diagnose AKI at 1 hour afe CPB. TIMP-2 and IGFBP7 may be recommended for supplementary criteria of AKI prediction.

[TIMP2]*[IGFBP7], three studies[6,8,9] utilized 0.3, while Pilarcyzk et al., and Mayer et al., utilized 0.817 and 0.41 respectively. Dusse et al., reported the best sensitivity and specificity (100% and 91%) while others[6–8,10] reported different values ranging from 60% - 89% and 58% - 81% respectively. As for the likelihood ratio, it was manually calculated and it was found that only Dusse et al., (11.1) reported a significantly favorable likelihood ratio. However, Meersch et al., and Oezkur et al., reported an unfavorable likelihood ratio, namely 1.74 and 1.94 respectively.

DISCUSSION

The diagnosis of AKI is usually performed by following the diagnostic criteria of Kidney Disease, such as Improving Global Outcome (KDIGO) of AKI [5] using creatinine elevation as the focus of the measurement. However, this diagnosis method poses limitations because of its incapability in diagnosing AKI at its earliest point before the disease becomes irreversible.[3] TIMP-2 and IGFBP7 are stress markers that are rapidly secreted during kidney injuries.[4] These biomarkers were used as detection methods of AKI at its earliest development.[9] Furthermore, all studies assessed within this case report presented similar findings such as increased levels of TIMP-2 and IGFBP7 within 24 hours of surgery. This is in line with previous findings that [TIMP2]*[IGFBP7] accurately identified patients with increased risk of AKI in an earlier time frame postoperatively.[3,11,12]

Dusse, et al.,[9] and Pilarczyk, et al.,[10] examined the diagnostic accuracy of [TIMP-2]*[IGFBP7] in diagnosing AKI by comparing it to the concentration of SCr level in several time points. Different from the other cohort studies, this study performs blinding on the investigators, which reduces information bias. However, Dusse, et al., and Pilarczyk et al., had a low sample size with 40 and 60 samples respectively, which was justified by their sample size calculator but risked a less representative result. In summary, both studies reported high diagnostic accuracy of [TIMP-2]*[IGFBP7] and were the only ones whose likelihood ratio indicated that [TIMP-2]*[IGFBP7] provided benefits in diagnosing AKI.

Mayer et al.,[7] employed a larger sample size of 110 subjects to examine the diagnostic capability of [TIMP-2]*[IGFBP7] in diagnosing AKI. The samples were consecutive patients which reduced selection bias. The measurement of urinary biomarkers was performed one hour after the surgery unlike other studies (within 24 hours), which tested their early capability in diagnosing AKI. However, this study poses limitations, such as being a single-center cohort and having low number of AKI events which lead to limited evidence for the obtained results.

In the study by Meersch, et al.[8] the samples were heterogeneous and had comorbidities associated with AKI, and the result showed TIMP2 and IGFBP7 performed well in predicting AKI. Both biomarkers had significantly higher specificity and sensitivity in diagnosing AKI. However, this study had a relatively small sample size of 50 from a single center. As a result, a larger population is required to validate the result despite the statistical and clinical significance.

Oezkur, et al.[6] had the largest sample when compared to other prospective cohort studies exploring AKI diagnosis in post-cardiac surgery patients. They excluded patients with chronic kidney diseases from their sample. Therefore, the use of TIMP2 and IGFBP7 as AKI diagnostic tools in these groups of patients is unknown.

In summary, the sensitivity and specificity results of Nephrocheck reported by the studies seemed to vary by quite a wide margin ranging from 60-100% for sensitivity and 58-91% for specificity. This indicates that the probability of a patient with AKI and being tested positive was 60-100% and those without AKI and tested negative was 58-91%. This variation may have occurred because the studies employed different [TIMP-2]*[IGFBP7] cut-off values. Therefore, further studies need to be carried out in order to assess the value of [TIMP- 2]*[IGFBP7] in diagnosing postoperative AKI, especially in determining a standardized cut- off value.

The highest likelihood ratio of 11.11 was reported by Dusse, et al., and this indicates that when Nephrocheck was used to detect AKI, the result was 11.11 times more suitable for patients suffering from this disease. Furthermore, they had positive results than those that were not suffering from AKI. This result is significantly higher than the LR from the other four studies included. There is a possibility of different methods and factors, including blinding, surrounding the population leading to this peculiarity. Therefore, there needs to be standardized and controlled study methods to ensure the credibility of this finding.

This case report posed several strengths which include the utilization of 3 renowned scientific databases to search for relevant studies. This was carried out using an independent screening process in order to ensure objectivity in the study selection process. On the other hand, the limitation of this case report was a language barrier that caused several studies to be excluded. Besides this case report also did not standardize the methodology of the appraised studies.

CONCLUSION

The [TIMP-2]*[IGFBP7] value was reported to be a promising AKI diagnostic tool that was able to diagnose postoperative AKI earlier. However, it seemed to be significantly influenced by the degree of severity, favouring moderate-to-severe AKI leading to a variety of results, and

more studies are required to ensure its credibility. Though [TIMP-2]*[IGFBP7] value is a promising diagnostic tool, it is not still recommended in diagnosing postoperative AKI.

