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Editorial

9vHPV (Nonavalent) Vaccine Policy in Indonesia

Yudi M. Hidayat, Gerry N. Reynaldi

The Human papillomavirus (HPV) vaccine is the most common sexually transmitted infection worldwide. In Indonesia, cervical cancer is the second most common cancer in women, and HPV infection is the main cause.

The distribution of HPV vaccine serotypes in Indonesia according to a study by Utomo et al. showed that out of 11,224 women, the most prevalent infections were types 52, 16, 18, and 58. The nonavalent HPV vaccine consists of virus-like particles that trigger an immune response against five additional high-risk HPV types (31, 33, 45, 52, and 58), in addition to the four types (6, 11, 16, and 18) protected by the quadrivalent vaccine. The vaccine works by triggering the production of antibodies that can fight against the targeted HPV virus, thus preventing HPV-related infections and diseases.

The nonavalent HPV vaccine has the potential to accelerate the elimination of cervical cancer. The 90-70-90 strategy is a global target set by the World Health Organization (WHO) to achieve the elimination of cervical cancer by 2030. This target includes three main indicators: 90% of all women must be vaccinated against HPV, 70% of women infected with HPV must be treated, and 90% of women diagnosed with cervical cancer must receive appropriate treatment. Implementation of the 90-70-90 strategy is expected to accelerate the global elimination of cervical cancer and provide significant health benefits for women worldwide.

In line with this WHO strategy, by providing protection against five additional high-risk HPV types, the nonavalent HPV vaccine can protect both men and women against genital warts and cancer caused by 9 HPV types, which account for nearly 90% of cervical cancer cases worldwide. Additionally, this vaccine can reduce the incidence of other HPV-related cancers such as vulvar, vaginal, and anal cancer.

A study involving 14,215 women aged 16-26 years that compared the quadrivalent HPV vaccine with the nonavalent HPV vaccine showed that the nonavalent vaccine provided 94.4% efficacy against the occurrence of CIN2 (95% CI: 78.8, 99.0) and 100% efficacy (95% CI: 46.3, 100) against the occurrence of CIN 3, compared to the quadrivalent HPV vaccine.

HPV Immunization Program

Australia is one of the countries that has implemented a national HPV vaccination program since 2007. Initially, this program was only intended for women, but since 2013, the program has been expanded to include men. This decision was made after research showed that HPV vaccination in men can reduce the risk of HPV transmission and prevent some types of cancer, such as anal and penile cancer.

One comparative study in women aged 18-24 years who came to the clinic for Pap testing reported that the prevalence of 4vHPV decreased from 28.7% before the vaccination program from 2005 to 2007 to 2.3% in vaccinated women ($p < 0.0001$) from 2010 to 2012. In 2018, Australia switched from using the quadrivalent vaccine to the nonavalent vaccine as part of their national vaccination program. This decision was made after research showed that the nonavalent vaccine provides protection against more extensive HPV serotypes.

Next Question, What About the HPV Immunization Program in Indonesia?

In Indonesia, the government has launched a national HPV immunization program in 2023, with the main target being girls in the 5th and 6th grades of elementary school, using a two-dose quadrivalent HPV vaccine and carried out during the School Children Immunization Month (BIAS).

Since the introduction of the nonavalent HPV vaccine, the trend has been for many countries to switch from the quadrivalent to the nonavalent HPV vaccine. This could also happen in Indonesia. It is possible that in the future, the government will switch to the nonavalent HPV vaccine and even consider expanding the target cohort to include not only girls, but also boys of the same age (5th and 6th grades of elementary school).

While a high adolescent vaccine coverage rate should be the main focus, another important question is whether older women and men - potentially up to the age of 45 or 50 - will be part of the HPV immunization program carried out by the government? (for example, female workers, and high-risk populations such as HIV populations?).

How About the Synergy Between the HPV Vaccine and Screening?

Regarding the national HPV immunization program, if we have high national vaccine coverage, in the future in Indonesia, a woman who has received the HPV vaccine may not need to be screened as frequently. However, the challenge is how to accurately identify those who have received vaccination (when they were in the 5th and 6th grades of elementary school), because the need for screening will only generally arise a few years later.

The role of screening in women who have been vaccinated against HPV needs to be further examined. Considering the excellent protection of the HPV vaccine, screening may be reduced to only three tests throughout their lifetime (for example, at the ages of 30, 40, and 60), but this must be verified in larger studies using HPV DNA tests.

Screening carried out in Indonesia can be done simultaneously with HPV vaccination. However, screening should not be a requirement to be able to receive HPV vaccination. Therefore, both vaccine coverage and screening coverage will increase equally.

HPV Vaccination in Men

Men are considered to be a 'reservoir' for HPV and can spread it to their partners. In addition, some HPV-related cancers, especially head and neck cancers, are increasing in men, and these are cancers caused by HPV. Currently, there is no HPV screening available for men. The role of an obstetrician and gynecologist is not only to provide services related to women's health but also to educate patients' husbands (even their sons) about HPV vaccination in men and the importance of HPV vaccination in men.

In conclusion, the nonavalent HPV vaccine is a potential solution to prevent the spread of HPV types, accelerate the elimination of cervical cancer, and reduce the incidence of other HPV-related cancers. Australia's success in implementing a national nonavalent HPV vaccination program is an example of how this vaccine can be used to protect the population from HPV-related diseases.

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Research Article

Qualitative Study on Maternal and Perinatal Health Services in Primary Health Care Facility in Banten Province

Kajian Mutu Pelayanan Kesehatan Maternal Perinatal Fasilitas Kesehatan Tingkat Primer di Provinsi Banten

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Abstract

Objective: To determine the effect of clinical governance in PHCs on maternal and perinatal health in Banten Province, Indonesia.

Methods: This was an observational analytic study with cross sectional method performed on the PHCs on Banten Province, Indonesia. All PHCs in Banten Province having maternal and perinatal health services were included in the study. Clinical governance and services were measured using a self-made questionnaire filled by the representative of the PHC. Characteristics analyzed in this study were age, education level, completed training, and occupation. Clinical governance aspects analyzed in this study were leadership, culture, competence, governance, and readiness. The services analyzed in this study were antenatal, pathology, and emergency service.

Results: There were 117 PHC representatives who were recruited to the study. The PHC which were categorized as "outstanding" for leadership, culture, competence, governance, and readiness were 23.1%, 41%, 98%, 81.2%, and 83.8%, respectively. The PHC which were categorized as having "good" antenatal, pathology, and emergency services were 92.3%, 51.3%, and 90.6%, respectively. The PHCs with better clinical governance aspects delivered better antenatal, pathology, and emergency services for maternal and perinatal care.

Conclusions: Primary health cares with better clinical governance aspects delivered better antenatal, pathology, and emergency services for maternal and perinatal care. Therefore, improving clinical governance is essential to improve maternal and perinatal health services quality in Banten Province, Indonesia

Keywords: clinical governance, health service, maternal health, quality assurance.

Abstrak

Tujuan: Untuk mengetahui pengaruh tata kelola klinik di Puskesmas terhadap kesehatan ibu dan perinatal di Provinsi Banten, Indonesia.

***Metode:** Penelitian observasional analitik dengan metode potong lintang yang dilakukan di Puskesmas di Provinsi Banten, Indonesia. Semua Puskesmas di Provinsi Banten yang memiliki layanan kesehatan ibu dan perinatal diikutsertakan dalam penelitian ini. Tata kelola dan layanan klinis diukur menggunakan kuesioner buatan sendiri yang diisi oleh perwakilan Puskesmas. Karakteristik yang dianalisis dalam penelitian ini adalah usia, tingkat pendidikan, pelatihan yang diselesaikan, dan pekerjaan. Aspek tata kelola klinis yang dianalisis dalam penelitian ini adalah kepemimpinan, budaya, kompetensi, tata kelola, dan kesiapan. Pelayanan yang dianalisis dalam penelitian ini adalah pelayanan antenatal, patologi, dan gawat darurat.*

***Hasil:** Terdapat 117 perwakilan Puskesmas yang direkrut untuk penelitian. Puskesmas yang dikategorikan "sangat baik" untuk kepemimpinan, budaya, kompetensi, tata kelola, dan kesiapan masing-masing adalah 23,1%, 41%, 98%, 81,2%, dan 83,8%. Puskesmas yang memiliki pelayanan antenatal, patologi, dan gawat darurat yang tergolong "baik" berturut-turut adalah 92,3%, 51,3%, dan 90,6%. Puskesmas dengan aspek tata kelola klinis yang lebih baik memberikan layanan antenatal, patologi, dan darurat yang lebih baik untuk perawatan ibu dan perinatal.*

***Kesimpulan:** Pelayanan kesehatan primer dengan aspek tata kelola klinis yang lebih baik menghasilkan pelayanan antenatal, patologi, dan kegawatdaruratan yang lebih baik untuk pelayanan ibu dan perinatal. Oleh karena itu, peningkatan tata kelola klinis sangat penting untuk meningkatkan kualitas pelayanan kesehatan maternal dan perinatal di Provinsi Banten, Indonesia*

***Kata kunci:** kesehatan ibu, pelayanan kesehatan, penjaminan mutu, tata kelola klinik.*

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INTRODUCTION

The Millennium Development Goals (MDGs) ended in 2015 with only 7 out of 75 countdown countries, countries with the highest rates of maternal and child mortality, successfully achieving the target for maternal mortality.[1] The maternal mortality rate in Indonesia is estimated at 189 per 100,000 live births according to the last survey published by Long Form Survey in 2020.[2] This figure is still far from the WHO target for the 2030 Sustainable Development Goals (SDGs) which is 70 per 100,000 live births.[3] According to Indonesian Family Health Directory Performance Report in 2021, the scope of antenatal care has reached 88.13% from the target 85% population. The scope of labour in health care facility is 101.44%. Even though the number shows an adequate scope of both antenatal and labour management, the maternal mortality rate is still high compared to other South East Asian countries.[2,4] Maternal deaths can occur due to delays in seeking, reaching, and getting adequate health services at health facilities.[5,6]

Maternal deaths due to poor health systems can be assessed in terms of services and macro health. The study conducted shows that the main factor of maternal death occurs due to the poor quality of health system services.[7] Several factors were found in the form of inadequate risk management, ineffective use and recording of medical records, poor interpersonal communication, poor monitoring of complications, delayed referrals, and ineffective communication between levels of health services.[7]

Several studies showed that countries that prioritize their health systems to primary health facilities have a higher probability of achieving SDGs than countries that focus on hospitals.[8-11] This happens because primary health facilities cover broader health determinants, strengthen communities, promote justice social services, and emphasizing synergistic action.[12] Several roles of primary health facilities in reducing maternal mortality include the effectiveness of antenatal visits, community empowerment, and strengthening determinants of maternal health such as education, sanitation, nutrition, and women's empowerment.[9]

Banten is a province in Indonesia which is directly adjacent to Jakarta, the capital of Indonesia. As one of the provinces with the largest population and directly adjacent to the capital, health services in Banten Province should be better than other provinces. However, the 2017 Indonesian Demographic and Health Survey data shows that the province of Banten is one of the provinces with the highest neonatal mortality in Indonesia.[4] In addition, Banten is also the province with the highest percentage of deliveries not assisted by skilled medical personnel on the island of Java.[4] These phenomena has raised various questions regarding the readiness of primary level health facilities (FKTP) in Banten Province in dealing with mother and child problems.

This study aims to assess the readiness of primary health facilities' clinical governance in managing and improving the quality of perinatal maternal services in Banten Province.

METHODS

This was an observational analytic study with cross sectional method performed in the PHCs in Banten Province, Indonesia, during the period of January to November 2022. All PHCs in Banten Province having maternal and perinatal health services were included for the study.

Clinical governance and services were measured using a self-made questionnaire filled by the representative of the PHC. The questionnaire was validated by evaluating the percentage of filled questions by chosen subjects, compiling input-suggestions from subjects, and analyzing the inputs, suggestions, and the questionnaire itself followed by revision accordingly. Construct validity was done by evaluating the analyzing factor from each questions from the three types of outcome with a minimum value of 0.4. The questionnaire was then given to 100 respondents to further follow up weather it was understandable of not, and from the trial, all questions was filled and there was no missing data. That conclude that the questionnaire is ready to be distributed. For reliability test, *Cronbach's alpha* was used for every dimension. Characteristics analyzed in this study were age, education level, completed training, and occupation. Clinical governance aspects analyzed in this study were leadership, culture, competence, governance, and readiness. The services analyzed in this study were maternal care, pathology, and emergency service.

The data was collected by direct interview, to help minimize the understanding bias. Questionnaire was given to primary health care facility in sub-district level which has an active practice in maternal and perinatal health services.

This study used 5% error margin and 95%

confidence interval limit. The variables included in this study were measured using a self-filled questionnaire which had already validated in Indonesia (Appendix 1). The questionnaire was filled by the representative in each of PHC. Clinical governance aspects analyzed in this study were categorized into "outstanding", "fair", and "poor". Meanwhile, the services analyzed in this study were categorized into "good" and "poor".

This study had been approved by the Research Ethics Committee of Faculty of Medicine, Universitas Indonesia. All the PHC representatives who were included in this study had given the informed consent prior to the study. Collected data were then analyzed using SPSS for Macintosh ver. 24. Characteristics of subjects and the clinical governance effects on the health services quality were analyzed using Chi-square test.

RESULTS

A total of 117 representatives from 128 PHCs in Banten, Indonesia were included in this study. The other PHCs not included was PHCs was the currently inactive one in giving maternal and perinatal health services. It was found that the median age of the subjects was 32 years, with the majority of subjects being female, having undergraduate education or higher, and were doctors. In addition, there was variability in the training such ad PONED training, that has been received by the representatives. Baseline characteristics of the representatives can be found in Table 1.

Table 1. Characteristics of the Representatives

Variables	Frequency, n (%)
Sex	
Male	36 (30.8)
Female	81 (69.2)
Education	
Primary	0
Secondary	34 (29.1)
Undergraduate	83 (70.9)
Training	
Labor and delivery	59 (50.4)
Antenatal care	52 (44.4)
Basic obstetrics and neonatal services	56 (47.9)
Occupation	
Doctor	83 (70.9)
Nurse	0 (0)
Midwife	12 (10.3)
Apothecary	1 (0.9)
Others (witch doctor)	21 (17.9)

Following the analysis of the baseline characteristics of subjects, the clinical governance aspects of the PHCs were measured and categorized. On the service capability factor of PHC, it was found that the most services had "outstanding" competency (83.8%). Based on the results of the questionnaire, it was found that the dimension that was most often classified as "poor" was leadership and management (6.0%).

Table 2. Clinical Governance of the PHCs

Variables	Frequency, n (%)
Leadership	
Outstanding	27 (23.1)
Fair	83 (70.9)
Poor	7 (6)
Culture	
Outstanding	48 (41)
Fair	64 (54.7)
Poor	5 (4.3)
Competence	
Outstanding	98 (83.8)
Fair	13 (11.1)
Poor	6 (5.1)
Governance	
Outstanding	95 (81.2)
Fair	15 (12.8)
Poor	7 (6)
Readiness	
Outstanding	98 (83.8)
Fair	14 (12.0)
Poor	5 (4.3)

Meanwhile, the maternal and perinatal health services were measured and categorized. Based on the results of the questionnaire obtained, it was found that 92.3% of PHCs had good maternal care services, 51.3% of PHCs had good pathology management service, and 90.6% of PHCs had good emergency services. The results of the analysis can be found in Table 3.

Table 3. Maternal dan Perinatal Services of the PHCs in Banten Province, Indonesia

Variables	Frequency, n (%)
Maternal care	
Good	108 (92.3)
Poor	9 (7.7)
Pathology management	
Good	60 (51.3)
Poor	57 (48.7)
Emergency service	
Good	106 (90.6)
Poor	11 (9.4)

The effects of respondents' characteristics and PHCs' clinical governance on each maternal and perinatal health service were analysed. the results of the analysis can be found in Table 4.

Table 4. The Effects of Characteristics and Clinical Governance on Maternal and Perinatal Services in PHCs

Variables	Maternal care	Pathology management	Emergency Service
Characteristics			
Education	0.718	0.524	0.050
Training			
Labor and delivery	0.490	0.783	0.528
Antenatal care	0.293	0.804	0.944
Basic obstetrics and neonatal services	1.000	0.635	0.209
Occupation	0.495	0.225	0.096
Clinical governance			
Leadership	<0.001	0.015	<0.001
Culture	<0.001	0.008	<0.001
Competence	<0.001	<0.001	<0.001
Governance	<0.001	<0.001	<0.001
Readiness	<0.001	<0.001	<0.001

Based on our analysis, it can be inferred that the individual characteristics did not significantly improve maternal and perinatal health services provided by the PHCs. However, better clinical governance aspects significantly improve the provided health services.

DISCUSSION

In this study, there were 117 subjects who were PHC staff in Banten who were willing to become PHC representatives as respondents to the questionnaire given. All subjects came from PHCs who provided maternal and child health services and filled out a complete questionnaire.

In assessing the ability to serve PHCs, it was found that most of the PHCs had relatively good leadership. The leadership factor in PHC can have various interpretations. In several studies, the leadership factor was assessed not only from the leader of a service unit, but also related agencies, to the regional leader of the PHC in question. by PHC.[13] Meanwhile, it was found that most of the PHCs has an outstanding culture. The cultural scope of PHC is very broad, starting from the culture of cooperation, communication, to the relationship between staff in a PHC. Culture is a very important factor in the services provided by PHCs. Previous research showed that communication between team members at a PHC was directly related to the implementation of tiered referrals for universal health coverage in Indonesia.[14] In PHCs with poor communication from their human resources, the implementation of tiered referrals was found to be still not optimal, causing service delays and poor outcomes.

In the competency assessment, it was found that most of the PHCs had a satisfactory competence. In previous research, it was found that increasing the competence of PHC service staff through the provision of training is one of the efforts that can have the greatest impact on the quality of services provided for mothers and children.[13] In a previous study conducted at the Puskesmas in Depok, it was found that the competence of medical service staff who are evenly distributed in maternal and neonatal emergency services is the most important factor in the quality of the maternal and neonatal services provided.[15] increasing the competence of PHC service staff through training is one of the efforts that can have the greatest impact on the quality of services provided for mother and children because it can improve the knowledge, skills, and attitudes of healthcare providers. This, in turn, can lead to improved clinical decision-making, better management of complications, and overall better quality of care for mother and children. Numerous studies have shown that training and education programs can significantly improve the competencies of healthcare providers and positively impact maternal and perinatal outcomes. A study conducted in Ethiopia found that healthcare providers who received training on emergency obstetric and neonatal care showed significant improvements in their knowledge, skills, and attitudes, resulting in a reduction of maternal and neonatal mortality rates.[16] Similarly, a study conducted in Nigeria found that training programs for midwives resulted in improved clinical decision-making and increased use of evidence-based practices during childbirth.[17] Therefore, increasing competency through regular training and evaluation important to do in PHC.