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Indonesian Geriatrics Society Consensus on COVID-19 Management in Older Adults

I Gusti Putu Suka Aryana,[1] Siti Setiati,[2*] Roza Mulyana,[3] Noto Dwimartutie,[2] Aulia Rizka,[2] Bayu Basuki Wijaya,[4] Nur Riviati,[5] Rensa,[6] Dina A. Ariestine,[7] Bambang Joni Karjono,[8] Eko Aribowo,[9] Hildebrand Hanoch V. Watupongoh,[10] Ridzqie Dibyantari,[2] Wayan Giri S. Putra,[1] Fandi Triansyah,[2] Rahmi Istanti,[2] Dian Daniella[1]

[1]Division of Geriatric, Department of Internal Medicine, Udayana University - Prof. Dr. Ngoerah General Hospital, Denpasar, Bali, Indonesia.
[2]Division of Geriatric, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusomo Hospital, Jakarta, Indonesia.
[3]Division of Geriatric, Department of Internal Medicine, Faculty of Medicine, Andalas University - Dr. M. Djamil General Hospital, Padang, Indonesia.
[4]Division of Geriatric, Department of Internal Medicine, Faculty of Medicine, Sebelas Maret University - Universitas Sebelas Maret Hospital, Surakarta, Indonesia.
[5]Division of Geriatric, Department of Internal Medicine, Faculty of Medicine, Sriwijaya University - Dr. Moh. Hoesin Hospital, Palembang, Indonesia.
[6]Department of Internal Medicine, Faculty of Medicine, Atmajaya University - Atmajaya Hospital, Jakarta, Indonesia.
[7]Department of Internal Medicine, Faculty of Medicine North Sumatera University - H. Adam Malik Hospital, North Sumatera, Indonesia.
[8]Division of Geriatric, Department of Internal Medicine, Faculty of Medicine, Diponegoro University - Kariadi General Hospital, Semarang, Indonesia.
[9]Division of Geriatric, Department of Internal Medicine, Faculty of Medicine, Gadjah Mada University - Sardjito General Hospital, Semarang, Indonesia.
[10]Department of Internal Medicine, Faculty of Medicine, Kristen Indonesia University, Jakarta, Indonesia.

**** Corresponding Author:***
Prof. Siti Setiati, MD., PhD. Division of Geriatric, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusomo Hospital. Jl. Diponegoro no. 71, Jakarta 10430, Indonesia.
Email: s_setiati@yahoo.com.

ABSTRACT

More than 80% of death cases and 95% of severe COVID-19 occur in patients aged over 60 years. Atypical clinical manifestations with high morbidity and mortality further emphasize the importance of COVID-19 management in older adults. Some older patients may appear asymptomatic while other may present with acute respiratory distress syndrome and multi organ failure. Fever, higher respiratory rate and crackles may present. The most common chest x-ray finding is ground glass opacity. Other imaging modalities that are often used are pulmonary computed tomography scan and lung ultrasonography. COVID-19 management in older adults should be comprehensive, starting from oxygen, fluid, nutritional, physical rehabilitation, pharmacology and psychosocial therapy. In this consensus, we also discuss about management of older adults with special condition such as diabetes mellitus, kidney disease, malignancy, frailty, delirium, immobilization and dementia. In post COVID-19 phase, we believe that physical rehabilitation is important as it is done to improve fitness.

***Keyword**: COVID-19, older adults, consensus, management.*

INTRODUCTION

Older adults are highly susceptible to infection. They are more prone to morbidity and mortality caused by Coronavirus Disease-19 (COVID-19) infection. More than 80% of death cases and 95% of severe COVID-19 occur in patients aged over 60 years.[1–3] Data in Indonesia about COVID-19 infection suggested that most positive cases are found at the age of 31-45 years (28.7%), while only 11.8% in older adults aged 60 years and older, but the mortality rate in the older adults is the highest at 46.8% among others group of age.[4]

Research and clinical experience show that diagnosing COVID-19 in the older adults is not always easy. The atypical clinical appearance, accompanied by functional and cognitive status decline and the presence of comorbidities make it quite difficult to detect COVID-19 infection at an early stage.[5] In addition, the administration of drugs to the older adults must consider the dose and side effects related to various organ function decline and interactions between drugs. The management of COVID-19 in the older adults should be comprehensive by paying attention to medical physical aspects and comorbidities, functional and mental status, cognitive function, and adequate fluid and nutrition.[6] Moreover, COVID-19 survivors can suffer from COVID-19 symptoms after the infection is healed which is called as the post-COVID syndrome. Post-COVID syndrome is associated with age. A multicenter study reported an incidence of 9.9% in the 18 to 49 year age group and increased to 21.9% in the 70 year age group.[7]

Given the magnitude of the problem of COVID-19 infection in the older adults, the Indonesian Geriatrics Society (*Perhimpunan Gerontologi Medik Indonesia* (PERGEMI)) made a Consensus on COVID-19 Management in Older adults. This consensus is based on the latest guidelines issued by health institutions or professional organizations. This consensus also refers to published articles in the form of systematic reviews, primary studies, consensus guidelines or other official guidelines. To obtain this data, several electronic data sources have been searched such as Pubmed, Scopus, bioRxiv/medRxiv with the keywords: COVID, diagnosis, older adults or their synonyms. This search is not limited to a particular time, place or research design.

METHODS

The COVID-19 consensus in the older adults was made based on the latest guidelines issued by health institutions or professional organizations. Compilation also refers to published articles in the form of systematic reviews, primary studies, consensus guidelines or other official guidelines issued by professional organizations by including citation sources.

This document provides guidelines only, does not set rules that require to be followed. This consensus is the compiler's statement based on the evidence and the authors' views on the handling of COVID-19 in older population. Clinicians who use this consensus should consider individual clinical conditions for treatment. This consensus will be reviewed and updated (if necessary) at least four years from the date it was made, in accordance with developments in medical science and technology.

Etiopathophysiology

There are three factors that influence the Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) infection, which are agent (virus), host and environmental factors. The cytopathic effect of the virus and the host's ability to respond to the immune response determine the severity of the infection. In the older adults, it is very common to find comorbid chronic diseases such as diabetes mellitus (DM), hypertension, cardiovascular disease, kidney failure and chronic obstructive pulmonary disease (COPD) which increase the severity of COVID-19, by increasing the risk of acute respiratory distress, respiratory failure, sepsis and hypercoagulability.[8,9] Environmental factors can be overcome by maintaining health protocols, such as washing hands, keeping distance and avoiding crowds (**Figure 1**).

The life cycle of SARS-CoV-2 consists of attachment, penetration, biosynthesis, maturation and release. After the virus binds to the host cell receptor, the virus enters the host cell through the process of endocytosis or membrane fusion. The spike protein present in the structure of SARS-

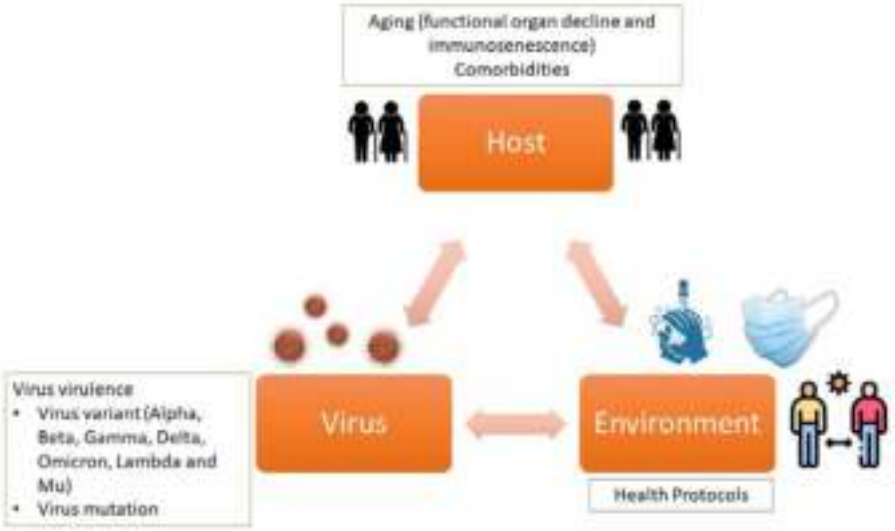

Figure 1. COVID-19 Epidemiology Trilogy in the Older Adults.

CoV-2 is activated by Type 2 Transmembrane Serine Protease (TMPRSS2) which is present in epithelial type 2 pneumocytes.[10] The activated SARS-CoV-2 spike will bind to the Angiotensin Converting Enzyme 2 (ACE 2) receptor on the apical side of the type II pulmonary alveolar epithelium, causing lung epithelial cell damage.[11]

The entry of the virus is followed by viral replication, destruction of infected cells and the induction of non-adaptive/innate cell responses. Viral antigens will be presented by antigen presenting cells (APCs) found on macrophages and dendritic cells that function to phagocytize epithelial cells that are apoptotic due to SARS-CoV-2. APCs further stimulate the body's humoral and cellular immune responses mediated by virus-specific T and B cells. CD4+ and CD8+ T cells play an important role. CD4+ T cells will activate B cells to increase the production of specific antibodies against SARS-CoV-2, while CD8+ is in charge of killing cells infected with SARS-CoV-2. In the humoral immune response, IgM and IgG are formed against SARS-CoV-2.[11]

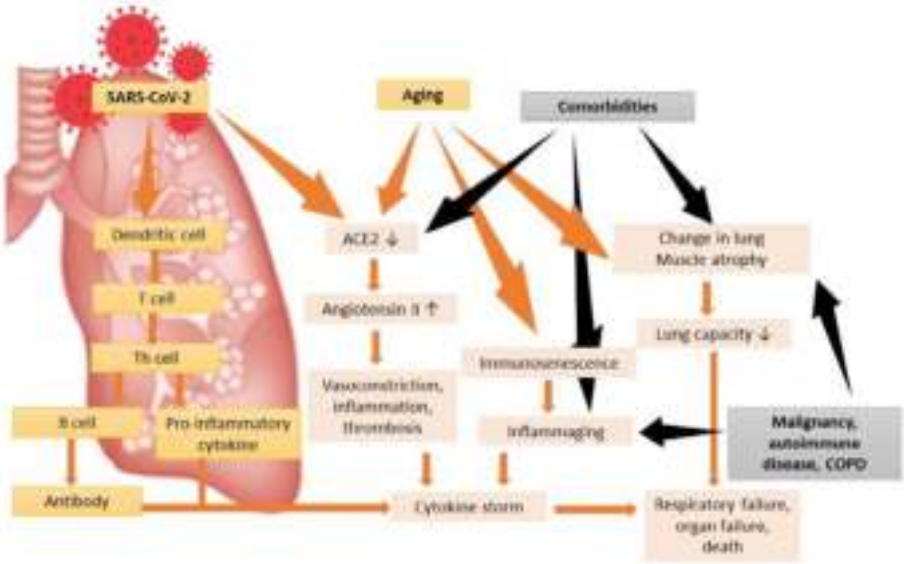

Figure 2. Pathophysiology of COVID-19 Infection in the Older Adults.