In the PHC governance assessment, it was found that most of the PHCs had a very good culture. It is important to evaluate this further

because previous research has shown that countries in Asia and Africa have the most problems in providing maternal and child health services, namely the management of PHCs, including staff and finances, which is not good enough.[13]

In the assessment of the maternal and perinatal services in PHC, it was found that the pathology management were the service that received the most unfavorable category. Pathology cases in maternal and perinatal care require a multidisciplinary approach and collaboration among healthcare providers, including midwifes, obstetricians, neonatologists, and pathologists. Timely and accurate diagnosis and management of pathology cases can significantly improve maternal and perinatal outcomes. In some cases, referral to higher-level facilities may be necessary for specialized care.

Several studies have investigated how healthcare providers in Indonesia manage complication or pathology cases. A study published in the Indonesian Journal of Obstetrics and Gynecology in 2020 found that obstetricians in urban areas faced challenges in managing maternal dan neonatal complications due to high patient volume and inadequate staffing. This study recommended strengthening referral system and providing training to improve obstetricians' clinical skills and knowledge.[18]

Another study published in the Journal of Public Health in Indonesia in 2017 examined midwives experiences in diagnosing and managing complication during pregnancy and childbirth in rural areas. The study found that midwives faced challenges such as inadequate training, limited resources, and difficulty referring patients to higher-level facilities. The study recommended improving midwives' clinical skills and knowledge, strengthening referral systems, and providing additional resources and support.[19]

Similar conditions were also found in studies conducted on PHC in other countries such as Nepal and Nigeria.[13,20] The least sufficient knowledges were the knowledges on prevention of vertical transmission from mother to child (<10%), examination and diagnosis of severe preeclampsia (59%), as well as neonatal sepsis examination (62%).[20] These results tend to be lower compared to existing knowledge on other maternal and neonatal emergencies.[20]

Even if healthcare providers have good competencies, they may face challenges in managing pathology cases due to various factors such as limited resources, inadequate training, and high workload. The 48.7% of respondents in this study had poor pathology case management may reflect these challenges. It would be helpful to examine the specific reasons why these healthcare providers struggled with pathology cases. Additional training, supervision, and support may be necessary to improve their skills and knowledge in managing pathology cases. Quality improvement initiatives that focus on improving teamwork and communication among healthcare providers can also improve pathology case management.

It was found that most of the PHCs had a relatively good emergency service category. The results obtained in this study were essential, bearing in mind that PHC as the first health care facility that deals directly with patients is expected to be able to provide emergency management needed by maternal and perinatal patients prior to referral to reduce the time between patient arrival and receive treatment thereby improving the patient's prognosis.

Assessing that the individual variables assessed in this study did not have a significant relationship with all maternal and perinatal outcomes, but all PHC variables (leadership, culture, competence, and governance) had a significant relationship with all maternal and perinatal outcomes, it can be concluded that the factors that shared factors (team factors) are more influential on PHC readiness and quality of maternal and perinatal services provided. Therefore, it is not enough to only improve the quality of one individual in the PHC, but it is better to focus on the quality improvement in a team, for example by providing joint training or improving the standard procedure owned by the PHC.

CONCLUSIONS

Primary health cares with better clinical governance aspects delivered better antenatal, pathology, and emergency services for maternal and perinatal care. Improving the quality of PHC has a significant positive relationship with better maternal and perinatal outcomes. When primary health care services are of higher quality, they can provide better antenatal, delivery, and postnatal care, which can result in improved maternal and perinatal health outcomes. All the PHC variables such as leadership, culture, competence, and governance had a significant relationship with

all maternal and perinatal outcomes because the factors that shared factors (team factors) are more influential on PHC readiness and quality of maternal and perinatal services provided. Therefore, efforts to improve the quality of primary health care services should address these factors and focus on providing team-based rather than to only improve the quality of one individual in the PHC.

DECLARATIONS

The data used in this study can be requested from corresponding author upon reasonable request.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest in this study.

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Research Article

Mode of Delivery and Neonatal Outcomes in Preterm Pregnancy

Metode Persalinan dan Luaran Neonatus pada Kehamilan Prematur

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Abstract

Objectives: To explain that mode of delivery has a correlation with neonatal outcomes in preterm pregnancies.

Methods: This study is a retrospective cohort conducted at Dr. Moewardi Surakarta Hospital, with total sampling of 484 cases divided into early preterm and late preterm from 2019 to 2021. Bivariate analysis was carried out to determine the correlation between Mode of Delivery (MOD) and neonatal outcomes.

Results: In the early preterm group, mode of delivery had a significant correlation ($p<0.05$) with birth weight, APGAR score, and neonatal mortality, however a significant correlation ($p<0.05$) in the late preterm group was only found in the APGAR score and neonatal mortality outcomes. Cesarean delivery, in both early and late preterm births had a lower rate of asphyxia and neonatal death ($p<0.05$).

Conclusion: There is a significant correlation between mode of delivery and neonatal outcomes in preterm birth. Caesarean delivery by indication can significantly reduce the risk of asphyxia, and neonatal mortality due to premature birth.

Keywords: mode of delivery, neonatal outcome, preterm.

Abstrak

Tujuan: *Menjelaskan bahwa metode persalinan sangat berkorelasi dengan luaran neonatus pada kehamilan prematur, oleh karena itu penelitian ini dapat menjadi bahan pertimbangan untuk memilih metode persalinan.*

Metode: *Penelitian ini merupakan penelitian kohort retrospektif yang dilakukan di RSUD Dr. Moewardi Surakarta, dengan total sampling sebanyak 484 kasus yang terbagi menjadi early preterm dan late preterm dari tahun 2019 hingga 2021. Analisis bivariat dilakukan dengan menggunakan analisis Chi-square dan Kruskal-Wallis untuk melihat korelasi antara metode persalinan (MOD) dan luaran neonatus*

Hasil: *Pada kelompok early preterm, metode persalinan memiliki hubungan yang signifikan ($p<0,05$) dengan luaran neonatus baik berat lahir, skor APGAR, dan kematian neonatus. Pada kelompok late preterm, hubungan yang signifikan ($p<0,05$) hanya didapatkan pada luaran skor APGAR dan kematian neonatus. Persalinan Caesar baik early maupun late preterm memiliki angka yang lebih rendah terhadap asfiksia dan kematian neonatus ($p<0,05$).*

Kata kunci: *metode persalinan, luaran neonatus, preterm.*

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INTRODUCTION

Mode of delivery is one of the essential factors in managing preterm labor. Preterm birth occurs in 5% to 10% of all pregnancies and is the most common cause of maternal and fetal morbidity and mortality worldwide. The risk to the fetus is mainly due to respiratory distress syndrome, sepsis, and low birth weight.[1,2] Recommendations for the delivery method for preterm birth are still controversial and have not been clearly defined.[2-4]

The rate of cesarean delivery (CS) has increased significantly over the past two decades, particularly in preterm deliveries. Cesarean delivery for premature babies has become an option in preterm labor. This is due to better management of postnatal care for Very Low Birth Weight (VLBW) infants. Several studies have also demonstrated significant beneficial effects of cesarean delivery (CS) in preventing neonatal death.[4,5] However, obstetricians and perinatologists still debate the optimal delivery method in preterm labor.

Studies evaluating the correlation between delivery method and neonatal outcome in preterm delivery are still lacking. Two previous studies have shown that vaginal delivery is associated with an increased likelihood of neonatal death with preterm delivery.[4,5] Several previous studies have also examined the outcome of Very Low Birth Weight neonates (<1,500g), comparing cesarean delivery with vaginal delivery and giving contrast results regarding the likelihood of intraventricular hemorrhage and neonatal death.[5,6]

A retrospective study is one of the reliable studies comparing neonatal outcomes in preterm birth related to the choice of delivery method. Therefore, this study aimed to determine the correlation between mode of delivery and neonatal outcomes in premature births at Dr. Moewardi Surakarta Hospital.

METHODS

This study is a retrospective cohort study conducted at Dr. Moewardi Surakarta Hospital. Samples were all patients with premature births, both early preterm and late preterm, at Dr. Moewardi Surakarta Hospital. A total sample of 484 patients were taken using the total sampling technique from 2019 to 2021. All samples were assessed and analyzed for characteristics and correlations between variables. The characteristics evaluated were gestational age, gravidity, maternal age, and mode of delivery.

The independent variable in this study is the mode of delivery, which is assessed on a categorical scale. The mode of delivery variable is classified into two, vaginal and Cesarian Section. In comparison, the dependent variables in this study were neonatal outcomes in Birth Weight (BW), APGAR score, and neonatal mortality. Birth Weight (BW) was assessed on an ordinal scale, classified as follows. VVLBW (Very Very Low Birth Weight, <1000 grams), VLBW (Very Low Birth Weight, 1000 - <1500 grams), LBW (Low Birth Weight, 1500 - < 2500 grams) and normal BW (Birth Weight > 2500 grams).

The outcome of neonatal was assessed based on the APGAR score at 5 minutes. The measurement of the APGAR score is in the form of an ordinal scale, with the following classification; Severe asphyxia (0-3), Moderate asphyxia (4-6), Mild or normal asphyxia (7-10) also categorical death, and still alive.

Bivariate analysis was conducted to determine the Correlation between mode of delivery and neonatal outcomes. Bivariate analysis was performed using the Chi-square to analyze the correlation between mode of delivery and neonatal mortality. In addition, the Kruskal Wallis was used to analyze the Correlation between mode of delivery and APGAR also birth weight scores. Data analysis using SPSS version 23 software.

RESULTS

A total of 484 samples were assessed and analyzed in this study. Table 1 describes the characteristics of each variables. The table explains that the total number of samples who received the vaginal delivery method was 173 people, while the other 311 received the Cesarean delivery method (CS).

Table 1. Characteristics of the Research Sample

Variable	Vaginal		Cesarean Section	
	N	%	N	%
Gestational age				
early	98	56.6	136	43.7
late	75			
Mother's age				
< 35	124	71.7	188	60.3
35	49	28.3	123	39.7
Gravide				
primigravida	51	29.5	59	19.0
multigravida	122	70.5	252	81.0
Total	173	100.0	311	100.0

source: primary data 2021

The sample was divided into early preterm (n=234) and late preterm (n=250) based on gestational age. This gestational age category was compared between early and late preterm, and bivariate was carried out to determine the Correlation between mode of delivery and neonatal outcomes in each group.

The characteristics of the sample maternal age were divided into <35 years (n=312) and ≥35 years (n=172). Meanwhile, the characteristics of gravidity were split into two categories, primigravida (n=110) and multigravida (n=374). After analyzing the characteristics of the sample, bivariate analysis was carried out to determine the correlation between the mode of delivery and neonatal outcomes. The results of the bivariate analysis are described in Table 2.

Tabel 2. Correlation Mode of Delivery and Neonatal Outcome

Variable	Early Preterm					Late Preterm				
	Vaginal		Cesarean Section		*P-value*	Vaginal		Cesarean Section		*P-value*
	N	%	N	%		N	%	N	%	
Birth Weight										
<1000	27	27.6	10	7.4	0.000	0	0.0	3	1.7	0.600
1000-<1500	33	33.7	53	39.0		5	6.7	15	8.6	
1500-<2500	34	34.7	69	50.7		48	64.0	113	64.6	
>2500	4	4.1	4	2.9		22	29.3	44	25.1	
APGAR										
Mild asphyxia	24	24.5	45	33.1	0.000	54	72.0	143	81.7	0.002
Moderate asphyxia	24	24.5	57	41.9		7	9.3	22	12.6	
Severe asphyxia	28	28.6	30	22.1		1	1.3	4	2.3	
Neonatal death										
Yes	22	22.4	4	2.9	0.000	13	17.3	6	3.4	0.000
No	76	77.6	132	97.1		62	82.7	169	96.6	
Total	98	100.0	136	100.0		75	100.0	175	100.0	

The study results showed that in the early preterm group, mode of delivery had a significant correlation ($p<0.05$) with neonatal outcomes, including birth weight, APGAR scores, and neonatal mortality. In addition, the data shows that cesarean delivery has a lower incidence of low birth weight, respiratory distress syndrome characterized by asphyxia and has a statistically lower mortality rate than vaginal delivery.

Meanwhile, a significant correlation ($p<0.05$) was only found in the APGAR score and neonatal mortality in the late preterm group. Cesarean delivery had a lower rate of asphyxia and neonatal death ($p<0.05$). The output in birth weight did not show a significant correlation with the mode of delivery in this study.

DISCUSSION

This study aims to determine the Correlation between mode of delivery on neonatal outcomes with preterm birth, which was determined by birth weight, APGAR score at 5 minutes, and neonatal mortality. This study found that cesarean delivery had a better benefit on neonatal outcomes in preterm delivery. Previous similar studies have shown mixed results and have not fully explained how the advantages of cesarean delivery are compared to vaginal delivery in preterm delivery.

This study showed a significant value in all groups, both early preterm and late preterm. Both groups showed that cesarean delivery significantly reduced the risk of neonatal death in preterm delivery. In addition, significant results were also shown in the APGAR score variable, where the group of cesarean delivery was lower in experiencing asphyxia than vaginal delivery. However, significant results were only shown in the early preterm group for the Birth Weight variable.[7,8]

Preterm pregnancies that need to be delivered may become the sign of indication that the fetal well-being is disrupted inside the womb or the fetus will have a harmful impact on the pregnancies, therefore delivering the baby as soon as possible is a preferred choice to prevent neonatal morbidity. Cesarean delivery has relatively faster than vaginal delivery in patients who have had no cervical dilation before; therefore, cesarean delivery becomes effective in saving infants' lives, including neonatal asphyxia, when they are required for medically indicated reasons. However, the assertion that cesarean delivery can reduce the risk of neonatal death in

preterm labor is still debated. Cesarean delivery is associated with increased short and long-term threats to the mother, including infection, bleeding, and future surgical complications from scarring. In addition, Cesarean deliveries are more expensive for the health care system.[9,10]

The results of this study are in line with a retrospective study and several other studies which showed that cesarean delivery was associated with a reduced probability of death within 28 days of life for neonates born before 31 weeks of gestation. In addition, the findings of this study are also in line with the study who found that cesarean delivery was associated with reduced neonatal mortality in singletons.[11,12]

This study is more complete compared to previous studies considering the relatively large sample size for rare conditions and the variation in the outcomes measured. In addition, this study also distinguished between the early preterm group and the late preterm group. This uses APGAR score at 5 minutes as the best available parameter, as it is easy to use and well known to both obstetricians and neonatologists. This parameter has also been shown to have adequate applicability in previous studies.

The nonrandomized retrospective cohort study used in this study lead to some limitations. We could not adjust the indication for delivery or classify spontaneous preterm delivery from indicated preterm birth. Thus, some of cesarean deliveries can occur in patients who require more urgent delivery and will be far worse if they undergo vaginal delivery. It is also conceivable that some cesarean deliveries may result from failed induction or trials of labour during health care. In addition, the data in this study did not contain information about the administration of steroids and antenatal pre-medication to reduce intraventricular hemorrhage and respiratory distress syndrome.

CONCLUSION

This study showed a significant correlation between mode of delivery and neonatal outcomes with preterm birth. Cesarean delivery can significantly reduce the risk of asphyxia, and neonatal death due to premature birth, but cesarean delivery should be consider those who need a more urgent indication than vaginal delivery, therefore the selection of the delivery method must be appropriate according to clinical conditions.

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Research Article

Attitude towards COVID-19 Vaccine among Pregnant Women

Sikap terhadap Vaksinasi COVID-19 di Kalangan Ibu Hamil

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Abstract

Objective: To assess the perceptions and intentions of pregnant women regarding COVID-19 vaccination and to explore the reasons for vaccine hesitancy as well as acceptance.

Methods: This prospective cross-sectional study was conducted in tertiary care hospital in Karnataka. Around 811 pregnant women attending the antenatal clinic were recruited into the study. Data were collected using a face-to-face, anonymous questionnaire written in local language.

Results: Eighty six point two percent participants were aged 20 to 30 years and 64.6% had completed their schooling. 94.3% of them were homemakers and 58 % of the participants were in their third trimester. 65.5% of study group members lived in a COVID-supportive environment. Participants with COVID-19 vaccination awareness accounted for 87.4% and 65.4% were willing to receive the same whole heartedly. Our study found that 65.4% of participants were willing to receive covid-19 vaccine. The reasons for refusal were Lack of sufficient information regarding the vaccine, may be harmful to foetus and mother and lack of data proving its quality and efficiency.

Conclusion: Pregnant women in the North Karnataka region were highly receptive to COVID-19 immunization. Although a high level of awareness was apparent, the lack of data and fear of side effects were two major concerns for refusal. Confidence in the government and the availability of free vaccines for all have demonstrated a massive impact on vaccination.

Keywords: antenatal, covid-19, pregnancy, vaccination.

Abstrak

Tujuan: *Menilai persepsi dan keinginan perempuan hamil terkait vaksin COVID-19 dan alasan terkait penerimaan dan juga keraguan terhadap vaksin.*

Metode: *Penelitian ini merupakan penelitian potong lintang dengan pendekatan prospektif yang dilakukan di Rumah Sakit Karnataka. Perempuan hamil sebanyak 811 menjadi subjek penelitian. Data dikumpulkan menggunakan kuesioner yang dituliskan dalam Bahasa lokal dan pengumpulan data dilakukan secara tatap muka.*

Hasil: *Sebanyak 86,2 % sampel penelitian berusia 20 hingga 30 tahun dan 64,6% telah lulus sekolah. Sebanyak 94,3% sampel adalah ibu rumah tangga dan 58% dari sampel dengan usia kehamilan pada trimester ketiga. Sebanyak 65,5% grup tinggal pada kondisi. Sebanyak 87,4% sampel sadar akan keutamaan vaksin dan 65,4% bersedia mendapatkan vaksin. Alasan penolakan terhadap vaksin adalah kurangnya pengetahun mengenai vaksin dan ketakutan akan efek sampingnya.*

Kesimpulan: *Perempuan hamil di Karnatakan Utara terbuka terhadap imunisasi COVID-19. Disamping tingginya angka kesadaran akan imunisasi COVID-19, penolakan umumnya didominasi akibat kurangnya pengetahuan dan ketakutan akan efek samping. Keyakinan pada pemerintah dan ketersediaan vaksin secara gratis sangat berpengaruh pada vaksinasi.*

Kata kunci: *antenatal. covid-19, kehamilan, vaksinasi.*

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INTRODUCTION

Worldwide, coronavirus disease 19 (COVID-19) has imposed large burdens of morbidity and mortality among the general population [1]. In the absence of an effective treatment for COVID-19, non-pharmaceutical interventions are the only available methods of disease control such as social distancing, face masks, and personal hygiene, however maintaining these actions are difficult on the long run. As a result, herd immunity by vaccination becomes the most effective eradication method, like in any viral epidemic diseases in the past. Vaccination has undoubtedly made a significant impact to human and animal health, notably in developing countries [2].

Pregnant women possess an increased risk of severe illness, intensive care unit admission and invasive ventilation when compared with non-pregnant patients of the same age. Therefore, Pregnant women are regarded as high-risk population in COVID-19 infection [3-6]. The incidence of covid 19 in our institute was 1.3% during our study period. Data the Covid-19 registry on pregnant and postpartum women shows that symptomatic cases were also significantly higher than that in the second wave with percentage of 28.7% and 14.3% respectively.

Given the risk-reward balance, a judgment call was taken in June 2021 by the ministry of health and family welfare of India to include the pregnant women in COVID 19 vaccination programme. The decision was based on the preponderance of probability and potential benefit of the mass use and protection towards pregnant women, especially given that COVID-19 infection is more severe in pregnancy. However, few studies revealed low acceptance rates for covid vaccine3.

Vaccine hesitancy is the reluctance of people to accept a vaccine in spite of its proven safety measures, efficiency and availability [7]. Vaccination coverage would be more extensive if we could better identify vaccination barriers in the population, especially among vulnerable groups, like pregnant women. Even the most effective vaccine would have a limited impact on the spread of a disease if people refused to take it. Few studies have been done in the recent past regarding the acceptance of covid vaccine but most of them were done in developed nations which are different in terms of family structure, income, education and awareness, accessibility to mass media, etc [6,7]. To identify the barriers of the acceptance of the vaccination, a questionnaire was designed differently by keeping all those differences in the mind. Identifying barriers among priority groups will help in planning vaccination strategies to increase uptake and contribute in fighting the pandemic 3. Our study aimed to assess the perceptions and intentions of pregnant women regarding COVID-19 vaccination and explore the reasons for vaccine hesitancy as well as acceptance.

METHODS

This prospective cross-sectional study was conducted in tertiary care hospital in Karnataka between September 2021 to December 2021. The hospital was a referral centre for covid infection as well as vaccination. Around 811 pregnant women attending the antenatal clinic were recruited into the study after the clearance by institutional ethical committee approval. Data were collected using a face-to-face, anonymous questionnaire written in local language and data collection preceded by a written informed consent. Participants were reassured about the confidentiality of the data. COVID-19 precautions were taken including the use of face mask, hand sanitation and physical distancing as preventive measures. Inclusion criteria were all pregnant women attending the out-patient clinic and willing to participate in the study. Exclusion criteria were patients with specific contraindications for COVID 19 vaccine, mental disabilities and communication disabilities.

As the infection and vaccination against covid-19 was a new of its kind, there was a paucity of validated tool during the study period. The questions prepared by the researchers themselves were used in the questionnaire. The questionnaire was created by the researcher contained 30 questions about sociodemographic characteristics, vaccination history, perception of risk related to the COVID-19 pandemic, the impact of the COVID-19 pandemic, and reasons for acceptance and denial for COVID-19 vaccination. The stage of pregnancy of the patients was based on the last menstrual period or first-trimester crown-rump length. High-risk pregnancy category includes preterm labor, hypertensive diseases of pregnancy, gestational diabetes mellitus, multifetal pregnancy, epilepsy, and placenta previa.

Sample size was calculated by convenient sampling method using openepi.com; an online sample size calculator. The sampling method

considered the pregnant population attending the out-patient clinic as well as the case load of covid 19 infection in pregnancy. With the 95% confidence interval and 5% margin of error the calculated sample was 400, we inflated the number to 811 to ensure an accurate and generalizable result. Discrete variables were represented as number and percentages. Statistical analysis was performed using SPSS 17 (SPSS Inc.,Chicago, IL, USA). The Shapiro–Wilk test and Kolmogorov–Smirnov test were used to determine the distribution of normality, and $\chi 2$ test was used to compare categorical data. Groups were compared with Spearman's rho test for correlation between sociodemographic variables and COVID-19 vaccine acceptance. A type-1 error below 0.05 was considered statistically significant.

RESULTS

The survey population represented a random sample of pregnant women attending the antenatal clinic in a tertiary care hospital. A total of 811 responses were obtained. Sociodemographic and clinical characteristics are presented in table 1. The largest group of participants (86.2%) was 20 to 30 years old and 64.6% had completed their schooling. Most of them were homemakers (94.3%) and 58 % participants were in their third trimester. 98.5% had no related comorbidities and 97.4 %were already vaccinated against tetanus. 44.9% had less than four family members and 71.4% denied the presence of school/college going children at home. Many participants (90.5%) were below the poverty line and 35.5% had no elderly people at home. 65.5% of study group members said that they live in a COVID-supportive environment. However, 98.2% denied any recent contact with COVID-19 patients. Participants with COVID-19 vaccination awareness accounted for 87.4% and 65.4% were willing to receive the same whole heartedly.

Table 1. Socio-demographic and Clinical Characteristics of the Participants

Characteristics	Category	No (%)
Age (years)	<20	38(4.7)
	20-30	699(86.2)
	30-40	72(9.1)
Education	Uneducated	33(4.1)
	school	524(64.6)
	college	172(21.2)
	Graduate	82(10.1)
Profession of the participant	Homemaker	765(94.3)
	Govt Job	18(2.2)
	Private job	28 (3.5)
Gestational age(wks)	<12	52(6.4)
	12-28	293(36.3)
	>28	465(57.4)
School/college going children at home	nil	579(71.4)
	1-2	121(14.9)
	>2	101(13.7)
Senior citizens at home	nil	288(35.5)
	1-2	231(28.5)
	>2	291(35.8)

Among participants who were willing to receive vaccine, 66.2% were 20-30 years old, 55.5% were educated with 66.5% home makers and 64% had low family income. Majority of them were in second and third trimesters with 81.8% being aware of the covid vaccine (Table 2).

Table 2. Attitude towards COVID-19 Infection and Vaccination

Characteristics	Categories	Willing to take vaccine/ vaccinated No(rows%)	Hesitant to take vaccine/ unvaccinated No(rows%)	*P-value*
		530(65.3)	281(34.7)	
Age (years)	18-20	27(71.1)	11(28.9)	0.06
	21-30	463(66.2)	236(33.8)	
	Above 30	38(52.8)	34(47.2)	
Education	Uneducated	15(45.5)	18(54.5)	0.29
	Schooling	334(63.7)	190(36.3)	
	College	124(72.1)	48(27.9)	
	Graduate	57(69.5)	25(30.5)	
Profession of the participants	Home maker	508(66.4)	257(33.6)	0.37
	working	22(47.8)	24(52.2)	
No of household members	<4	245(67.3)	119(32.7)	0.449
	4-6	160(61.5)	100(38.5)	
	>6	125(66.8)	62(33.2)	
Annual income	BPL	469(63.9)	265(36.1)	0.007
	APL	61(79.2)	16(20.8)	
Gestational age(weeks)	<12	38(73.1)	14(26.9)	0.063
	13-28	179(60.9)	115(39.1)	
	>29	313(67.3)	152(32.7)	
Covid prone working environment	Yes	185(66.1)	95(33.9)	0.754
	No	345(65)	186(35)	
is social distance possible?	yes	435(65.6)	228(34.4)	
	No	95(64.2)	53(35.8)	
Awareness of covid vaccine in pregnancy	yes	484(70.8)	200(29.2)	0.0
	No	46(36.2)	81(63.8)	

Among participants who denied vaccine, 76% were unaware of it and 94.3% lived below poverty line. Uneducated constituted 6.4% and 91.5% were home makers (Table 2). Number of household members were more than six in 23.6% of participants among willing to receive vaccine and 22% among denying pregnant women (Table 2). Among reasons for not taking vaccine, lack of sufficient information accounted for 54.4% with fear of adverse effects to mother rating second (20.3%). Other reasons were advice from the family members against vaccination (10.7%), fears of adverse effects to the foetus (8.9%), fear of injection (3.2%), worries regarding the side effects (1.4%) and unlikely to get covid infection (0.7%) and doubts regarding the vaccine (0.4%) 57.5% of participants opined that Asha workers informed them regarding vaccine and 31.6% received the same by their treating doctors. Source of information via media, family and friends constituted about 6.4% and 1% respectively.

DISCUSSION

This study was conducted in the declining phase of the second wave of COVID-19 infection in our region, where COVID-19 immunization has been approved for pregnant women in India. Our research uncovers the complex and interdependent factors around COVID-19 vaccine acceptance, denial, and the analysis of the underlying reason. Respondents who were unwilling to receive vaccine expressed three reasons for the refusal. The first one is the lack of sufficient information regarding the vaccine [3, 8,] teratogenic potential [9, 10] and insufficient data regarding the quality and efficiency. [8, 11-13]. Vaccination programmes can only be considered effective if they have a high level of acceptance and coverage. Our study found that 65.4% of participants were willing to receive covid-19 vaccine. This number exceeded the results in some of the studies[9-11,13-16] but not in some studies [17]. Few studies showed an acceptance rate of less than 25% [3,18-20]. The reasons behind this difference might be due to differences in access to health care services, different level of awareness regarding severity of infection and vaccination and different study population.

In contrast with some studies and consistent with the skjefte study, we found that women aged 20 to 30 years were more likely to get vaccinated [3,13,18,21]. In some part of the study showed that

the participants with low income were more inclined towards acceptance of the vaccine. This could be explained by, first, the fact that our government hospital is a tertiary care facility and that it provides services to low-income people to a greater extent. Secondly, the Government of India's decision to make the COVID-19 vaccine free for all Indian citizens could have played a huge role. A positive correlation was found between COVID-19 vaccine acceptance and number of school/college going children at home. This could be due to greater anxiety of transmission of infection to other family members via children [3,12]. Pregnant women in their second and third trimester were more willing to get vaccinated as compared with pregnant women in first trimester, these observations were in contrast. [3,22,23]

Unlike few studies, the majority of the participants willing to receive vaccines were educated (86%) and a few (10%) were graduates [9,12,13,24]. This may be due to the increased chances of being exposed to different media for an educate which fosters the awareness regarding vaccination. Our study discovered that immunization awareness among participants achieved through Asha workers accounted most followed by the same created by treating consultants. 30% of participants unwilling to get vaccinated were unaware of COVID-19 vaccine and 10% of them said they had family and friends who were objected to vaccination. This study brings the fact into light that in this world of rapidly spreading and easily available wrong information, it is imperative that awareness should be spread not only to the pregnant women but also to wider population who influences them effectively [9,25]. This could be done via mass communication media, reception areas in the hospital and so on.

Our findings show that trust in government is strongly associated with vaccine acceptance and can contribute to public compliance with recommended actions [18,26]. Clear and coherent communication from government representatives is crucial to strengthening public confidence in immunization programmes. Maternal mortality accounts for 2% of total covid deaths in India from February 2020 to June 2021, comprising one fifth of usual maternal deaths. The decision to allow vaccination of pregnant women against COVID-19 is a step towards achieving target 3.1 of sustainable development goals which aims to reduce global MMR (maternal mortality rate) of less than 70 per one lakh live births [27].

This study had some limitations. This survey is a snapshot taken at a point in time and was conducted in the context of a highly dynamic and changing landscape with rapid changes in the clinical presentation of the disease threat as well as fast track development of COVID vaccine itself. The strength of the study is its novelty and inclusion of large number of parameters.

CONCLUSION

Pregnant women in the North Karnataka region were highly receptive to COVID-19 immunization. Although a high level of awareness was apparent, the lack of prior knowlede and fear of having side effects were two major concern for the refusal. Continued public health efforts and vaccine safety awareness campaign would help overcome these barriers and encourage pregnant women to get vaccinated. Confidence in the government and the availability of free vaccines for all have demonstrated a massive impact on vaccination.

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Research Article

Antibiotic Use in Cesarean Section among Obstetricians and Gynecologists in the Second Largest City in Indonesia

Penggunaan Antibiotik pada Operasi Sesar oleh Dokter Obstetri dan Ginekologi di Kota Terbesar Kedua di Indonesia

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Abstract

Objective: To evaluate the pattern of antibiotic use in cesarean section `by obstetricians in Surabaya, Indonesia.

Methods: This was a descriptive observational study with a cross-sectional method. Study data were obtained from online interviews using electronic forms. This study used a total sampling method taken from obstetricians and gynecologists in Surabaya, Indonesia. The primary outcome of this study was a pattern of antibiotic use, including prophylactic use, selection of antibiotics, the timing of administration, additional antibiotics during and after surgery, and consideration of choice.

Results: The majority of antibiotics used in CS are in line with the guidelines. The types of prophylactic antibiotics (iv) used are varied; the majority were cefazoline (74.5%), ceftriaxone (14.5%), and cefotaxime (11.6%). Most antibiotics were administered <30 minutes before surgery. 2.5% of obstetricians routinely added antibiotics during a cesarean, while 33% were based on a particular condition such as prolonged surgery, massive bleeding, or risk of infections. The selection of antibiotics by obstetricians was based on protocols followed in the hospital (44.5%).

Conclusion: This study demonstrates that most obstetricians utilized antibiotic prophylaxis appropriately and followed guidelines for Cesarean Section.

Keywords: antibiotic, cesarean section, maternal health, obstetricians.

Abstrak

Tujuan: *Untuk mengevaluasi pola penggunaan antibiotik pada seksio sesarea oleh dokter kandungan di Surabaya, Indonesia.*

Metode: *Ini merupakan studi deskriptif observasional dengan metode pengambilan data potong lintang. Data studi diperoleh dari wawancara online dengan menggunakan formulir elektronik. Studi ini menggunakan total sampling dari dokter obstetri dan ginekologi di Surabaya, Indonesia. Hasil utama dari penelitian ini adalah pola penggunaan antibiotik, termasuk penggunaan profilaksis, pemilihan antibiotik, waktu pemberian, antibiotik tambahan selama dan setelah operasi, dan pertimbangan pilihan antibiotik tersebut.*

Hasil: *Mayoritas antibiotik yang digunakan pada seksio sesarea sesuai dengan pedoman. Jenis antibiotik profilaksis (iv) yang digunakan bervariasi, mayoritas adalah cefazoline (74,5%), ceftriaxone (14,5%), dan cefotaxime (11,6%). Sebagian besar antibiotik diberikan <30 menit sebelum operasi. 2,5% dokter kandungan rutin menambahkan antibiotik saat operasi sesar, sedangkan 33% didasarkan pada kondisi tertentu seperti operasi yang berkepanjangan, perdarahan masif, atau risiko infeksi. Pemilihan antibiotik oleh dokter kandungan berdasarkan protokol yang diikuti di rumah sakit (44,5%).*

Kesimpulan: *Studi ini menunjukkan bahwa sebagian besar dokter kandungan menggunakan profilaksis antibiotik dengan tepat dan mengikuti pedoman untuk operasi seksio sesaria.*

Kata kunci: *antibiotik, dokter kandungan, kesehatan ibu, operasi sesar.*

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INTRODUCTION

Cesarean section (CS) is the most frequently performed surgery in obstetrics and gynecology, and the trend is increasing annually. The World Health Organization (WHO) has released that the number of caesarean sections performed worldwide has increased and now accounts for more than 1 in 5 (21%) deliveries. According to the study, this number is expected to rise over the next ten years, with nearly a third (29%) of all newborns projected to be delivered via cesarean section by 2030[1]. The incidence of SC is much higher in private hospitals than in public hospitals. In Indonesia, a demographic health study involving 56,462 pregnant women showed that the CS prevalence rate had increased rapidly from 4% in 1998 to 18.5% in 2017. These figures in urban areas (22.9%) were double those in rural areas (11.8%).[2]

Prophylactic antibiotics are highly recommended for CS.[3–6] Antibiotic prophylaxis has been shown to reduce maternal morbidity, healthcare costs, and antibiotic overuse.[7,8] Prophylactic antibiotics can also reduce the risk of postoperative and surgical wound infections.[5–7,9,10] According to the Scottish Intercollegiate Guidelines Network's guidelines on antibiotic prophylaxis in surgeries, surgical antibiotic prophylaxis must be used appropriately thus, they must be supported by evidence of their efficacy and the effect of these antibiotics on the patient's normal bacterial flora and the patient's immune system must be minimized.

The inappropriate use of antibiotics can cause severe health problems, including antibiotic resistance.[11–16] Bacteria, viruses, fungi, and parasites can develop immunity to antibiotics, reducing the effectiveness of antibiotics.[14,15,17] According to the Centers for Disease Control and Prevention, approximately 2.8 million people in the US exhibit antibiotic resistance, and more than 35,000 people die yearly[16]. Antibiotic resistance develops when bacteria and fungi develop the ability to resist medications initially intended to eradicate them. Due to the otherwise surviving and growing bacteria, physicians must be cautious about this phenomenon.[18] Inappropriate antibiotic use, in terms of drug selection and administration timing, dose, and duration, is a critical factor in the emergence of antibiotic resistance.

Although recommendations for the use of antibiotics have been advised, in practice, antibiotic use in CS varies according to the attending obstetricians' judgment. Apart from following the guidelines, personal experience influences how obstetricians provide antibiotics during cesarean section. The purpose of this study was to describe the pattern of antibiotics use by obstetricians in Surabaya, including prophylactic use, selection of antibiotics, the timing of administration, additional antibiotics during and after surgery, and consideration of choice.

METHODS

This descriptive study was conducted in Surabaya, Indonesia, from July to August 2021. The ethical clearance was approved by the Ethical Committee of Universitas Airlangga Hospital (No. 138/KEP/2021), Surabaya, Indonesia. The research sample comprised obstetricians working in all hospitals in Surabaya. All obstetricians in Surabaya were included in this study based on the inclusion and exclusion criteria (total sampling size). Obstetricians who are practicing and performing cesarean sections actively mostly meet the inclusion requirements. The exclusion criterion was a participant who did not fully complete the questionnaire.