The non-adaptive and adaptive immune responses in the older adults are less effective, making them susceptible to severe COVID-19 exposure. Changes in the immune system due to aging along with multimorbidity make older adults more susceptible to complications from COVID-19.[12,13]

DIAGNOSIS

History Taking

Older adults patients with COVID-19, had unspecific main complaints, such as fever (83.6%), cough (62.7%), of shortness of breath (25.5%), fatigue (19.9%), productive cough with sputum production (17.7%), chest tightness (15.3%), diarrhea (13.0%), muscle pain (4.6%), no appetite (8.4%), nausea and vomiting (4.4%).[14–16] Some patients may present with minimal or no symptoms, while others may present with severe pneumonia or complications such as acute respiratory syndrome, septic shock, acute myocardial infarction, venous thromboembolism, or multi-organ failure.[17]

Older adults patients with COVID-19 are likely to have atypical symptoms, such as delirium, postural instability or diarrhea. They can also be found with behavioral changes, impaired balance, functional decline, reduced mobility, syncope, and falls. In older adults with frailty, The Clinical Frailty Score (CFS) was used to determine grading of frailty at the time of hospital admission. [18–21]

Physical Examination

On physical examination, slight increase of temperature (more than 37.3 ^{0}C) may be found. The presence of hypothermia is a sign of a life-threatening infection. Other findings include increased respiratory rate (>25 breaths/min) dan oximetry measurements (oxygen saturation <90%). Lung auscultation revealed wet crackles or coarse crackles. Bronchial breath sounds and egophony suggest pneumonia. Pulmonary percussion revealed a dull sound in the affected area. In addition, there is an increase in pulmonary fremitus.[22]

Laboratory Findings

Laboratory abnormalities show elevated white blood cell and neutrophil counts as well as C-reactive protein. In addition, a decrease in lymphocytes (lymphopenia) in older patients. In moderate to severe cases, there is an increase in procalcitonin levels. In severe cases of COVID-19, or in the presence of cardiac complications and elevated levels of troponin I, d-dimer,and lactate dehydrogenase may be found.[15,21,23]

Chest X-Ray

The most common chest X-ray findings are patchy or diffuse asymmetric, indistinct borders, airspace opacity, either described as consolidation or as an area of ground glass opacity (GGO), peaking on day 10-12 since symptom onset. The most common distribution found in lungs is bilateral, peripheral, and predominantly basal.[24]

Pulmonary Computed Tomography-scan (CT-scan)

Pulmonary CT-Scan may be of diagnostic aid to confirm COVID-19 in addition to RT-PCR examination. However, the sensitivity of CT also depends on the time course of symptoms. Findings on CT-scan for diagnosing COVID-19 infection based on time course of symptoms are described as follows:[22]

1. Initial stage (0–4 days): GGO, either isolated or contiguous with consolidation (in 57% of older patients). Main findings: pulmonary vasculature (84%), intralobular septal thickening (50%), adjacent pleural thickening (40%), air bronchogram (40%), subpleural lines (25%), and crazy-paving pattern (15%). Consolidation pattern, linear opacity, and crazy-paving pattern in severe/critical patients.
2. Progressive stage (5-8 days): Increased GGO and crazy-paving appearance; sometimes accompanied by an inverted halo. Young patients tend to have more GGO, while older patients tend to have consolidation on multiple lobes. The proportion of multiple lobe involvement in older adults group is higher.
3. Peak stage (9–13 days): Progression of GGO into a consolidated and mixed pattern, superimposed intralobular reticulation produces a crazy paving pattern. The lesions

show mild to moderate progression during the initial 2 weeks from symptom onset. A thin line of hypoattenuation between the visceral pleura and the high-density lesion or subpleural sparing.

4. Absorption stage (>14 days): Enlarged subpleural arch fibrotic area/line and architectural distortion seems to be appear. Either the abnormalities will disappear in 1 month or more, or linear fibrosis streaks persist. As many as 80% of patients had residual lesions at discharge, the majority of which were GGO and residual linear opacities.

Lung Ultrasonography (LUS)

LUS primary findings in COVID-19 infection include all findings that are known as acute respiratory distress syndrome (ARDS). Relevant findings found in LUS for diagnosing COVID-19 infection may include:[22]

a. Pleural line thickening with pleural line irregularity; The serosa can be uneven, disjointed, and fragmented, as is found in ARDS.

b. B-lines are visualized as B-line group storms (B-patterns), either in separate or converging forms, sometimes giving the appearance of glowing white lungs. These lines correspond to the initial appearance of the "ground glass" area detected on CT, and can often arise from a single point of thickened pleural line and from small peripheral consolidations.

c. Multifocal posterior subpleural small consolidations in different patterns, including non-translobar and translobar consolidation.

d. Massive consolidation of such tissue without a bronchogram (obstructive atelectasis).

e. Large pleural effusions (simple or complex) are not common..

THERAPY

Comprehensive Geriatric Assessment (CGA)

Analysis of the data in CGA is very important and useful in management, especially in older patients with geriatric syndromes who have COVID-19 infection. The assessment aspects that need to be considered in the CGA are described in Table 1.