The trial's primary outcome was the pattern of antibiotic use in CS in cases of infection and non-infectious. The definition of infection cases was any type of infection in the mother that occurred during childbirth, both systemic infections and those localized to the uterus (e.g., chorioamnionitis and endometritis). The primary outcome of this study was the pattern of antibiotic use, including prophylactic use of antibiotics, selection of prophylactic antibiotics, the timing of antibiotic administration, the addition of antibiotics during and after surgery, and consideration of antibiotics. The clinical characteristics of the respondents were assessed based on age, work experience, and workplace. The healthcare center is divided into primary, secondary, and tertiary hospitals and exceptional hospitals for mothers and children. The type of hospital is distinguished based on the completeness of the type of health services that can be provided and the number of beds and health personnel available.

The research data were obtained from online electronic forms (Google Forms) interviews. We provided a questionnaire of 27 questions related to general characteristics and the use of antibiotics

in cesarean section. The research team contacted prospective study participants, provided information regarding the study, and obtained informed consent from participants. The study participants then completed the questionnaire, and the researcher collected the data. The data were displayed as descriptive statistics as some participants were affected and percentages from total participants. The data collected from the questionnaire is then cleaned to separate biased data. For easier reading results, the data is visualized as a table containing the amount and percentage of each question variable. Then, the data were analyzed descriptively by interpreting the percentage of respondents' answers.

RESULTS

Two hundred obstetricians agreed to participate (50% response rate) in this study. The remaining obstetricians (211 people) did not respond due to the pandemic. Most participants (93.5%) were between the ages of 30 and 60 and had less than ten years of work experience (53.5%). Participants worked in various hospital settings, with the majority (39%) working in secondary and primary hospitals (33%). Classification "others" on hospital type refers to the obstetrician's practice outside the hospital, such as in the inpatient clinic (Table 1).

Table 1. Background Characteristics

Characteristics	N (%)
Ages (years old)	
30 – 39	67 (33.5)
40 – 49	65 (32.5)
50 – 59	55 (27.5)
60 – 69	11 (5.5)
70 - 79	2 (1)
Working Experience (years)	
>20	14 (7)
10 – 20	79 (39.5)
< 10	107 (53.5)
Hospital Type	
Tertiary care hospital	30 (15)
Secondary care hospital	78 (39)
Primary care hospital	66 (33)
Mother and Child hospital	4 (12)
Others	2 (1)

The pattern of antibiotics used in non-infected CS by an obstetrician is shown in Table 2. In non-infectious CS, all obstetricians used prophylactic antibiotics. The types of antibiotics (iv) varied; the majority were cefazoline (74.5%), ceftriaxone (14.5%), and cefotaxime (11.6%). The doses of prophylactic (iv) antibiotics used varied from 500 to 3000 mg, and most were administered <30 min before the surgery (79.5%). Of the participants, 2.5% administered additional antibiotics routinely during surgery. The other 33% were administered additional antibiotics during surgery based on prolonged surgery, bleeding > 1.5 liters, and a risk of infection. Of the participants, 28.5% continued IV antibiotics after CS for 1 to 7 days, although there was no sign of infection. Some participants continued to give oral antibiotics after surgery (32.8%). The most common oral antibiotics administered postoperatively were cefadroxil and amoxicillin for 3–7 days (Table 2).

Table 2. Antibiotics Used in Cesarean Section

Antibiotics Used	N (%)
Cesarean Section on Non-infected Cases	
Antibiotics Prophylactic used	
Yes	200 (100)
No	0
Type of Prophylactic Antibiotics Used (iv)	
Cefazoline	149 (74.5)
Ceftriaxone	29 (14.5)
Cefotaxime	23 (11.6)
Cefoperazone	2 (1)
Amoxicillin Clavulanic Acid	2 (1)
Cefuroxime	5 (2.5)
Ampicillin Sulbactam	4 (2)
Gentamycin	1 (0.5)
Erythromycin	1 (0.5)
Phosphomycin	1 (0.5)
Timing of antibiotics administration	
>30 minutes before CS	35 (17.5)
<30 minutes before CS	159 (79.5)
After abdominal incision	5 (2.5)
After cord clamping	1 (0.5)
Addition of antibiotics during surgery	
Yes	5 (2.5)
No	129 (64.5)
If necessary, based on the clinical judgment during surgery	66 (33)
What condition necessitates the addition of antibiotics?	
Prolong surgery	52 (78.7)
Bleeding > 1.5 L	29 (43.9)
Infection risk	11 (16.6)
Others	15 (22.7)
Antibiotics (IV) continued after cesarean	
Yes	57 (28.5)
No	143 (71.5)
Duration of antibiotics (IV) continued after cesarean (days)	
1	29 (50.8)
2	8 (14.0)
3	14 (24.5)
5	5 (8.7)
7	1 (1.7)

Antibiotics oral continued after cesarean without infection risk or complications	
Yes	65 (32.8)
No	133 (67.17)
Type of oral antibiotics given after cesarean	
Cefadroxil	38 (58.4)
Amoxicillin	14 (21.5)
Ciprofloxacin	6 (9.2)
Amoxicillin Clavulanic Acid	1 (1.53)
Cefixime	4 (6.1)
Levofloxacin	1 (1.5)
Duration of oral antibiotics continued after cesarean (days)	
3	18 (27.6)
4	1 (1.5)
5	39 (60)
6	1 (1.5)
7	6 (9.2)

In cases of CS with infection, all participants were administered antibiotics during surgery. The most common types of antibiotics administered during cesarean delivery in cases of infection were ceftriaxone, metronidazole, cefazolin, and cefotaxime by an IV line. These antibiotics can be administered alone or in combination with several antibiotics. Postoperative oral antibiotics were continued in 96% of participants, the most common being ceftriaxone, metronidazole, cefotaxime, and amoxicillin-clavulanate for 1–7 days (Table 3).

Table 3. Antibiotics Use in Infected Cases Caesarean Section

Antibiotics Used	N (%)
Cesarean Section on Infected Cases	
Type of Antibiotics (iv) administrated	
Ceftriaxone	93 (46.5)
Cefotaxime	36 (18)
Cefazoline	43 (21.5)
Ampicillin Sulbactam	12 (6)
Amoxicillin Clavulanic Acid	7 (3.5)
Gentamicin	15 (7.5)
Metronidazole	51 (25.5)
Cefuroxime	2 (1)
Meropenem	1 (0.5)
Amikacin	2 (1)
Amoxicillin	1 (0.5)
Antibiotics continued after cesarean	
Yes	192 (96)
No	8 (4)
Type of Antibiotics administrated	
after cesarean	88 (45.8)
Ceftriaxone	33 (17.1)
Cefotaxime	7 (3.6)
Cefazoline	15 (7.8)
Ampicillin Sulbactam	25 (13.1)
Amoxicillin Clavulanic acid	17 (8.8)
Gentamycin	69 (35.9)
Metronidazole	1 (0.5)
Cefuroxime	1 (0.5)
Cefixime	3 (1.5)
Cefadroxil	2 (1.0)
Amikacin	
Duration of Antibiotics administrated after cesarean (days)	
1	1 (8.3)
2	19 (9.8)
3	91 (47.3)
4	1 (0.5)
5	57 (29)
7	8 (4.1)

The selection of antibiotics by obstetricians was based on protocols followed in the hospital (44.5%), the latest scientific evidence (20%), followed by specialist doctor education (16%), recently attended scientific seminars (10%), and other reasons (9.5%).

DISCUSSION

This study showed that all obstetricians administered prophylactic antibiotics before CS. Cochrane studies have shown that using prophylactic antibiotics in a CS can reduce superficial perineal wound infection, deep perineal wound infection, and probably wound breakdown.[19] However, the choice of prophylactic antibiotic administered is still highly varied. Most obstetricians used cefazoline (1st generation cephalosporin) as an intravenous prophylactic antibiotic at a dose of 2 g (93.2%); the remaining used 1–2 g. The rest use various antibiotics, including 2nd and 3rd generation cephalosporins. The pattern of prophylactic usage of broad-spectrum antibiotics is predominantly prevalent in Asian countries. Most obstetricians administered prophylactic antibiotics either less or more than 30 min before CS. Only five obstetricians gave antibiotics after the abdominal incision, and one received antibiotics after cord clamping. The WHO recommends administering prophylactic antibiotics for CS using a single dose of 1st generation cephalosporin between 30 and 60 minutes before surgery.[5] Using a single dose of CS prophylactic antibiotics can reduce costs, potential toxicity, and the risk of colonization by resistant microorganisms. The American College of Obstetricians and Gynecologists also recommends using a first-generation cephalosporin as the antibiotic of choice for SC prophylaxis.[4] The guidelines from the American Society of Health-System Pharmacists and the Society of Obstetricians and Gynecologists of

Canada also recommend using a single dose of a first-generation cephalosporin 15–60 minutes before incision.[20] A meta-analysis involving 16,328 pregnant women who underwent CS showed that cefazolin prophylaxis might reduce the risk of post-SC surgical site infection (SSI).[21] Two obstetricians still used amoxicillin-clavulanate as prophylactic antibiotics, contrary to the WHO recommendations, which prohibit amoxicillin-clavulanate as a prophylactic antibiotic because it increases the risk of necrotizing enterocolitis, particularly in preterm infants.[5] Most obstetricians used cefazolin as a prophylactic antibiotic at 2 g intravenously. The ACOG recommends determining the dose of prophylactic antibiotics based on maternal weight. Pregnant women with weights < 80 kg can be administered 1 g of cefazolin, while women with weights > 80 kg can be administered 2 g intravenously.[4] However, in Indonesia, The Indonesian Society of Obstetricians and Gynecologists (POGI) recommends the prophylactic use of cefazolin at a 2 g for all CS. The Indonesian Ministry of Health and the WHO's Indonesian wing have issued the latest guidelines on antimicrobial stewardship, aiming to regulate the use of antibiotics at the hospital.[22]

Regarding the timing of antibiotics, it was found that 3% of obstetricians administered antibiotics after abdominal incisions. The WHO recommends administering prophylactic antibiotics 30–60 minutes before surgery, while the ACOG recommends it within 60 minutes.[4,5] A systematic study showed that administering antibiotics within 60 minutes before an incision can reduce the risk of postpartum endometritis (43%), surgical wound infection (38%), and morbidity due to infection (28%) without interfering the neonatal outcomes, compared to mothers who were administered antibiotics after cord clamping.[23]

This study found that five obstetricians routinely administered additional antibiotics during CS. Meanwhile, 33% administered additional antibiotics only in special conditions (bleeding, prolonged surgery, or cases of high risk of infection), following the applicable recommendations. The WHO recommends special conditions that may increase the risk of postoperative infection (high body mass index (BMI)) prolonged labor, prolonged operative time, profuse bleeding, or extensive surgical manipulation). Antibiotics can be adjusted at higher or additional doses.[5] In one study, the administration of an additional antibiotic at the time of CS surgery (azithromycin 500 mg IV for 1 h) significantly reduced the risk of endometritis, surgical site infection, and other postoperative infections (relative risk (RR) 0.51, $p<0.001$).[24] However, this has not been officially recommended in international guidelines[3–5]

The present study found that some obstetricians continued routinely administering antibiotics after CS, either IV (28%) or orally (32.8%). The type of IV antibiotics used is the same as the prophylactic antibiotics; only the duration is extended postoperatively; the majority are between 1–3 days. Furthermore, some obstetricians and gynecologists also administer routine oral antibiotics post-SC, although there is no risk of infection. Cefadroxil and amoxicillin are the main types of oral antibiotics administered, lasting 3–5 days. To date, there is no substantial evidence supporting this routine protocol. A study involving 301 patients who underwent elective CS showed that prolonging IV antibiotics after CS for 72 h does not reduce the SSI risk.[25] The routine use of oral antibiotics after CS may not be appropriate because they are not prophylactic or therapeutic and are not based on the presence or absence of infection. Prolonging prophylactic antibiotic use can also trigger the emergence of antibiotic-resistant bacteria. The main rationale for antibiotic use is the shortest possible time to minimize side effects, develop bacterial resistance, and reduce hospital costs.[26] The use of antibiotics after CS may be considered under special conditions, such as obesity. This is supported by a randomized controlled trial involving 402 obese women who received additional antibiotics, metronidazole 500 mg and cephalexin 500 mg, for 48 h after CS. The intervention group had a lower risk of surgical wound infection than the control group (RR, 0.41; 95% CI: 0.22-0.77; $p=0.01$).[27]

We also evaluated the administration of antibiotics in CS surgery in cases of infection. All obstetricians provided therapeutic antibiotics during surgery with various choices, mainly by IV ceftriaxone, metronidazole, cefazoline, and cefotaxime. Similar oral antibiotics were administered postoperatively for 1–7 days. Antibiotics are only administered empirically before bacterial culture and antibiotic sensitivity tests are available. After surgery, generally, only the initially administered antibiotics are continued because the examination of bacterial cultures is complex in Indonesia. Not all health

facilities can perform bacterial cultures; some have to refer samples to a higher health center or external laboratory for cultures. Moreover, antibiotics were continued post-surgery based on the results of the germ culture.

This study shows that most antibiotic selections by obstetricians do not follow the hospital protocol (<50%). This may be attributed to the prevailing health system in Indonesia, where most obstetricians are not permanent employees of the hospital where they work; as a result, obstetricians' involvement and compliance with hospital service protocols are typically lower. In many countries, compliance with hospital protocols, specifically with the administration of antibiotics, remains a challenge.[26,28] In Indonesia, most obstetricians practice in more than one hospital (including private practice/clinic), lowering their adherence to the service protocol. This research reflects the mindset of obstetricians providing services at three hospitals.

Physician awareness and compliance with antibiotic use protocols in hospitals must be strengthened, as well as education, supervision, and hospital law enforcement. Additionally, professional organizations (POGI, IDI) should promptly develop standards for the use of antibiotics in obstetrics and gynecology; as a result, all obstetricians, regardless of the location, can practice uniformly.

One of the study's limitations was the study design. The data collection methods used were interviewing or conducting an online survey on daily practice, which are subjective and vulnerable to bias. Additionally, the details of cases were not analyzed, which may affect the antibiotics chosen by obstetricians. Additional trials using a prospective cohort design should be conducted to verify the findings of this preliminary study. This study also just included obstetricians in one big city in Indonesia.

CONCLUSIONS

This study showed that most obstetricians in Surabaya had used antibiotic prophylaxis for CS surgery appropriately and according to the guidelines. However, postoperative antibiotics should be corrected as it was not under the guidelines. Physician knowledge and compliance with antibiotic protocol in hospitals and professional organization (POGI, IDI) guidelines, education, supervision, and hospital law enforcement, must all be increased.

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Research Article

Ovarian Reservation in Women with Ovarian Endometriosis Cyst after Laparoscopic Cystectomy and Leuprorelin Acetate Administration

Cadangan Ovarium pada Perempuan dengan Kista Endometriosis Ovarium setelah Laparoskopi Kistektomi dan Leuprorelin Asetat

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Abstract

Objective: To determine differences in ovarian reserve in women with ovarian endometrial cysts after laparoscopic cystectomy and leuprorelin acetate administration

Methods: Single cohort prospective pre and post-test design study with 25 research subjects. The independent variables were interventional laparoscopic cystectomy, and leuprorelin acetate administration. The dependent variable is ovarian reserve as measured by AMH levels.

Results: The research subjects had a mean age of 31 years and a BMI of 23.55 kg/m2. There were 8 subjects with unilateral cysts and 17 subjects with bilateral cysts. Preoperative AMH levels had a median value of 1.32 ng/mL (0.88-5.13), postoperative AMH levels had a median value of 1.07 ng/mL (0.60-4.53), and postoperative AMH levels + Leuprorelin Acetate injection had a median value of 1.06 ng/mL (0.50-4.65). There was a significant difference between preoperative AMH and postoperative AMH ($p<0.001$). There was no significant difference between postoperative AMH and postoperative AMH + Leuprorelin Acetate injection ($p=0.149$). BMI has a relationship ($p=0.048$) with pre-operative AMH levels with a weak and opposite relationship ($r= -0.399$).

Conclusion: There was a statistically significant 18.9% decrease between pre-operative post-operative AMH levels. Body Mass Index (BMI) and pre-operative AMH levels are associated with one another.

Keywords: anti-müllerian hormone, leuprorelin acetate, ovarian cystectomy, ovarian reserve.

Abstrak

Tujuan: *Mengetahui perbedaan cadangan ovarium pada perempuan dengan kista endometrium ovarium setelah laparoskopi kistektomi dan injeksi leuprorelin asetat.*

Metode: *Desain penelitian pre dan post-test prospektif kohort tunggal dengan 25 subjek penelitian. Variabel bebas adalah intervensi laparoskopi kistektomi, dan injeksi leuprorelin asetat. Variabel terikat adalah cadangan ovarium yang diukur dengan kadar AMH.*

Hasil: *Subjek penelitian memiliki rerata usia 31 tahun dan IMT 23,55 kg/m2. Terdapat 8 subjek dengan kista unilateral, 17 subjek dengan kista bilateral. Kadar AMH praoperasi memiliki nilai median 1,32 ng/mL (0,88-5,13), kadar AMH pascaoperasi memiliki nilai median 1,07 ng/mL (0,60-4,53), dan kadar AMH pascaoperasi + injeksi Leuprorelin Asetat memiliki nilai median 1,06 ng/mL (0,50-4,65). Ada perbedaan yang signifikan antara AMH pra operasi dan AMH pascaoperasi ($p<0,001$). Tidak ada perbedaan bermakna antara AMH pascaoperasi dengan injeksi AMH + Leuprorelin Asetat pascaoperasi ($p=0,149$). IMT memiliki hubungan ($p=0,048$) dengan kadar AMH pra operasi dengan hubungan yang lemah dan berlawanan ($r= -0,399$).*

Kesimpulan: *Terdapat penurunan 18,9% antara tingkat AMH pra-operasi dan tingkat AMH pasca-operasi yang signifikan secara statistik. Ada hubungan antara BMI dan tingkat AMH pra-operasi*

Kata kunci: *cadangan ovarium, hormon anti-müllerian, kistektomi ovarium, leuprorelin asetat.*

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INTRODUCTION

Endometriosis is defined as the presence of endometrial-like tissue outside the uterus. Approximately 30-50% of women with endometriosis are infertile and ovarian endometrial cysts are commonly found during infertility screening examinations.[1–3] Ovarian endometriosis cysts can cause chronic pelvic pain and infertility.[1]

Treatment of endometriosis consists mainly of hormonal therapy or surgical therapy. Milder forms of endometriosis can be treated with oral contraceptive pills, whereas severe forms of endometriosis, such as ovarian endometriosis cysts, require surgical management with ovarian cystectomy.[1] It is suspected that ovarian cystectomy surgery can cause damage to the ovarian follicles resulting in decreased ovarian reserve. Hormonal therapy using GnRH agonist is thought to also affect ovarian reserve.[4]

One of the reliable markers of ovarian reserve is the measurement of Anti-Müllerian Hormone (AMH) levels. AMH is thought to play a role in the transition phase from inactive primordial follicles to follicles which then develop due to gonadotropin stimulation.[5]

This study aims to assess the ovarian reserve by measuring AMH levels in patients with ovarian endometriosis cysts before surgery, after laparoscopic cystectomy surgery, and three months after administration of leuprorelin acetate injection.

METHODS

This research was a prospective single cohort study of pre and post-test design conducted at the Gynecology Polyclinic of the Merpati Installation RSUP Dr. Kariadi Semarang. The research subjects consisted of 25 patients with ovarian endometrial cysts who underwent laparoscopic cystectomy followed by injection of leuprorelin acetate. All subjects met inclusion criteria and had no exclusion criteria. The inclusion criteria were women aged 18-45 years, diagnosed with endometrial cysts through history taking, physical examination, and supporting examinations according to the protocol, desiring a pregnancy, and willing to participate in the study by signing an informed consent. Exclusion criteria were pregnant or breastfeeding women, a woman using hormonal contraception, with a history of taking Vitamin D in the past year, a history of previous ovarian surgery, and currently having a malignancy. The drop-out criteria for the study were the mismatched in anatomical pathology results with ovarian endometriosis cysts, and lost follow-up during the study.

The research sample was taken using consecutive sampling technique. All patients with clinical endometriosis cysts who would undergo surgery were included in the prospective research subjects. Prospective research subjects who met the inclusion criteria and did not have the exclusion criteria, would be subjected to blood collection and pre-operative AMH serum examination. Following the surgery, an anatomical pathology examination was done. If the results of anatomical pathology did not show endometriosis cysts, the prospective research subjects would be excluded. Prospective research subjects with anatomical pathology results in accordance with endometriosis cysts would be included as research subjects. The subjects received leuprorelin acetate injection every month. Blood samples from the subjects were taken for AMH serum examination upon receiving leuprorelin acetate injection and two months after. Data collected was analyzed and reported.

The data with a numerical scale was tested for the distribution of Shapiro Wilk's data. If the data distribution was normal, the data would be presented using the mean ± SD, while if it was not normal it would be presented in the median (minimum-maximum). Nominal scale data was presented as a percentage in each category.

Differences in serum AMH levels before and after laparoscopic cystectomy, before laparoscopic cystectomy and after leuprorelin acetate injection, after laparoscopic cystectomy and after leuprorelin acetate injection were tested by the Wilcoxon difference test. The relationship between age and BMI on AMH levels was tested by the Spearman test.

The research was carried out after obtaining ethical clearance from Dr. Kariadi Semarang with ethical clearance no. 938/EC/KEPK-RSDK/2021.

RESULTS

Table 1. Characteristics of Research Subjects

Variable	n (%)	Mean ± SD	Median (min-max)
Age (years)	-	31.04±6.20	-
Body Mass Index (kg/m2)	-	23.55±2.14	-
Cysts Number		-	-
Unilateral	8 (32)		
Bilateral	17 (68)		
AMH before Surgery (ng/mL)	-	-	1.32 (0.88-5.13)
AMH after Surgery (ng/mL)	-	-	1.07 (0.60-4.53)
AMH after Surgery + Leuprorelin Acetate (ng/mL)	-	-	1.06 (0.50-4.65)

Table 1 shows the characteristics of research subjects. Research subjects had an age with a mean ± standard deviation: 31.04 ± 6.20 years. Body mass index had a mean ± standard deviation: 24.19 ± 2.19 kg/m2. Based on the number of cysts, 8 subjects (32%) had unilateral cysts and 17 subjects (68%) had bilateral cysts. AMH before surgery had a median value of 1.32 ng/mL, the smallest value of 0.88 ng/mL and the largest value of 5.13 ng/mL. AMH after surgery had a median value of 1.07 ng/mL, the smallest value of 0.60 ng/mL and the largest value of 4.53 ng/mL. AMH after surgery + leuprorelin acetate had a median value of 1.06 ng/mL, the smallest value of 0.50 ng/mL and the largest value of 4.65 ng/mL.

Table 2. Differences in AMH levels before surgery, after surgery, and after surgery + leuprorelin acetate injection

Variable	*P-value*
AMH before Surgery AMH after Surgery AMH after Surgery + Leuprorelin Acetate	<0.001 0.149

Table 2 shows there was a significant difference (p<0.001) between preoperative AMH levels and postoperative AMH levels. There was a decrease in AMH value of 17% between AMH before surgery and AMH after surgery. There was no significant difference (p=0.149) between postoperative AMH levels and postoperative AMH + leuprorelin acetate levels.

Table 3. Age and BMI on AMH Levels before Surgery

	AMH before Surgery	
	r	***P-value****
Age	0.074	0.725
BMI	-0.399	0.048

Table 3 shows Body mass index had a relationship (p=0.048) with AMH levels before surgery with a weak and opposite relationship (r= -0.399). This means that an increase in body mass index value is associated with a decrease in AMH levels before surgery. There was no relationship between age and AMH levels before surgery (p=0.725).

DISCUSSION

The average age of the subjects in this study was 31 years. Most of the subjects had bilateral cysts (68%). The mean of subjects with BMI more than 24 kg/. Thirty- fifty percent of women with endometriosis will experience infertility. The incidence of endometriosis mainly occurs between the phases of menarche and menopause. The peak incidence occurs between the ages of 25 and 45 years. Recent data show that there is generally no association between BMI and the incidence of endometriosis, but there has been a significant increase in the incidence of endometriosis in obese women compared with normal weight women.[6]

When compared with healthy ovaries, ovaries with endometrial cysts had a reduced response to exogenous gonadotropin stimulation, lower antral follicle counts, lower follicle density in the cortex, and increased follicular atresia. Further assessment showed that the density of the primordial follicles was reduced, and the general morphology and vascular network were also distorted. Follicular loss may occur even in the early stages of cyst development.[7]

Ovarian reserve reflects, both qualitatively and quantitatively, reproductive potential and oocyte function, in a patient. The most reliable and widely used quantitative marker of ovarian reserve is serum anti-Müllerian hormone (AMH) levels. Endometriotic cysts can affect ovarian reserve in two ways: impairing circulation in the ovarian

cortex by compression of the cyst and thereby causing follicle loss and/or through regulation of inflammation within the cyst wall leading to follicular destruction.[1] A systematic review and meta-analysis examining 17 articles with a total sample of 968 women with endometriotic ovarian cysts found that AMH levels were significantly lower in the endometriotic cyst group compared to women with non-endometriotic benign ovarian cysts or healthy ovaries (mean difference: -0.84 ng/ml; CI: -1.16 to -0.52; P<0.01). In the secondary analysis, women with endometriotic cysts were separately compared with women with non-endometriotic ovarian cysts (mean difference: -0.61 ng/ml; CI: -1.37 to -0.32) and women with normal ovaries. (mean difference: -0.61 ng/ml; CI -0.99 to -0.24). Both comparisons showed a significantly lower AMH in patients with endometrial cysts. These findings suggest that the presence of endometrial cysts is associated with reduced AMH levels in women.[8]

In this study, there was a significant difference between AMH levels before and after surgery. There was no significant difference between postoperative AMH levels and postoperative AMH + leuprorelin acetate.

Regarding the assessment of ovarian reserve through measurement of follicular density based on ovarian biopsy and Anti-Müllerian Hormone (AMH) in endometriosis patients, it was found that AMH levels were shown to decrease with age in untreated endometriosis patients, but AMH levels were significantly lower in endometriosis cases than in endometriosis patients with the control group only occurred in patients over the age of 36 years. The decrease in AMH was more rapid in endometriosis than that in controls. The number of primordial follicles based on ovarian biopsy decreased as AMH levels decreased in the intervention and control groups. Therefore, it can be concluded that AMH is a reliable marker of ovarian reserve in endometriosis patients.[9]

The effect of endometrial cysts and laparoscopic cystectomy on AMH hormone levels by assessing 1,642 patients who were divided into 4 groups, namely group 1 (1,232 infertile patients without endometriosis cysts), group 2 (141 patients with cysts). endometriosis), group 3 (147 patients who underwent unilateral or bilateral laparoscopic cystectomy due to endometrial cysts for more than 6 months), and group 4 (31 patients who underwent cystectomy during the study phase). The mean level of AMH was significantly lower in patients with bilateral cystectomy compared with patients with unilateral cystectomy. The mean serum AMH level was also significantly lower in patients with bilateral endometrial cysts compared with patients with unilateral endometrial cysts. In group 4, the mean AMH level decreased significantly from 3.95 + 0.42 preoperatively to 2.01 + 0.21 ng/ml at 3 months postoperatively.[10]

Laparoscopic ovarian cystectomy in cases of endometrial cysts has a negative effect on ovarian capacity.[11] Similar to previous studies that have been shown, ovarian cystectomy can affect ovarian reserve.[10,12–14] This side effect occurs immediately after surgery and affects patients over the medium term, at least 6 months.[15] The cause of decreased ovarian reserve not only due to loss of follicles during endometriotic cyst removal but also blood loss during surgery. The additional effect of total abdominal hysterectomy on serum AMH is the loss of ovarian reserve more than 30%. Surgery, hysterectomy, can reduce the ovarian blood supply and result in a temporary decrease in ovarian reserve.[16]

In a meta-analysis that discussed the effect of unilateral and bilateral laparoscopic surgery in endometriosis cases on AMH hormone levels after 3 months and 6 months found that unilateral and bilateral laparoscopic endometriosis surgery affected AMH levels. AMH levels were reduced in both comparisons, and the decrease in AMH levels was influenced more by the effect of laparoscopic surgery for bilateral endometriosis than laparoscopic surgery for unilateral endometriosis. In addition, the number in AMH will increase after 6 months.[17]

There are several studies that discuss the effect of using leuproline acetate or GnRH agonists (GnRHa) on women's AMH levels. AMH decreased 7 days after GnRHa administration by a median of 24% (1.7 ng/mL) and then increased above the pre-intervention level 14 and 30 days after GnRHa by 13% (2.5 ng/mL) and 32% (3.1 ng/mL). Significant changes in AMH levels occurred in the first 4 weeks after GnRHa administration, suggesting that AMH may not be used as a good marker of ovarian reserve during this interval. Administration of GnRHa causes an initial increase in LH and FSH, which can stimulate follicular development and E2 secretion (flare), followed by gonadotropin desensitization and continued suppression of gonadotropins and ovarian steroids. The GnRH receptor is expressed by human granulosa cells and is up regulated, in part, by GnRH. The decrease in AMH after 7 days

of treatment was due to upregulation of the GnRH receptor combined with the antiproliferative and apoptotic effects of short-term GnRHa exposure on granulosa cells.[18] Studies in rodents have shown that GnRH receptor expression in granulosa cells is decreased with prolonged administration of GnRHa. This explains the continued increase in AMH levels starting from day 14 of GnRHa administration. The decrease in AMH that occurred at day 7 allowed the expansion of the pool of small preantral and antral follicles that secrete AMH, resulting in an increase in AMH levels on days 14 and 30. The initial decrase in AMH resulted from an undeectable gonadotropin flare which'll then mature follicular development in antral follicles. gonadotropin-reponsive bulk, thereby reducing AMH[19]. Following a flare, suppression of FSH and LH inhibits FSH-dependent maturation of follicles, thereby increasing the total number of AMH-secreting gonadotropin-responsive follicles.

An increase in body mass index has a relationship with a decrease in AMH levels before surgery. The effect of obesity on AMH levels in women of reproductive age (18-48 years) stated that from 13 studies (n = 1214 women consisted of 811 non-obese women (body mass index; BMI <30 kg/m2) and 403 obese women. (BMI > 30 kg/m2), five of which reported a reduction in AMH levels with obesity, whereas eight showed comparable AMH levels between groups.[20] Environmental changes that occur in ovarian follicles have been confirmed in obese women and involve disturbances in several system, including steroidogenic, metabolic, and inflammatory, all of which can impact folliculogenesis and ovulation potential.[21] A single meta-analysis demonstrated a negative association between AMH and BMI.[22]

Obesity is generally associated with systemic insulin resistance and compensatory hyperinsulinemia. Excessive insulin levels have been shown to alter granulosa cell uptake, and subsequently, AMH production.[23] In addition, the increased leptin production associated with obesity may directly suppress AMH production. This condition stems from the inhibitory effect of leptin on the expression of the AMH receptor gene.[24] Obesity may lead to increased apoptotic effects at the ovarian follicle level, which is a mechanism that occurs in animal models. Although this mechanism could explain the decrease in ovarian follicle count and AMH levels, this seems less likely based on available data.[25] The limitations of this study include ovarian reserve in this study was only measured by a single marker, namely AMH levels. The antral follicle count (AFC) is another useful marker for assessing ovarian reserve. European Society of Human Reproduction and Embryology (ESHRE) strongly recommends that to predict the response to ovarian stimulation, AFC or AMH assessment is recommended over other ovarian reserve tests, the size of the endometriosis cyst in this study was not specifically measured using a standard measuring instrument, the measurement is only subjectively carried out by the operating operator during the operation therefore the variable size of the cyst is invalid to be used.

CONCLUSIONS

Differences in ovarian reserve in women with ovarian endometrial cysts after laparoscopic cystectomy and injection of leuprorelin acetate had a median value of 1.06 ng/mL, the smallest value of 0.50 ng/mL and the largest value of 4.65 ng/mL. A decrease of 18.9% between AMH levels before surgery and AMH levels after surgery was statistically significant. There is a relationship between BMI and AMH levels before surgery.

This study suggests that a significant decrease in AMH occurs after cystectomy surgery, therefore it is necessary to perform cystectomy surgery to better maintain ovarian reserves and educate patients about the risk of decreased ovarian reserves after cystectomy surgery. Further study is required to measure the number of antral follicles (AFC) as an alternative in assessing ovarian reserve.

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Research Article

Depo Medroxyprogesterone Acetate (DMPA): Long-Term Effects on Menstrual Cycle Disorders

Depo Medroksiprogesteron Asetat (DMPA): Efek Penggunaan Jangka Panjang terhadap Gangguan Menstruasi

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Abstract

Objective: To determine the effect of the duration of using Depo Medroxyprogesterone Acetate (DMPA) on the Menstrual Cycle

Methods: This research is an analytical study of 102 patients who used DMPA for less than 1 year and more than 1 year and then analyzed the types of menstrual disorders experienced while using DMPA contraception at RSIA Masyita Makassar Tahun 2022. Data collection was carried out by direct observation at RSIA. Masyita was then arranged in a checklist that has been prepared.Data is processed using SPSS version 25.0.

Results: There is a significant effect between the length of use of Depo Medroxyprogesterone Acetate (DMPA) and the Menstrual Cycle (p=0.000).

Conclusion: There are long-term effects of using DMPA on menstrual cycle disorders, the most common being hypermenorrhea. These results showed that the use of Depo Medroxyprogesterone Acetate (DMPA) for > 1 year has shown many effects on Menstrual Disorders and can be used by midwives to providing knowledge and counseling to patients who wll to use DMPA regarding the side effects and risks of using longer contraceptives.

Keywords: Depo Medroxyprogesterone Acetate (DMPA), duration of use, menstrual cycle.

Abstrak

Objektif: *Untuk mengetahui pengaruh lama penggunaan Depo Medroksiprogesteron Asetat (DMPA) terhadap siklus menstruasi.*

Metode: *Penelitian ini merupakan jenis penelitian analitik terhadap 102 pasien yang menggunakan DMPA kurang dari 1 tahun dan lebih dari 1 tahun kemudian dianalisis jenis gangguan menstruasi yang dialami selama menggunakan kontrasepsi DMPA di RSIA Masyita Makassar. Pengumpulan data dilakukan dengan metode observasi langsung di RSIA Tahun 2022. Masyita kemudian disusun dalam checklist yang telah disiapkan.Data diolah menggunakan SPSS versi 25.0.*

Hasil: *Ada pengaruh yang signifikan antara lama penggunaan Depo Medroksiprogesteron Asetat (DMPA) dengan siklus menstruasi (p=0,000).*

Kesimpulan: *Adanya Efek dari Jangka Panjang Penggunaan DMPA terhadap gangguan siklus Mentsruasi yang terbanyak yaitu Hipermenore. Hasil ini menunjukkan bahwa Penggunaan Depo Medroksiprogesteron Asetat (DMPA) selama > 1 tahun telah menunjukkan banyak efek pada gangguan menstruasi dan selanjutnya dapat digunakan oleh bidan dalam memberikan pengetahuan dan konseling kepada pasien yang akan menggunakan DMPA mengenai efek samping dan resiko penggunaan kontrasepsi yang lebih lama.*

Kata kunci: *Depo Medroksiprogesteron Asetat (DMPA), lama pemakaian, siklus menstruasi.*

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INTRODUCTION

Family planning is one of the government's strategies to reduce maternal mortality, especially for mothers with 4T conditions; giving birth too young (under the age of 20 years), giving birth too often, giving birth too close and giving birth too old (over the age of 35 years).[1]The achievement of KB participation continues to increase from time to time, in 2016 it showed that the number of KB participants in Indonesia was around 74.8% of KB users.[2]Around 49.7% of women in Indonesia chose hormonal injection contraception as their contraceptive method, including the Depo Medroxyprogesterone Acetate (DMPA) type.[3]

The type of contraception that is most widely used in the world today is the DMPA injection[4]. DMPA ranks highest in the selection of contraceptives to prevent pregnancy. DMPA is known as the most effective contraceptive, where the pregnancy rate of using DMPA is only 0.3% in the first year of use.[5,6]and is a contraceptive option that can be recommended for women who wish to delay pregnancy[6].

Side effects of a contraceptive method are factors that need to be considered in determining the decision to continue using the contraceptive method. Changes in the menstrual cycle are one of the side effects that are often complained of by Depo Medroxyprogesterone Acetate (DMPA) injectable birth control acceptors, the normal menstrual pattern becomes amenorrhea, regular bleeding, spotting, changes in frequency, duration and amount.[7]

A study conducted[8] in 2020 stated that there was a difference in the average length of the shortest and longest menstrual cycle days in the 1-month hormonal injection (combination) and 3-month hormonal injection (DMPA) groups (p-value <0.001). Where clinically the shortest and longest menstrual cycles was found in the 3-month hormonal injection contraception (DMPA) group compared to 1-month hormonal injection contraception (combined) (60 vs 28) and (90 vs 35).