Oxygen Therapy

Oxygen therapy can be initiated via a nasal cannula or mask for patients with an oxygen saturation of 92% and a respiratory rate of 24 breaths/min (or a saturation of 88% in those with a history of type II respiratory failure). If clinical improvement does not occur within one hour or clinical deterioration occurs, increase oxygen therapy using a High Flow Nasal Cannula (HFNC).[25]

Fluid Therapy

Adequate hydration is very important in the management of COVID-19 infection in the older adults, because they are at high risk for dehydration. Improving the condition of dehydration can reduce the risk of mortality in the older adults with pneumonia.[26] Hypovolemia is common in critically ill COVID-19 patients. A simple examination to determine hypovolemia is performed bedside in the isolation room with

Table 1. CGA of Older Adults with COVID-19.

CGA Components	Descriptions
Medical Assessment	Complete history including history of organ systems and socio-economic status. A thorough physical examination includes vital signs, oxygen saturation, anthropometry, neurologic and musculoskeletal.
Functional Status	Assessment using Activity Daily Living (ADL) Barthel or KATZ, and Lawton Instrumental Activities of Daily Living (IADL).
Cognitive Status	Abbreviated Mental Test (AMT), the Mini-Mental State Examination (MMSE), MiniCog or Indonesian version of the Montreal Cognitive Assessment (MoCA InA) instruments.
Emotional/Psycho-Affective Status	Geriatric Depression Scale (GDS) instrument.
Nutritional Status	Mini Nutritional Assessment (MNA), Short Form-MNA (SF-MNA), Malnutrition Screening Tool (MST), Subjective Global Assessment (SGA) instruments.

passive leg raising (PLR), lactate clearance, pulse pressure variation (PPV), and collapse or absence of the inferior vena cava. Symptoms of dehydration include changes in mental status, thirst, oliguria, and capillary refilling time.[27] The maintenance fluid requirement for the older adults is around 25-30 ml/kg body weight per day. This need will increase if there is a fever, which will increase fluid requirements by about 12.5% for every 100C increase in temperature. Conditions of hyperventilation and sweating will increase fluid requirements by 10-60% and 10-25%, respectively.[28] Crystalloid fluids may be given for fluid resuscitation of critically ill COVID-19 patients. Vasopressors may be used if septic shock persists after fluid resuscitation, in order maintaining a MAP of 60-65 mmHg.[29]

Nutritional Therapy

Nutritional management in older patients with COVID-19 includes screening for nutritional status, diagnosis of nutritional status and nutritional therapy. Seven nutritional screening and assessment tools used were Nutritional Risk Screening 2002 (NRS-2002), Mini Nutritional Assessment (MNA), MNA short form (MNA-SF), Malnutrition Universal Screening Tool (MUST), Nutritional Risk Index (NRI), Geriatric NRI (GNRI), and Modified Nutrition Risk in the Critically ill (mNUTRIC).[30–32] To establish the diagnosis, there are three phenotypic criterias (presence of weight loss (%), low body mass index (kg/m2), and reduced muscle mass) and two etiological criteria (decreased food intake and presence of inflammation). The diagnosis of malnutrition is established when there is at least one phenotypic criteria and one etiologic criteria.[33] Nutritional therapy should be started as soon as the patient is hospitalized (within the first 24-48 hours). Calorie needs range from 27-30 kcal/kg/day. This caloric requirement must be adjusted to nutritional status, level of physical activity and tolerance. In addition, protein needs of 1 gram/kg/day must be met. The addition of 400 kcal of energy and 25 grams of protein can improve physical fitness and the prognosis of COVID-19 in older adults.[24,34] The need for fat and carbohydrates in a ratio of 30:70 and a ratio of 50:50 if the patient is on mechanical ventilation. The use of oral nutritional supplements (ONS) should be given to all older adults admitted outside the ICU. The parenteral route is used if the enteral route cannot achieve the target nutritional needs of older adults.[24]

Physical Rehabilitation Therapy

Physical rehabilitation program should be started as soon as possible, because recent meta-analysis showed physical training can improve older patients'outcome during acute, critical, and post-COVID phase,[22] which includes special needs related to the aging process, such as disability, geriatric syndrome, frailty, cognitive impairment and decreased sensory abilities.[35] On recent meta-analysis, physical training proven to improved patients' outcome in acute phase, critical phase and post- COVID-phase.[36] Rehabilitation options are more directed towards the respiratory system, cardiovascular system and psychological aspects of the patient.[37,38] Geriatric rehabilitation carried out in post-COVID-19 patients such as self-management, swallowing exercises, cognitive exercises, breathing exercises, physical exercises and stress management.[39] Cough exercises can be done using the active cycle breathing technique and the self-air stack technique[40] to help in airway clearance.[41–43]

Routine screening for sarcopenia in the older adults following diagnosis of COVID-19 should be started as early as possible as acute sarcopenia is a common complication. Cytokine storm will induced various pro-inflammatory cytokine causing acute sarcopenia.[44] Acute sarcopenia was associated with immune dysregulation and cytokine storm, length of stay and readmission, and ICU admission and mechanical ventilation.[45] The prolonged duration of bed rest causes a decrease in the physical activity of the older adults which can decrease protein synthesis, resulting in a decrease in skeletal muscle mass and strength.[39,46] Muscle mass was assessed by magnetic resonance imaging (MRI), CT-scan, ultrasound, anthropometric calculations and bioelectric impedance analysis (BIA). Muscle strength can be assessed with a grip strength test.[47,48]