Other studies [9]regarding the side effects of using DMPA found that 22 acceptors (44%) experienced severe side effects and most of the side effects were experienced after using DMPA injectable contraception for more than 3 months to 1 year (60%). There is a positive and significant relationship between duration of use of DMPA injection contraception and the incidence of side effects (ρ = 0.000 <0.05). From this study it can be concluded that the occurrence of side effects is strongly influenced by the duration of using DMPA injectable birth control.

The results of another study related to DMPA contraception found a relationship between the use of hormonal contraception and menstrual cycle disorders, acceptors of progestin hormonal contraceptives have a 1.6 times greater risk of experiencing menstrual cycle abnormalities than combined hormonal contraceptive acceptors. Most of the progestin hormonal contraceptive respondents 36 (85.7%) and more than half of the combined hormonal contraceptive respondents 20 (52.6%) experienced menstrual cycle abnormalities.[10]

The results of the preliminary survey showed number of users of Cyclofem and DMPA hormonal contraception, namely 589 acceptors where DMPA was quite high in the last 5 months from January to May 2022, namely 296 acceptors (Depo Progestin) and direct interview results from 10 women using contraception, 6 of them experienced problems and complaints in the menstrual cycle. Some mothers also chose to drop out when experiencing this problem. The results of the study also stated that most mothers experienced menstrual cycle disorders, namely amenorrhea[6,11]. Therefore researchers are interested in seeing how "The Influence of Old Use of Depo Medroxyprogesterone Acetate (DMPA) on Menstrual Cycle Disorders at RSIA Masyita Makassar".

METHODS

This research is a type of analytic observational research with a cross sectional design. The research variable is the independent variable, the duration of DMPA injection contraception use and the dependent variable, menstrual cycle disorders.12 This research was conducted at RSIA Masyita Makassar Tahun 2022. Inclusion criteria are DMPA acceptors who do not experience history of hormonal diseases and disorders and Exclusion criteria are Cyclofem acceptors and DMPA acceptors who are not willing to be respondents. The study was conducted from October to December 2022. Sampling in this study used Total Sampling, 102 DMPA acceptor respondents consisting of a group with a duration of use < 1 year and a group with a duration of use > 1 year using the direct observation method at RSIA Masyita then compiled in a checklist that had been prepared. Menstrual disorders variable,

mother's data, information on menstrual disorders experienced by the mother whether there are no complaints, amenorrhea, polymenorrhea, hypermenorrhea, hypomenorrhea, metroragia or spots. Furthermore, it is presented in the form of cross tabulation, the duration of DMPA use has an effect on menstrual cycle disorders. Data analysis using (frequency distribution), bivariate (Mann-Whitney U).12 The research data were analyzed using the Statistical Product and Service Solutions (SPSS) version 25.0 statistical test.

RESULTS

Univariate analysis

Frequency Distribution of DMPA Duration and Menstrual Disorders at RSIA Masyita Makassar in 2022.

Table 1. Frequency Distribution

Variable	Frequency	%
Age Characteristics		
<20	5	4.9
20-35	68	66.7
>35	29	28.4
Educational Characteristics		
Elementary	2	2.0
Junior high school	41	40.2
Senior high school	56	54.9
Bachelor	3	2.9
Job Characteristics		
IRT	96	94.1
Businessman	6	5.9
Government officials	0	0
Balance		
≤2	93	91.2
>2	9	8.8
Length of Use (year)		
<1	40	39.2
>1	62	60.8
Types of Menstrual Disorders		
No abnormalities	31	30.4
Polimenore	5	4.9
Amenorrhea	8	7.8
Hypermenorrhea	20	19.6
Metroragia	9	8.8
Spotting	15	14.7
Hypomenorrhea	14	13.7
Disturbance Category		
Normal	28	27.5
Abnormal	74	72.5

Bivariate Analysis

Effect of duration use of DMPA on Menstrual Disorder Category at RSIA Masyita.

Table 2. The Effect of the Duration use of DMPA on Menstrual Disorders at RSIA Masyita Makassar in 2022

Length of Use	Menstrual Disorders category				Total		*P-value* (mark)
	Normal		Abnormal				
(year)	F	%	F	%	F	%	
<1	26	92.9	14	18.9	40	39.2	0.000
>1	2	7.1	60	81.1	62	60.8	

Multivariate Analysis

The Effect of DMPA Use on Types of Menstrual Disorders at RSIA Masyita.

Table 3. The Effect of Duration of DMPA Use on Menstrual Disorders at RSIA Masyita Makassar in 2022

Length of Use (year)	Types of Menstrual Disorders														Total		*P-value*
	No abnormalities		Polime-nore		Ame-norrhea		Hyperme-norrhea		Metroragia		Spotting		Hypome-norrhea				
	F	%	F	%	F	%	F	%	F	%	F	%	F	%	F	%	
<1	28	90.3	1	20.0	3	37.5	1	5.0	2	22.2	4	26.7	1	7.1	40	39.2	
>1	3	9.7	4	80.0	5	62.5	19	95.0	7	77.8	11	73.3	13	92.9	62	60.8	0.000

Menstrual Disorders category

This study showed that acceptors who used DMPA <1 year were more in the category of normal menstrual disorders, with total of 92.9%, compared to those who were not normal, of 18.9%. Even though it is used <1 year, there are also categories of abnormal menstrual disorders.

This happens on average to acceptors who have used DMPA for almost 12 months and normally new DMPA acceptors. Meanwhile, only 7.1% of acceptors who used DMPA more than 1 year were included in the normal menstrual category and 81.1% were included in the abnormal category. Hormonal factors greatly affect menstrual disorders experienced by DMPA acceptors so that acceptors are still found even though they have been using it for more than 1 year but there are no complaints related to menstruation, in this case it is still in normal condition.

Types of Menstrual Disorders

This study showed that acceptors who used DMPA in less than 1 year at most, about 90.3% of the total respondents who did not experience menstrual disorders or disorders, while acceptors who had used DMPA more 1 year experienced the most types of menstrual disorders Hypermenorrhea, with total of 95.0% and only about 9.7% did not experience menstrual disorders or menstrual disorders. This indicates that DMPA acceptors experienced menstrual cycle disturbances in more than 7 days.

DISCUSSION

A study by Westh off discussed the contraceptive effectiveness of DMPA, long-term safety in relation to cardiovascular risk, gynecological and breast malignancies, and ostopenia. Moreover, it discussed clinical comparison of hormonal contraceptives. This research is in line with the research on the effect of duration of DMPA use on menstrual cycle disorders because one of the most frequent complaint from the results of this study is menstrual cycle disorders Amenorrhea. Researchers have further studied the effect of duration of DMPA use on each Menstrual Cycle disorder. Disorders of the menstrual cycle according to.[13] There are several types, namely polimenorrhea, amenorrhea, hypermenorrhea, metorargia spotting and hypomenorrhea. From the results of the study it was found that the most common type of menstrual disorder experienced was Hypermenorrhea, namely menstrual disorders where menstruation occurs for more than 7 days or the duration is longer.

The study entitled Effect of long-term progestin treatment on endometrial blood vessels in cycling normal rats.[14] This study used a mouse model to look at the effects of long-term exposure to progesterone on the endometrium and vascular structure. Normal mice were given Silastic implants containing medroxyprogesterone acetate (MPA) or levonorgestrel (LNG) and dissected after 1, 3 or 6 weeks. This study demonstrated changes in the microvascular structure of the endometrium after long-term progestin use. There were significant effects of MPA and LNG administration on normal cycle mouse endometrium, including changes in blood vessel density, stromal cell density, epithelial cell height, and VEGF immunoreactivity. Further studies are needed to investigate the physiological mechanisms by which progestin influences endometrial microvascular changes and endometrial bleeding. This study shows that the use of DMPA which only contains progestin hormones can affect the endometrium and bleeding that occurs in the endometrium with the emergence of various complaints of menstrual disorders experienced by DMPA acceptors after more than 1 year of use. This is the basis for researchers to look at the side effects of prolonged DMPA use on menstrual cycle disorders. In theory we know that the menstrual process mostly involves the endometrium in women, physiological changes occur in the endometrium when women experience menstruation. With the mechanism evoked by the two hormones above against endometrial cells, this study showed that the use of DMPA which only contains progestin hormones can affect the endometrium and bleeding that occurs in the endometrium with the emergence of various complaints of menstrual disorders experienced by DMPA acceptors after more than 1 year of use.[15]

The study entitled Weight gain and menstrual abnormalities between Depoprovera and Noristerat users[11]. This study used the following methods: A retrospective comparative study conducted over 3 years and involving 237 subjects who saw comparators using injectable hormonal contraceptives (DMPA or Noristerat). This study consisted of 2 groups; the group that used DMPA contraception for 3.6 and 12 months and the group that used Noristerat type contraception which had used it for 2.4 and 12 months. Then the effects of weight gain, length and pattern of menstrual cycles are examined as well as the side effects. The results showed that 10% of DMPA users did not experience changes in menstrual patterns and only 7% of Noristerat users did not experience changes in menstrual patterns whereas 60% of DMPA users experienced amenorrhea

and only 57% of Noristerat users experienced amenorrhea at the end of the one year period indicating an increase in the number of mothers experiencing amenorrhea. Amenorrhea is one of the most common menstrual disorders and the most common reason for contraceptive drop out. The results of this study were different because the most common type of menstrual disorder is hypermenorrhea compared to Amenorrhea with the use of DMPA for 0.1 year. Researchers used this information as a basis to determine the duration of DMPA use, by looking at more than 1 year and in this study took the samples at intervals of 1, 3 and 6 months. The results of this study also provide information and show how DMPA has more influence on the menstrual cycle, namely Hypermenorrhea.

The study entitled The Effect of Long Term Depo Medroxyprogesterone Acetate (DMPA) on Serum and Plasma Nitric Oxide (NO) Levels in Female Wistar Rats (Rattus norvegicus)[16] carried. This study explains that DMPA use in the long term inhibits the protection of estrogen as a vasoprotective molecule that can increase NO production in blood vessels. The results showed that there was a significant difference in the mean serum NO levels between the control group and the group that was given DMPA at the 8th week. The results of this study were continued on higher research subjects, namely humans, it turned out that the use of DMPA in humans more than 1 year showed significant changes in menstrual disorders. We know that Nitric Oxide is a vasodilator substance that plays a role in blood vessels so that NO is very closely related to human blood vessels. Nitric oxide (NO) levels in the endotheliumincreases during the follicular phase of the menstrual cycle concomitant with increases in 17β estradiol and decreases during the postovulatory progesterone phase, when the progesterone phase is high.[17] The vasoprotective effects of NO are endothelial cell proliferation, protection of endothelial cells from apoptosis, and inhibition of cell inflammation.[18]

Relationship between long-term use of Depo Medroxyprogesterone Acetate (DMPA) and changes in blood pressure at RSIA Masyita Makassar. This research is an analytic survey research with a case-control study approach where this research was conducted by first measuring the sample's blood pressure and then retrospectively tracing the history of blood pressure measurement results while being a DMPA injection acceptor. This research was conducted at RSIA Masyita Makassar.

The results of this study are in line with the results of research that was also conducted at RSIA Masyita Makassar. There are quite a lot of users or acceptors of DMPA hormonal contraception in Masyita in 2022 and are increasing. This study also provides showed that the use of DMPA for more than one year has shown the effect of an increased blood pressure and the effect of menstrual disturbances experienced by acceptors who use DMPA for more than 1 year.

An article of the Effect of DMPA Injecting KB on Menstrual Cycle Disorders in KB Acceptors[19] by Lilis Candra Yanti, Annisa Lamaindi in the Scientific Journal of Health Sandi Husada Year 2021 showed the effect of prolonged use of DMPA contraception on complaints of menstrual disorders. Longer use of DMPA contraception can affect menstrual cycle changes. An analytic observational study with a total sample of 35 research subjects using DMPA with a duration of use of DMPA on 5 years of data conducted in the Work Area of the Health Center.

The results of this study are limited to the influence of regular and irregular menstrual cycles. This is the material for comparison in this study because it is proven that more women experience irregular menstruation. This research was developed because it not only saw the effect of regular and irregular menstrual cycles but also saw the types of menstrual disorders experienced by acceptors after using DMPA both for more than 1 year and less than 1 year with a total sample of 102 research subjects.

CONCLUSION

The use of Depo Medroxyprogesterone Acetate (DMPA) for more than 1 year showed that acceptors experienced more menstrual disorder and hypermenorrhea compared to acceptors who only used it for less than 1 year. These results indicate that the use of Depo Medroxyprogesterone Acetate (DMPA) for more than 1 year has shown to cause menstrual disturbance. This can further be used by midwives in providing knowledge and counseling to patients who will use DMPA regarding side effects and risks in longer use contraception.

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Research Article

In Utero Ultrasonography Parameters as a Children Growth Prediction at Age 2 – 3

Parameter Ultrasonografi in Utero sebagai Prediksi Pertumbuhan Anak Usia 2-3 Tahun

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Abstract

Objective: To determine the relationship between ultrasound parameters during pregnancy and the growth of children aged 2 – 3 years.

Methods: This was an analytic observational study with a longitudinal approach using data from the first 1,000 days of life. Study conducted at the Faculty of Medicine, Diponegoro University. The research subjects were children aged 2 – 3 years old with good nutritional status and democratic parenting from 14 City Health Centers in Semarang. The ultrasound parameters obtained were Biparietal Diameter (BPD), Abdomen Circumference (AC), Head Circumference (HC), Femur Length (FL) at 20 – 24 weeks of gestation. Data were obtained during the second trimester. Data on the growth of children at the age of 2 years obtained are height, weight, and HC. Statistical test using Pearson correlation test, with $p<0.1$ is considered significant.

Results: The level of confidence used in this study was 90% and obtained 45 research subjects with a female gender of 26 children (57.8%) and 19 male children (42.2%). The Pearson test showed that there was no significant relationship between BPD and height (p=0.18; r=-0.20), AC and height (p=0.12; r=-0.23), and FL and height (p=0.17; r=-0.20). There was a significant relationship between HC and height (p=0.04; r=-0.29), BPD and weight (p=0.06; r=-0.28), HC and weight (p=0.01; r=-0,.5), AC and weight (p=0.08; r=-0.26), FL and weight (p=0.05; r=-0.29) and prenatal HC and postnatal HC (p=0.03; r=-0.32).

Conclusion: There is a significant relationship between ultrasound parameters during pregnancy and the growth of children aged 2 – 3 years.

Keywords: abdominal circumference, biparietal diameter, child growth, femur length, head circumference, pregnancy.

Abstrak

Tujuan: *Untuk mengetahui hubungan antara parameter ultrasonografi saat kehamilan dengan pertumbuhan anak usia 2-3 tahun.*

Metode: *Penelitian ini merupakan penelitian observasional analitik dengan pendekatan longitudinal menggunakan data penelitian 1.000 hari pertama kehidupan yang dilakukan di Fakultas Kedokteran Universitas Diponegoro. Subjek penelitian adalah anak usia 2 – 3 tahun dengan gizi baik dan pola asuh demokratis dari 14 Puskesmas Kota Semarang. Parameter USG yang diperoleh adalah diameter biparietal (BPD), lingkar abdomen (AC), lingkar kepala (HC), dan panjang femur (FL) pada usia kehamilan 20 – 24 minggu. Data diperoleh selama trimester kedua. Data tumbuh kembang anak usia 2 tahun yang diperoleh adalah tinggi badan, berat badan, dan lingkar kepala. Uji statistik menggunakan uji korelasi Pearson, dengan p<0,1 dianggap signifikan.*

Hasil: *Tingkat kepercayaan yang digunakan dalam penelitian ini adalah 90% dan diperoleh 45 subjek penelitian dengan jenis kelamin perempuan 26 anak (57,8%) dan 19 anak laki-laki (42,2%). Uji Pearson menunjukkan bahwa tidak ada hubungan yang signifikan antara BPD dengan tinggi badan (p=0,18; r=-0,20), AC dan tinggi badan (p=0,12; r=-0,23), dan FL dan tinggi (p=0,17; r=-0,20). Terdapat hubungan yang signifikan antara HC dengan tinggi badan (p=0,04; r=-0,29), BPD dan berat badan (p=0,06; r=-0,28), HC dengan berat badan (p=0, 01; r=-0,35), AC dan berat badan (p=0,08; r=-0,26), FL dan berat badan (p=0,05; r=-0,29) dan HC prenatal dan postnatal HC (p=0,03; r=-0,32).*

Kesimpulan: *Terdapat hubungan yang signifikan antara parameter ultrasonografi saat kehamilan dan pertumbuhan anak usia 2 – 3 tahun.*

Kata kunci: *diameter biparietal, kehamilan, lingkar abdomen, lingkar kepala, panjang femur, pertumbuhan anak.*

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INTRODUCTION

Intrauterine life is one of the determinants of the quality of child development. Growth is an increase in the number, size, dimensions at the level of cells, organs, and individuals. Physical growth is typically assessed using weight, height (length), head circumference, arm circumference, and skin folds. Weight is used as a parameter to assess the nutritional status of children, while head circumference is used to monitor children's brain growth and development.[1,2]

Infants experience rapid growth in the first 1000 days of life. The first 1000 days of life is a very important phase in documenting the growth of children to detect abnormalities as early as possible. The process of child growth takes place regularly, interrelated, and continuously starting from conception to adulthood. The stages of child growth are divided into two, namely the prenatal and postnatal periods, each of which has its characteristics and differences in anatomy, physiology, biochemistry, and character.[3,4]

Growth and development disorders can occur if there are both or either genetic and environmental factors. For example, stunting is a condition in which children under five experience limited or reduced growth and development. This condition is caused by chronic malnutrition so that children are too short for their age. Malnutrition can occur in the womb and can only be seen when the child turns 2 years old. Stunting has a negative impact on nutritional problems in Indonesia because in the future it will affect the physical and functional aspects of the body and will increase child morbidity. Basic Health Research (Riskesdas) showed the prevalence of stunting in Indonesia reached 37.2% in 2013 and decreased to 30.8% in 2018. However, this figure is still far from the target of the World Health Organization (WHO), namely 20%.[4–6]

Health services for pregnant women or Antenatal Care (ANC) are carried out during the mother's gestational age range which is grouped according to gestational age and is divided into three categories: the first trimester; second trimester; and third trimester. This service is carried out to monitor the pregnancy process to ensure the health of the mother and the growth of the fetus is good, to find out whether there are pregnancy complications that may occur during early pregnancy, and to improve and maintain the health of the mother and fetus. The health services provided for pregnant women include fetal examination, one of which is through ultrasound examination.[7] Ultrasound examination is a diagnostic method that uses ultrasonic waves that are non-invasive, safe, practical, and can provide information on fetal growth.[8] Fetal growth assessments such as biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), and femur length (FL) were used to measure the assessment of the estimated fetal weight (EFW).[9]

Based on the description above, pregnant women need to carry out ANC examinations, especially ultrasound, to find out pregnancy complications and detect the disturbances in the growth and development of children later because the process is continuous from conception to adulthood. One example of a child's growth and development disorders is stunting which can only be seen at the age of 2 years.[5,9] Therefore, it is necessary to research to determine the relationship between ultrasound parameters during pregnancy and the growth of children aged 2 years.