Pharmacologic Therapy

1. Multivitamin
   Some vitamins that play a role in the immune system, their effects on COVID-19 infection, dosage recommendations, and their functions are describe as follows:
   a. Vitamin A with recommended dose of 600 mcg/day plays a role in maintaining the integrity of the epithelial lining of the airways, including maintaining the number of airway cilia.[49–51]
   b. Vitamin B regulates intestinal immune system (gut barrier function), proliferation of T lymphocytes, B cells, and natural killer (NK) cell activity, reduce inflammation through homocysteine metabolism, reduce pro-inflammatory cytokines and prevent hypercoagulability. In severe COVID-19, it is recommended to give thiamine (B1) 1 ampoule / 24 hours.[52]
   c. Vitamin C maintains epithelial integrity, plays a role in leukocyte migration to the site of infection, phagocytosis, bactericidal activity, natural killer (NK) cell activity, T lymphocytes (especially CD8+ cells), and antibody production. Recommended administration of non-acidic vitamin C 500 mg/6-8 hours orally or lozenges 500 mg/12 hours in asymptomatic or mild COVID-19 infection and 200-400 mg/8 hours is recommended for moderate and severe cases.[53,54]
   d. Vitamin D may improve epithelial integrity, synthesis of antimicrobial peptides (e.g.: cathelicidin) by epithelial cells and macrophages, and enhance the non-specific immune system by increasing phagocytosis.[51,55] 400-1000 IU of vitamin D supplementation has been recommended for COVID-19 infection.[56]
   e. Vitamin E enhance chemotaxis and phagocytosis, NK cell activity, lymphocyte cell proliferation, and increase response to vaccination.[53] However, there is still not much information about the effect of vitamin E supplementation on COVID-19 infection.
2. Antiviral
   Antiviral therapy with the right time of administration is highly recommended in the early course of COVID-19 patients. Oseltamivir reduces mortality in influenza patients.[57] Remdesivir is a monophosphoramidate prodrug of an adenosine analog that affects viral ribonucleic acid (RNA) polymerase and may reduce viral RNA production.[58] Lopinavir/ritonavir (LPV/RTV) as a therapy for COVID-19 infection is still controversial.[59] Favipiravir (FPV), a purine nucleic acid analogue that targets RNA-dependent RNA polymerase (RdRP). Arbidol is a broad-spectrum antiviral molecule that inhibits both DNA and RNA viruses by altering the viral membrane structure.[60]
3. Anticoagulant
   Prophylactic dose heparin therapy should be given to prevent thromboembolic complications and is recommended in

Table 2. Dosage of Thromboprophylactic Anticoagulants in COVID-19 Patients.

COVID-19 Severity	Body Weight	Anticoagulant Dosage
Moderate	Not considering body weight	CrCL 60 mL/min: enoxaparin 1x40 mg/day, subcutaneously or fondaparinux 1x2.5 mg subcutaneously
		CrCL <60 mL/min: UFH 2x5000 units/day subcutaneously
Severe / Critical	Body weight ≥60 kg	CrCL 60 mL/min: LMWH (enoxaparin) 2x40 mg/day, subcutaneously
		CrCL <60 mL/min: UFH 3x7500 units/day subcutaneously
	Obesity (≥120 kg or BMI ≥35)	CrCL 60 mL/min: LMWH (enoxaparin) 0.5 mg/kgBW 2 times a day (maximum dose 2x100 mg/day)
		CrCL <60 mL/min: UFH 3x10000 units/day subcutaneously
	Body weight <60 kg	CrCL 60 mL/min: LMWH (enoxaparin) 2x30 mg/day, subcutaneously
		CrCL <60 mL/min: UFH 3x7500 units/day subcutaneously

hospitalized COVID-19 patients with elevated D-dimers.[61] In the absence of impaired renal function, the use of low molecular weight heparin (LMWH) is recommended over unfractionated heparin (UFH) or oral anticoagulants.[62] Administration of LMWH or oral anticoagulants given post-hospital from COVID-19 infection care for 14 - 30 days.[63]

4. Steroids
   Steroid administration improves survival and better outcomes in severe COVID-19 infection patients with hypoxia. It can be given to patients with severe COVID-19 infection and not to patients with mild or moderate degrees. Dexamethasone 6 mg per day intravenously equivalent dose can be given for 10 days.[25]

5. Anti IL-6
   Interleukin-6 plays a role in the differentiation of T cells into Th17 and also plays a direct role in systemic inflammation that occurs in autoimmune rheumatic diseases. Anti-IL-6 inhibits the binding of IL-6 to its receptors thereby preventing the inflammatory cascade.

6. Antibiotics
   WHO recommends giving antibiotics only in severe cases of COVID-19 infection and does not recommend routine antibiotics in cases of mild COVID-19 infection.

7. Convalescent Plasma
   According to the meta-analysis done by Aryana et al, convalescent plasma therapy was associated with lower mortality risk in older patients with COVID-19. It is recommended to administer convalescent plasma early due to more beneficial effect.[64] Contraindications to administration are history of allergy to plasma products, pregnancy, breastfeeding women, IgA deficiency, acute thrombosis and severe heart failure with the risk of fluid overload. Other contraindications are relative, such as septic shock, renal failure on hemodialysis, disseminated intravascular coagulation or comorbid conditions that may increase the risk of thrombosis.

6. Intravenous Immunoglobulin (IVIG)
   Therapy with IVIG gives promising results although there are still controversial results. The doses of IVIG used in these studies varied widely, but most of these studies used large doses of IVIG of about 0.3-0.5 grams/kg/day for 3 or 5 consecutive days.

Psychosocial Therapy

Psychological and mental health problems caused by COVID-19 infection are major problems because the older adults are physically and mentally more vulnerable.[65] During the COVID-19 pandemic, the older adults experienced social isolation that could increase the mental health problem prevalences such as depression, anxiety, stress, and insomnia.[29]

Supportive therapy for the older adults to overcome psychosocial problems during the COVID-19 pandemic including doing hobbies (such as light exercise, art, recreational activities, etc.) and distance psychotherapy.[66] Psychotherapy management can work together with health care workers (doctors, psychologists) by utilizing technology such as telephone, telemedicine or the internet called as Internet Cognitive Based Therapy.[67]

Table 3. Indications for Anti-IL-6 Therapy.

SOFA score < 3 with a score of CURB-65 > 2 **OR**
Oxygen saturation < 93% but it can be corrected by giving oxygen fraction <50% (equivalent to O2 no more than 6 L/min by nasal cannula or simple mask) **OR**
Respiratory rate > 30 per minute **OR**
The chest X-ray shows bilateral multilobed infiltrates, with one of the following biologic markers: - D-dimer ≥0,7 mcg/L - IL-6 ≥40 pg/mL - Lymphocyte < 800x10^9/L - Ferritin ≥700 mcg/L - Fibrinogen > 700 mg/dL

Table 4. Efforts to Maintain Mental and Physical Health of Older Adults During Acute COVID-19.[65]

Considerations	Practical Advice
Physical Health (COVID-19 infection inducing stress)	1. Stop reading, watching, and hearing news including social media about the pandemic because repeated exposure to information about the COVID-19 infection can induce stress and anxiety. 2. Refrain from spreading unofficial information. 3. Understand that it is normal to feel stressed and afraid in unexpected situations. 4. Take a deep breath, stretch and do yoga or meditation. 5. Pay attention to one's needs, emotions and thoughts. 6. Decide on actions after considering collective and social influences. 7. Refrain from discriminating against or blaming certain individuals or groups for the infection. 8. Take care and encourage yourself 9. Those with mental illness or substance abuse may be particularly vulnerable in an emergency and, as such, should continue for giving treatment. Pay attention to the symptoms, if such thing happen in this case seek medical help.
Social Support System (Social Health)	10. Maintain contact with family and friends. 11. Maintain regular religious activities and contact with local communities. 12. Be informed in advance of where and how to receive counseling and services. 13. Make sure any family member and close friends know when symptoms of sadness, depression and anxiety occur.
Physical Activity (Physical Health)	14. Maintain a daily schedule and exercise pattern to keep health y. 15. Have regular habits to maintain health. 16. Make time for some leisure activities and find fun activities. 17. Maintain a healthy and balanced diet. 18. Get enough sleep. 19. Avoid excessive drinking and drug use. 20. Take prescription drugs as usual.

Therapy Considerations for Special Conditions

1. Older adults with DM
   Older patients with uncontrolled diabetes mellitus are more susceptible to secondary bacterial infections that can worsen COVID-19 course of disease.[68] It requires monitoring of blood sugar, the use of intravenous and subcutaneous insulin, and multidisciplinary cooperation in the application of therapeutic regimens and patient education. Preprandial/fasting blood glucose target for frail older patients is higher (140-180 mg/dL).[69] In older adults patients with few comorbidities and life expectancy >10 years, the HbA1c target is <7.5%. In older patients with frailty and life expectancy <5 years, the HbA1c target is <8.5%.[69–71]
2. Older adults with Kidney Diseases
   The use of antivirals and immunosuppressant drugs used in the treatment of COVID-19 infection can trigger acute kidney injury (AKI).[72] In older patients with chronic kidney disease (CKD), each drug dosage should be adjusted. For the prevention of thrombosis, LMWH can be given at the same dose without the need for dose adjustment. Tocilizumab can be given at normal doses without the need for a dose adjustment. Remdesivir and favipiravir are not recommended for CKD patients with glomerular filtration rate (GFR) < 30 ml/min/1.73 m^2 except in severe cases where the benefits outweigh the risks. Hemodialysis (HD) patients must continue to come to the HD unit regularly with airborne isolation room facilities for COVID-19

Table 5. List of Drugs in Type 2 DM Patients Related to COVID-19 Infection.

Drugs of choices	Recommendations
Metformin	Not recommended in patients with severe/critical symptoms, with gastrointestinal disturbances or hypoxia. Can be continued on an outpatient basis if there are no complaints.
Sulfonylureas	Can be continued on an outpatient basis if symptoms are mild. Risk of hypoglycemia if food intake is not adequate.
Alpha Glucosidase Inhibitors	Can be used to control blood sugar after meals. Not recommended in patients with severe/critical symptoms or with gastrointestinal symptoms.
Thiazolidinediones (TZD)	Can be used during treatment with glucocorticoids on an outpatient basis. Risk of fluid retention and not recommended in hemodynamic compromise.
DPP-4 *inhibitor*	Can be continued if symptoms are mild.
SGLT-2 inhibitor	Not recommended for COVID-19 patients with moderate to severe symptoms due to the risk of dehydration and ketosis.
GLP-1 RA	Continue on an outpatient basis with no gastrointestinal symptoms
Insulin	Generally used in hospitalization with moderate to severe symptoms. Caution the risk of hypoglycemia.
Aspirin	Generally continued on an outpatient basis for secondary prevention of cardiovascular disease.
Statin	Generally continued on an outpatient basis. On inpatient individualization decisions needed along with another considerations.