METHODS

This research was done within both the Obstetrics and Gynecology Department, and Pediatrics Department. The study was conducted at 14 City Health Centers in Semarang from April to September 2021. This research has received permission from the Health Research Ethics Commission (KEPK) Faculty of Medicine, Universitas Diponegoro, Semarang with number 227/EC/KEPK/FK-UNDIP/VII/2021.

This research was an analytic observational study with longitudinal approach. The population of the subjects in this study was children aged 2 - 3 years old in 14 Semarang City Health Centers who met the inclusion and exclusion criteria of the study. The inclusion criteria in this study were children aged 2 - 3 years old, domiciled in Semarang, good child nutrition, democratic parenting, have complete ultrasound records, and ultrasound examination performed by Obstetrics and Gynecologists who are a certified basic ultrasound examiners. Meanwhile, the exclusion criteria were children with congenital abnormalities and mothers who have comorbidities during pregnancy. In the ultrasound examination, no kappa test was carried out, but those who did the examination were Obstetrics and Gynecology specialists who had a certificate of basic ultrasound standardization where to get

the certificate, they had to undergo training in which there was conformity with the results of the ultrasound examination.

The ultrasound parameters obtained were Biparietal Diameter (BPD), Head Circumference (HC), Abdomen Circumference (AC), and Femur Length (FL) at 20-24 weeks of gestation. Data were obtained in the second trimester. The child growth data obtained were height, weight, and HC.

The data obtained were analyzed statistically using the Shapiro Wilk test on data with a normal distribution (p>0.1) using the Pearson correlation test. The level of confidence in this study was 90% because from the existing data, not all of them have a complete ultrasound.

RESULTS

Table 1. Distribution of Research Subject Characteristics

Variables	n	%	Mean ± SD
Maternal Age			
14 – 19	2	4.4	
20 – 29	24	53.3	
30 – 39	18	40	28.36 ± 5.48
40 – 49	1	2.2	
Child's Gender			
Girl	26	57.8	
Boy	19	42.2	
USG Parameter			
BPD			5.51 ± 1.35
HC			19.63 ± 2.71
AC			17.45 ± 2.72
FL			3.79 ± 0.64
Child's Growth			
Height			83.48 ± 2.61
Weight			10.81 ± 1.35
HC			47.12 ± 1.19

The research subjects consisted of 45 mothers and children. Maternal age was categorized into four, the results obtained are 2 (4.4%) mothers categorized for 14 – 19 years, 24 (53.3%) mothers categorized for 20 – 29 years, 18 (40%) mothers categorized for 30 – 39 years , and 1 (2.2%) mother categorized for 40 – 49 years. The mean value for maternal age was 28.36 ± 5.48 years. In children, 26 (57.8%) children were girls and 19 (42.2%) were boys. On the results of ultrasound parameters, the mean BPD value was 5.51 ± 1.35 mm. The mean value for HC was 19.63 ± 2.71 mm. The mean value for AC was 17.45 ± 2.72 mm. The mean value for FL was 3.79 ± 0.64 mm. In the results of child growth, the mean value of height was 83.48 ± 2.61 cm, the mean value of weight was 10.81± 1.35 kg, and the mean value of HC was 47.12 ± 1.19 cm.

Table 2. Correlation between BPD, HC, AC, and FL with Height

Variables	Height	
	p-value	***r***
BPD	0.18	-0.20
HC	0.04*	-0.29
AC	0,12	-0.23
FL	0.17	-0. 20

Description: Pearson Correlation Test (p<0,1)

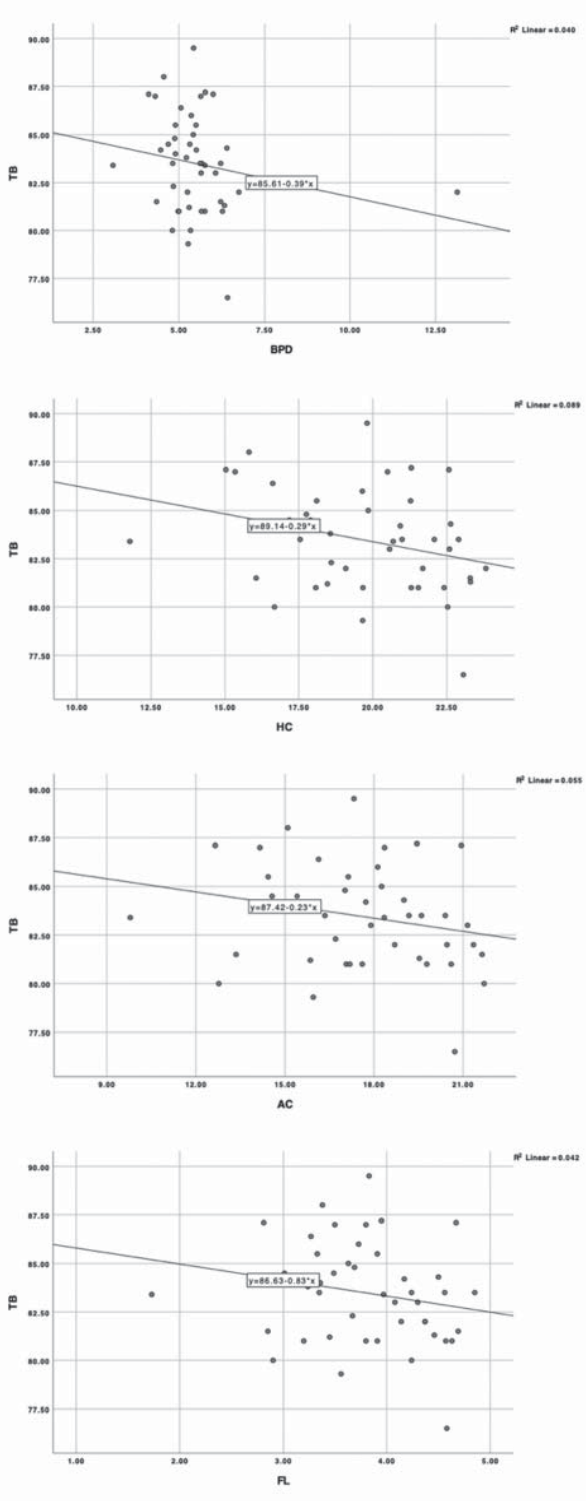


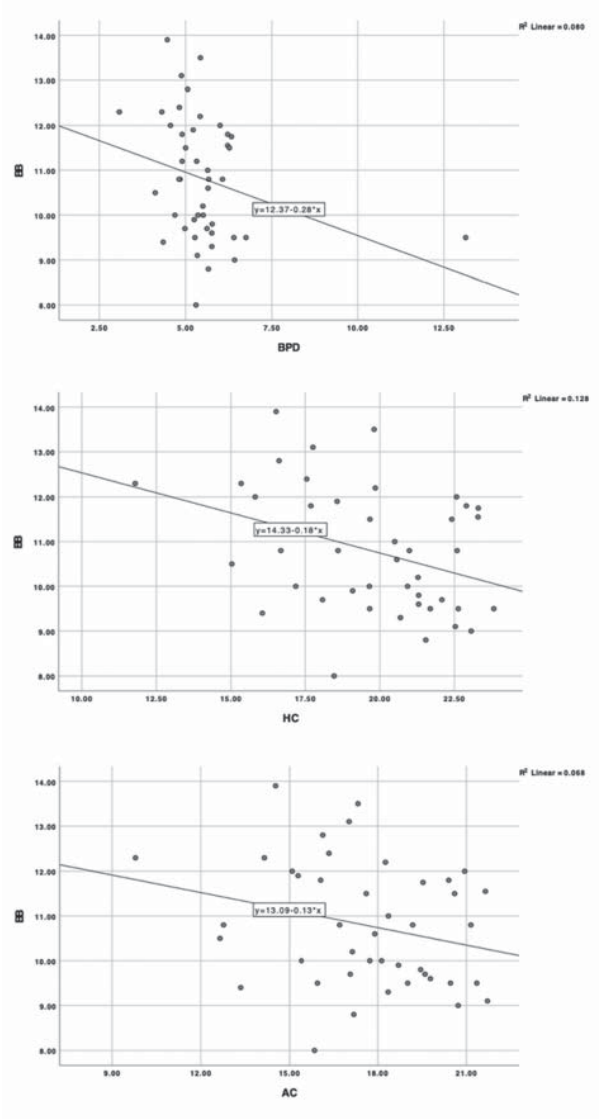
Figure 1. The scatter plot graph between BPD, HC, AC, and FL to Height

From the results of the bivariate analysis conducted with the Pearson correlation test on the relationship between BPD, HC, AC, and FL with height, the results showed no significant correlation (p> 0.1) between BPD and height (p=0.186; r=-0.201). There was a weak but significant negative relationship (p<0.1) between HC and height (p=0.046; r=-0.0299). There was no significant relationship (p>0.1) between AC and height (p=0.120; r=-0.235). There was no significant relationship (p>0.1) between FL and height (p=0.177; r=0.205).

Table 3. Correlation between BPD, HC, AC, and FL with Weight

Variables	Weight	
	p-value	***r***
BPD	0.06*	-0.28
HC	0.01*	-0.35
AC	0.08*	-0.26
FL	0.05*	-0.29

Description: Pearson Correlation Test (p<0.1)



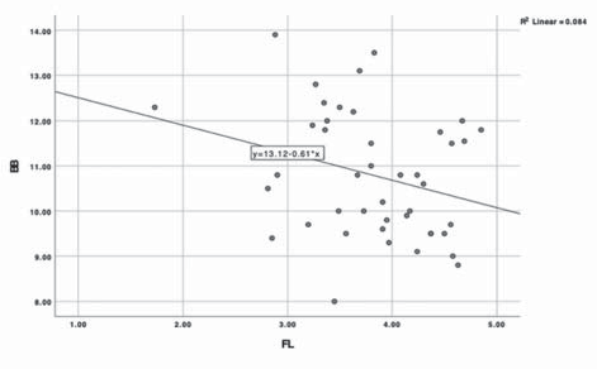


Figure 2. The scatter plot graph between BPD, HC, AC, and FL to Weight

Table 4. Correlation between prenatal HC and postnatal HC

Variables	Postnatal HC	
Prenatal HC	***p-value***	***r***
	0.03*	-0.32

Description: Pearson Correlation Test (p<0.1)

From the results of the bivariate analysis carried out with the Pearson correlation test on the relationship between prenatal HC and postnatal HC, the results showed that there was a weak but significant negative relationship (p<0.1) between prenatal HC and postnatal HC (p=0.03; r=-0.32).

DISCUSSION

Child growth takes place regularly, interrelated, and continuously starting from conception to adulthood. The growth experienced a rapid increase at an early age, especially at first 1,000 days of life. The growth stage of children is divided into two, namely the prenatal period and the postnatal period. In the prenatal period, growth monitoring can be done through ultrasound examination using BPD, HC, AC, and FL parameters. Meanwhile, in the postnatal period, height and weight monitoring of children can be carried out.[3,4,9] In this cross-sectional study, the researchers discussed the relationship between ultrasound parameters during pregnancy and the growth of children at the age of 2 – 3 years.

The results of this study showed a relationship between ultrasound parameters and child growth. Children's growth is interrelated starting from conception to adulthood.[3] In theory, if the femur is too short, the child will not grow too long despite a severe delay in bone age. On the other hand, if the femur is long, the risk of short stature is very small.[10] Meanwhile, FL is also used to calculate in the Hadlock formula to measure

EFW.[9] In a study conducted that EFW can be used to predict a child's weight at the age of 3 years.[11] The results of the Pearson test in this study showed that the relationship between FL and height was not significant, while the relationship between FL and weight was significant. This is not in accordance with previous research which reported a relationship between FL and height in children.[10] Meanwhile, in the same study, a relationship between FL and weight in children was also reported.[10] This discrepancy could be caused by several factors, such as the limitation of the number of research subjects, wherein this study the height of the children was normal. Another factor that can influence is the supplements consumed by the mother during the third trimester, where in a study was found that it could also increase the growth of children in the early 5 years of life.[12]

This study shows that there was a significant relationship between HC and height, BPD and weight, and HC and weight. However, BPD and height did not show a significant relationship. BPD and HC are two parameters used to assess fetal brain size as found.[13] Fetal brain size can predict a child's brain development related to somatic growth.[14,15] This is in accordance with research who stated that children's brain development is related to children's weight. In the same study, it was stated that children's height was significantly associated with HC.[14] BPD and HC were also used to assess EFW using the Hadlock formula, where EFW could be used to predict a child's weight at the age of 3 years.[11,16]

This study showed a significant relationship between AC and weight but did not show a significant relationship between AC and height. AC is not an indicator of growth in height or weight.[10] However, together with BPD, HC, and FL, AC is a parameter used to assess EFW using the Hadlock formula, where EFW can be used to predict a child's weight at the age of 3 years.[11,16]

In this study, we found a significant relationship between HC in the prenatal period and HC in the postnatal period. HC is a picture of brain development in which during the prenatal period, the brain is very vulnerable so that brain development is not only determined by congenital factors but also pregnancy conditions and postnatal environmental factors.[2,17] HC at birth reflects HC in prenatal period which is associated with cognitive and motor development at 2 years of correction.[18]

The growth of children in the prenatal and postnatal period might be influenced by several factors. In another study, it was stated that genetics affects fetal growth.[19] Maternal factors that affect a child's weight are diabetes mellitus, smoking, and hypertension. Meanwhile, fetal factors that can influence, namely gender, also affect growth in the prenatal period.[19] In the research that has been done, it was found that children who live in urban areas have better weight compared to children who live in rural areas because more children living in rural areas suffer from infectious diseases, this can be influenced by the mother's education. Mother's education may be directly proportional to mother's knowledge about nutrition and also healthy lifestyle.[20]

The discrepancy obtained in this study with previous research is due to limitations in the study. Limitations of the study include the level of confidence in this research was 90%, therefore the number of subjects involved in this study is less than previous studies. If the number of subjects was more varied and the sampling method was different, it is possible to obtain different results from this study. This study also did not pay attention to the nutritional intake of mothers during pregnancy and in the children, and did not pay attention to the history of the disease in children.

CONCLUSIONS

Based on the results of the study, there is a relationship between ultrasound parameters during pregnancy and the growth of children aged 2 – 3 years. Further research can be done on ultrasound parameters in the first 1,000 days of life using cohort or case-control research methods. In future research, it is important to consider the history of the child's disease and the nutritional intake.

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Research Article

Effect of Nanocurcumin in Combination with Methotrexate on Telomerase Activity, NF-kb Expression, and Proliferation Index of Bewo Choriocarcinoma Cells

Pengaruh Kombinasi Nanokurkumin dengan Methotrexate terhadap Aktivitas Telomerase, Ekspresi NF-kb, dan Indeks Proliferasi Sel Koriokarsinoma Bewo

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Abstract

Objectives: To determine the anti-cancer effect of nanocurcumin on choriocarcinoma.

Methods: This study observes the telomerase activity, NF-κB expression, and BrdU proliferation index in cultures of BeWo choriocarcinoma cell line (ATCC CCL-98) exposed to MTX and nanocurcumin in various doses. The sample used in this study consisted of 4 groups of BeWo cells that received a combination of MTX and nanocurcumin, and 2 other groups for positive and negative control.

Results: There was a decrease in telomerase activity, NF-κB expression, and BrdU proliferation index in the 4 treatment groups compared to the negative and positive control group ($p \leq 0.05$).

Conclusion: Nanocurcumin and MTX decrease telomerase expression, NF-κB expression, and the BrdU proliferation index in choriocarcinoma BeWo cell line culture faster than MTX alone.

Keywords: BrdU index proliferation, choriocarcinoma, methotrexate, nanocurcumin, NF-κB expression, telomerase expression.