confirmed, suspected, probable, and close contact patients. CKD patients undergoing Continuous Ambulatory Peritoneal Dialysis (CAPD) are advised to visit the CAPD unit as little as possible. Visit to the CAPD unit only if there is severe peritonitis or infection found at the existing site.[72]

AKI is common in COVID-19 infection and is associated with viral cytopathic effects, sepsis, and massive activation of the immune system with cytokine storm, hypoxia, rhabdomyolysis, and renal hypoperfusion. Extracorporeal therapies such as hemoperfusion or hemofiltration can be used as renal support therapy.[72]

3. Older adults with Malignancy

Some cancer therapy modalities and considerations for their administration during COVID-19 infection:

d. Surgical Treatment: surgical procedures on an elective or non-invasive basis may be delayed, but considering the risk of tumor progression and other therapeutic alternatives. Surgery aimed at reducing symptoms and reducing neurological complications is prioritized in surgical treatment procedures during COVID-19 infection.

e. Radiotherapy : need to be postponed if there is no significant benefit or progress. In curative radiotherapy, a short-term hypofraction regimen is given. Intraoperative radiotherapy is considered in the older adults undergoing surgery to reduce outpatient visits. Patients undergoing palliative radiotherapy need to be given a small fraction.

f. Systemic therapy (including chemotherapy, targeted therapy, hormonal therapy, and immunotherapy): need to consider the higher risk of infection in the treatment group who are myelosuppressed or require more frequent hospital visits.

g. Palliative Care: discussion of terminal patient care plans and goals. This can be done with the care by telemedicine assistance. It is required to assist communication between the patients and their family.

4. Older adults with Frailty (Pre-Frailty and Frailty)

Older adults patients with COVID-19 had a frailty rate of up to 78%.[73] Frailty was

associated with higher mortality risk in older adults with COVID-19.[74] Some of the interventions that can be chosen for older adults with COVID-19 are physical activity, nutrition, and pharmacology. Physical activity needed is a combination of resistance training, aerobics, coordination and balance training.[34,75] Recommended protein supplementation (15 grams of milk protein twice a day) for six months or protein consumption of at least 1-1.5 g/kg/day.[76] Other interventions are in the form of Mediterranean diet, prebiotics, supplementation of antioxidants and multivitamins help improve the condition of frailty.[77,78]

5. Older adults with Delirium
   Delirium is a symptom often found in various serious diseases in older people, including severe COVID-19 infection. Ultra-Brief Confusion Assessment Method can be used for rapid assessment and observation of cognition. With this method is only take less than 1 minute to complete the examination of delirium. Delirium interventions in the older adults who are being treated of COVID-19 infection include orientation interventions, therapeutic activities, improving sleep quality, early mobilization, hearing and vision adaptation, maintaining oral and fluid intake, social minimization, and providing psychoactive medication.[79]

6. Older adults with Dementia
   In this current situation of COVID-19 pandemic, isolation affects older people with dementia who have memory and orientation problems and behavioral disorders. Several strategies for treating older adults patients with dementia and COVID-19 infection are described as follows:[80]
   1. Mild cognitive impairment or mild dementia
      a. For patients: plan work and activities by day of the week to prevent time disorientation; have a regular sleep schedule; maintain physical health by following a healthy diet, doing sports according to ability, meditation and cognitive exercise; create contact lists and save emergency contact numbers; think positively and limit yourself to negative news about COVID-19.
      b. For families and nurses: pay more attention to the mental and physical health of patients; routine telecommunications and frequent frequency; seek medical help if there are symptomatic changes in the patient; make sure to only communicate positive things to patients.
      c. For doctors and health workers: maintain personal hygiene and always use personal protective equipment; check the patient regularly; provide guidelines for personal hygiene and directions for reducing stress; providing tele-counseling; and provide immediate assistance when there are symptoms of acute illness.
   2. Moderate and severe dementia
      Strategies that can be implemented are education on the use of hand sanitizing products; change public equipment to personal; carry out disinfection; periodically check the ventilation of the room; checking body temperature and any symptoms related to COVID-19; perform a COVID-19 test (if needed); provide guidelines for workers; carry out COVID-19 tests on workers regularly.

7. Older adults with Immobilization
   Prolonged immobilization causes a decrease in muscle mass, muscle strength, and changes in the structure of muscle contractile proteins, which will cause a decrease in functional status in the older adults. Some of the interventions that can be done to prevent such conditions are:[81]
   a. Early Strength Intervention: light to moderate resistance training to prevent loss of muscle mass, muscle strength, and functional capacity, started as soon as possible; regular limb mobilization; progressive resistance training after the acute phase; and active mobilization and

muscle strength training.

b. Neuromuscular Electrical Stimulation: performed on critically ill patients who cannot perform physical exercise, combined with dynamic mobilization and functional strength training.
c. Heat therapy: heat therapy throughout the body to aid muscle recovery, reduce cell damage and protein degradation.

PREVENTIONS

Patients in the convalescent phase often experience persistent symptoms. Several options that can be chosen to reduce the complaints include:[82]

1. Increase the intensity of activity and exercise gradually, as well as improve breathing technique to reduce shortness of breath.
2. Exercise to improve physical and mental health.
3. Speak unforcefully, opt for other communication media, and stay hydrated in the case of hoarse voice.
4. Regular meal and hydration time in the case of intubated patient, in which swallowing is temporarily or permanently impaired.
5. Seek help in the case of difficulties in returning to daily activities.

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