Abstrak

Tujuan: *Untuk mengetahui pengaruh anti kanker nanokurkumin pada koriokarsinoma.*

Metode: *Penelitian ini mengamati aktivitas telomerase, ekspresi NF-kB, dan indeks proliferasi BrdU pada kultur sel koriokarsinoma BeWo (ATCC CCL-98) yang dipapar MTX dan nanokurkumin dalam berbagai dosis. Sampel yang digunakan dalam penelitian ini terdiri dari 4 kelompok sel BeWo yang mendapat kombinasi dari MTX dan nanokurkumin, dan 2 kelompok lainnya untuk kontrol positif dan negatif.*

Hasil: *Terdapat peningkatan dalam aktivitas telomerase, ekspresi NF-kB, dan proliferasi BrdU pada empat kelompok perlakuan yang dibandingkan dengan kelompok kontrol positif dan negatif ($p<0.05$).*

Kesimpulan: *Nanokurkumin dan MTX meningkatkan aktivitas telomerase, ekspresi NF-kB, dan indeks proliferasi BrdU dalam kultur sel koriokarsinoma BeWo yang lebih cepat daripada MTX sendiri.*

Kata kunci: *Aktifitas telomerase, ekspresi NF-kB, koriocarsinoma, Metotreksat, Nanokurkumin, Proliferasi indeks BrdU.*

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INTRODUCTION

The incidence rate of gestational trophoblastic disease is 11.5 per 1,000 pregnancies in Indonesia. This has made Indonesia as one of the highest incidence rates of gestational trophoblastic disease in the world. Around 15-20% of patients with complete hydatidiform mole will experience malignant transformation into a malignant trophoblastic disease.[1,2]

Fifty percent of all gestational trophoblastic disease presented as hydatidiform mole, 25% cases are associated with abortion or ectopic pregnancies, and 25% occurred after term and preterm deliveries. Choriocarcinoma is the malignant form of the gestational trophoblastic diseases. Around 2.5% of complete mole cases advance into choriocarcinoma.[3,4] Choriocarcinomas can be distinguished into gestational and non-gestational choriocarcinoma. Gestational choriocarcinoma occurs mostly in reproductive women, usually 1 year after molar or non-molar pregnancy. Non-gestational choriocarcinoma originates from germ cells or trophoblast differentiation in endometrial carcinoma.[2]

Choriocarcinoma is the most common malignant trophoblastic disease compared to an invasive mole or placental site trophoblastic tumor (PSTT). Malignant trophoblastic disease is a malignancy with high levels of invasion and metastasis, in which invasion can alter the myometrium and cause uterine perforation. Distant organ metastases occur in about 19% of choriocarcinomas. The metastases occur hematogenously, most often to the lungs (80%), the vagina (30%), the brain (10%), the liver (10%), the kidneys, and gastrointestinal tract. However, choriocarcinoma is responsive to chemotherapy and its cure rate is 90%. One of the chemotherapy agents used to treat choriocarcinoma is methotrexate (MTX). The mechanism of MTX in choriocarcinoma sensitivity could be associated with the inhibition of RAD51 expression due to DNA damage and the suppressed homologous recombination in tumor cells induced by MTX.[5,6] Methotrexate itself comes with a variety of toxicities. The current development of research on natural drugs, such as curcumin is needed to reduce the toxicity caused by methotrexate and improve the effects of chemotherapy-resistant cases. In addition, the incidence of recurrence in post-chemotherapy patients with choriocarcinoma is about 27% in high risk cases.[1,2]

Human Telomerase Reverse Transcriptase (commonly referred to as hTERT) in humans, is a catalytic subunit of the enzymes telomerase that is associated with repetition of DNA sequences at the end of the eukaryotic chromosomes that work to prolong telomeres. The expression of hTERT is generally required for the development and proliferation of cancer cells.[7] Expression of hTERT has an important role in the continuity of the malignant treatment process. Activation of this enzyme often occurs simultaneously with malignancy. Telomerase expression can be found in choriocarcinoma and exhibits an active and strong properties in the pathogenesis of choriocarcinoma.[8]

NF-κB has physiological function as the main transcription factor for the regulation of immune response and inflammation on various tissue. NF-κB-induced gene expression plays an important role in the tumor and metastasis, where NF-κB-dependent transcription generates the protein which causes cells' resistance to apoptosis, stimulates oncogenic activity, angiogenesis and also stimulates the cells' proliferation.[9,10]

Curcumin (*Dipheruloylmethane*) [*1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5dione*] is a natural phytochemical that was considered has chemopreventive and chemotherapeutic activity by working on 'multiple signaling pathways', including cell survival regulation, proliferation, and angiogenesis process in cancer cells, without any toxic effect on healthy cells.[11] Some literatures have reported some biological aspects of curcumin. The biological aspect of curcumin that is currently being developed is its pharmacological properties such as anti inflammation, antihepatotoxic, and anti-cancer. Curcumin, as an anticancer, is associated with its activity as antiradical, antioxidant, antiproliferation, antiinflammation, and antiapoptotic compound. That mechanism goes by inhibiting hTERT activity that plays a role in cell proliferation, so it decreases BrdU index proliferation[12] and inhibits cell growth, stimulating apoptosis by suppressing NF-κB transcription activity. However, the curcumin in its oral preparation has a low bioavailability, hence the analog structure that has the feature of faster absorption and longer half-life is preferred. Oral administration of curcumin nanoparticles improves the bioavailability up to 5,6 times and longer half-life compared to its original form.[13]

Eventhough curcumin is considered as a novel chemotherapeutic agent, little is known

about its effect in choriocarcinoma. This study aims to investigate the effects of nanocurcumin in addition to methotrexate toward telomerase activity, NF-κB expression, and BrdU proliferation index in BeWo Choriocarcinoma Cell Line Culture (ATCC CCL-98), compared with methotrexate only.

METHODS

This study is an experimental study, conducted in vitro using BeWo Choriocarcinoma Cell Line Culture (ATCC CCL-98) that is intervented in selected doses of nanocurcumin 25,50,100 µg/mL and MTX 100 µg/mL and in telomerase activity, NF-κB expression and BrdU proliferation index were assessed. This study was held in cell/tissue culture laboratory, Faculty of Medicine, Universitas Brawijaya, Malang, from March to April 2018. The sample was taken from BeWo Choriocarcinoma from American Type Culture Collection CCL-98.

A preliminary study was done to determine the dosage of MTX and the result was 100µg/ml, which showed MTX suppresses the NF-κB expression faster compared to the other dosages (999.22±181.39 density/mm^2, p≤0.001). Repetition was done for 5-plo in different dosages. Five steps are 5 samples consist of BeWo Choriocarcinoma Cell Line Culture (ATCC CCL-98) without curcumin nor MTX, 5 samples consist of BeWo Choriocarcinoma Cell Line Culture (ATCC CCL-98) received MTX 100 µg/ml, 5 samples consist of BeWo Choriocarcinoma Cell Line Culture (ATCC CCL-98) received MTX 100 µg/ml + Nanocurcumin 25µg/ml, 5 samples consist of BeWo Choriocarcinoma Cell Line Culture (ATCC CCL-98) received MTX 100 µg/ml + Nanocurcumin 50µg/ml, 5 samples consist of BeWo Choriocarcinoma Cell Line Culture (ATCC CCL-98) received MTX 100 µg/ml + Nanocurcumin 100µg/ml, 5 samples consist of BeWo Choriocarcinoma Cell Line Culture (ATCC CCL-98) received MTX 100 µg/ml + Nanocurcumin 200µg/ml.

After receiving the intervention, BeWo Choriocarcinoma Cell Line Culture (ATCC CCL-98) were incubated for 6 hours. Each sample underwent 5 times of replication before being assessed. Telomerase activity was measured by a Real-Time PCR. A mixed component that was measured consisted of 12.5 µl *Premix* QTD 2x, 1.0 µl of cell or tissue, 11.5 µl of qualified PCR water, with the total volume of 25.0 µl. Upon adding 1 µl of the examined preparations, RT PCR detection program was arranged into 5 steps among others: telomerase action, PCR initial, 3-steps cycling, annealing, and extension. PCR quantification was shown on the data that was collected on screen during the PCR process in a real-time manner, with the cycle threshold or CT value after cycles are done.

NF-κβ was measured by immunohistochemistry and BrdU was measured by flow cytometry. The observation was made on each slide examining how many cells expressed NF-κB with immunohistochemistry staining, which was counted per 200 cells, captured with 400x magnification microscope, and Adobe Photoshop CS2 program to assess the NF-κβ immunohistochemistry thickness on the nucleus with RGBbv unit. Bromodeoxyuridine marking was equipped in the *in vitro* BrdU marking.

Data analysis was done using number of tests consecutively: Shapiro-Wilk test for normality test in data with ratio scale, Independent T-test to compare 2 groups of sample, One Way Anova Test to compare more than 2 groups of sample, and Dunnet test for multiple comparisons if the result from One Way Anova Test show significant differences and disapproval of H_0 (p-value ≤0,05). The data were analyzed using *Statistical Package for the Social Sciences* (SPSS) For Windows Version 23.

This study was reviewed and approved by the Institutional Review Board and Ethical Committee Dr. Cipto Mangunkusumo, a national reference and teaching hospital. The samples were maintained under applicable medical ethical standards.

RESULTS

Nanocurcumin effects on Telomerase Expression

This study showed the decreasing mean value of telomerase expression between the control group (-) (75.50±4.11%) and the control group (+) (68.22±3.34%) as depicted in Figure 1. The highest mean value of telomerase expression was found in the negative control group (no intervention), and followed by positive control group (received MTX 100 µg/mL), and the number was decreasing in the following group as the nanocurcumin received was increasing. Thus, the administration of nanocurcumin + MTX 100 µg/mL was able to decrease telomerase expression faster than only

MTX 100 µg in BeWo Choriocarcinoma Cell Line Culture (ATCC CCL-98).

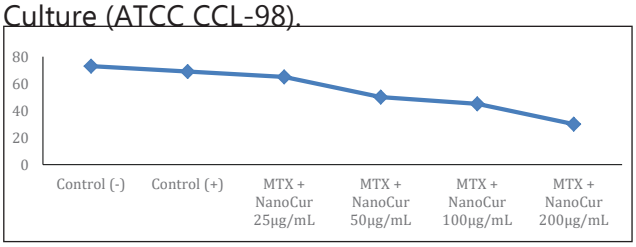

Figure 1. The mean value of Telomerase expression.
*p-value ≤ 0.05 versus negative control; *p-value ≤ 0.05 versus positive control.

Effect of Nanocurcumin on NF-κB Expression

This study showed the decreasing mean value of NF-κB in the culture of *cell line* choriocarcinoma *BeWo* (*ATCC CCL-98*) between the control group (-) (2455.27±124.12 density/mm^2) and control (+) (2065.29±264.02 density/mm^2) as depicted in Figure 2. The highest mean value of telomerase expression was found in the negative control group (no intervention) and followed by positive control group (received MTX 100), and the number decreased in the following group as the nanocurcumin received was increasing. Thus, the administration of nanocurcumin + MTX 100 µg were able to decrease NF-κB expression faster than only MTX 100 µg in BeWo Choriocarcinoma Cell Line Culture ATCC CCL-98).

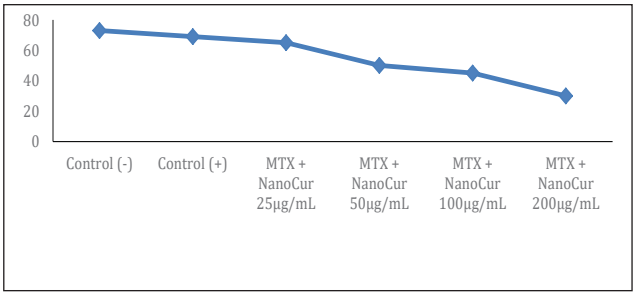

Figure 2. The mean value of NF-κB expression

*p-value ≤ 0.05 versus negative control; *p-value ≤ 0.05 versus positive control.

Effect of Nanocucumin on BrdU Proliferation Index

This study showed the decreasing mean value of proliferation of BrdU in the culture *cell line* choriocarcinoma *BeWo* (*ATCC CCL-98*) between the control group (-) (7.26±0.96%) with the control group (+) (5.81±0.72%) as depicted in Figure 3. The highest mean value of BrdU Proliferation index was found in the negative control group (no intervention) and followed by positive control group (received MTX 100), and the number was decreasing in the following group as the nanocurcumin received was increasing. Thus, the administration of nanocurcumin + MTX 100 µg was able to decrease BrdU proliferation index faster than only MTX 100 µg in BeWo Choriocarcinoma Cell Line Culture (ATCC CCL-98).

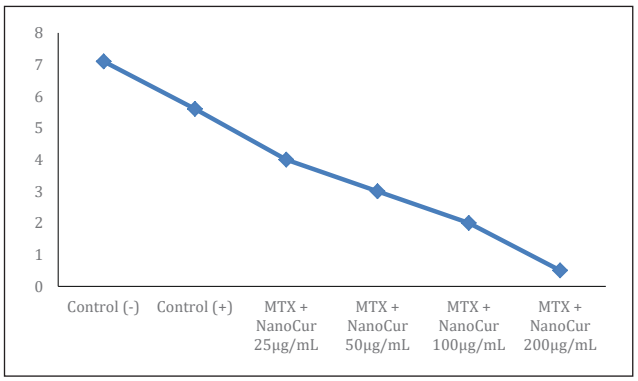

Figure 3. The mean value of BrdU proliferation index
*p-value ≤ 0.05 versus negative control; *p-value ≤ 0.05 versus positive control.

DISCUSSION

In this study, a preliminary study was conducted to determine the optimum dose of MTX that would be used for the intervention on BeWo choriocarcinoma cell line culture. The subject of the preliminary study received several different doses of MTX. In the observation group, MTX was given in low to higher doses. The study showed a 100 µg/mL MTX suppressed the NF-κB expression faster than that of other doses (999.22±181.39 density/mm2, p ≤ 0.001). These results are supported by previous studies showing the administration of medium-dose MTX 10 µg/mL - 100 µg/mL in cancer cells after an 8-hour incubation that could induce apoptosis through decreased cell viability.[14] Methotrexate activates the formation of polyglutamate, AICAR inhibitor, which increases extracellular adenosine secretion. Medium dose methotrexate induces apoptosis of dependent T cells via CD95 dependent pathway.[15] High dose methotrexate (> 100 µg/mL) suppresses the proliferation process, but it did not induce apoptosis. Suppression of proliferation through RNA and DNA barriers by inhibition of dihydrofolate reductase in phase cell cycle S. The suppression of proliferation occurs due to number of suppressors of inflammatory factors such as IL-1β, IL-6, TNF α, COX 2, and LOX.[1]

This study showed a decrease in telomerase expression by administering MTX on BeWo choriocarcinoma cell line culture. Previous

research has shown that methotrexate caused DNA replication in S phase to be halted so that no changes in telomerase expression occurred.[16] Inhibition of cell proliferation is a result of the cytotoxic effect of methotrexate by inhibition of dihydrofolate reductase (DHFR). This inhibition makes tetrahydrofolate depletion, resulting in inhibition of purine and pyrimidine synthesis precursors. Thymidylate can not catalyze dUMP methylation into dTMP without THF, resulting in *thymineless death* and inhibition of purine synthesis. Inhibition of pyrimidine synthesis precursors occurred because methotrexate also directly inhibits AICAR (ATIC) transfomilase and EAR (EART) transfomilase.[17] This results in chromosome instability so that the synthesis of hTR and hTERT at the onset of S phase is inhibited. The suppression of hTERT expression causes the downregulation of C-myc resulting in cell proliferation not occurring.[18–20] Other studies have shown that methotrexate could decrease the metabolism of folate and inhibited hTERT expression in cancer cells. Methotrexate inhibits methionine thereby decreasing S-adenosyl methionine, a methyl donor in protein methylation of folate metabolism. In addition, the decrease in folate metabolism is also due to decreased activation of ERK and AKT signal pathways that function as a proliferation factor.[17]

The results of this study showed that the administration of MTX + nanocurcumin decreased telomerase expression in BeWo choriocarcinoma cell line culture with the average of telomerase expression was smaller than that of the positive control group. This is in accordance with other studies showing that telomerase expression decreased after nanocurcumin administration for 48 hours. Downregulation of telomerase expression is attributed to the suppression of hTERT expression or local suppression of nucleotide translocation on independent hTERT cells.[21] This result is supported by a study by Shariati et al indicating that there was a change in telomerase expression after a low dose of nanocurcumin for 72 hours.[22] The decrease of telomerase expression is due to the accumulation of nanocurcumin inside the cell, leading to the increase of ROS accumulation, increasing of Smad3 and E2F1 causing a decrease in expression hTERT.[23]

This study showed that a combination of MTX + nanocurcumin had a synergistic effect on telomerase expression. Both drugs together decreased hTERT expression directly and by increasing oxidative stress in the cell, so telomerase expression got lower.

NF-κB transcription factor is suspected of having a role in the development of cancer caused by oncogenic mutations in some malignancies. NF-κB acts as a link between inflammation and cancer. The expression of NF-κB is the result of inflammation or the effect of the formation of an inflammatory microenvironment in the development of malignancy.

The results of this study showed a decrease in expression of NF-κB after MTX administration on BeWo choriocarcinoma cell line culture. Inhibition of expression NF-κB has a role in the proliferation process through multiple pathways. Methotrexate inhibits DHFR through two inflammatory pathways: BH2 inhibition that decreases BH4 expression, thus uncoupling the iNOS enzyme, increasing the production of ROS and activating JNK and the release of dependent adenosine, AICAR, activating of adenosine receptors. Both of these pathways results in a decrease in the production of NF-κB.[24] These findings are being reinforced by previous studies showing that methotrexate inhibited NADPH dehydrogenase dependent which are2-oxoglutarate and pyruvate dehydrogenase. This resulted in a decrease of glutathione, acytoplasmic antioxidants. In addition, ROS accumulation resulted in the disruption of potential mitochondrial membranes through activation of the JNK kinase. JNK kinase induced the proapoptotic target genes and activates apoptosis.[17] Another pathway that suppresses TNF is through the release of adenosine and its interaction with adenosine of the A3 receptor as well as the inhibition of pyrimidine synthesis. Methotrexate inhibits genes regulated by NF-κB ie COX-2, iNOS, MMP-9, IL-1, IL-2, IL-6, and GM-CSF.[25]

This study also showed that there was a decrease in NF-κB expression by giving MTX +nanocurcumin to BeWo coriocarcinoma cell line culture. Curcumin is a polyphenol that suppresses various anti-inflammation such as TNFα, IL-1, IL-2, IL-6, IL-8, and IL-12. Inactivation of TNF α led to inhibition of IKK activation, inhibition of IkB α phosphorylation, and translocation of p65 subunit from NF-κB.[26] Increased NF-κB expression was influenced by activation of protein kinase and protein C. In this study, administration of nanocurcumin resulted in ROS accumulation in cells which destroy TNF and H2O2. Both processes result in the inhibition of protein kinase.[27]

This study also showed that the administration of MTX + nanocurcumin greatly decreased NF-κB expression in BeWo coriocarcinoma cell line culture. MTX and curcumin inhibit NF-κB expression in the same pathway through inhibition of IkBα phosphorylation. In addition, both of these regimens increase ROS levels in the nucleus activating cytochrome C that induces apoptosis. This results in the simultaneous administration of MTX and nanocurcumin further decreasing the expression of NF-κB. Curcumin increases MTX and folate acid in KG-1 cells by increasing the sensitivity of B-mRNA and protein folate receptors. In addition to that, curcumin increases the cytotoxic effect of MTX. Combination of drugs (curcumin + doxorubicin) with nanoparticle formula inhibits drug resistance logging by inhibiting Bcl-2 expression and prolonging doxorubicin effect in cells.[28]

BrdU is a thymidine analog that joins a single-stranded DNA in a cell cycle. BrdU is used as a proliferation marker and in cell migration that is inserted in the S-phase cell cycle and persists in the cell.[29]

This study showed the decrease of BrdU index proliferation by the administration of MTX on BeWo choiocarcinoma cell line culture. Methotrexate interferes with the kinetic of cell cycle by discontinuing it on S-phase. Methotrexate inhibits dihydrofolate reductase, altering neutrophil formation in the hydrolysis of dUMP to dTMP in pyrimidine synthesis. In S-phase, p53 destroys thymidine and stranded DNA by DNA helicase. The disruption of synthesis of purines cause the "thymineless stress", an apoptosis process.[16,17]

The results of this study showed that the administration of MTX + nanocurcumin decreased the proliferation index in of BeWo choriocarcinoma cell culture with mean value of BrdU proliferation index smaller compared to the mean value of positive control group. The administration of nanocurcumin causes the accumulation of ROS in cells and the downregulation of apoptotic suppressor proteins (BcL-2 and BcL-xL). The inreased ROS also causes downregulation of the API-1 binding factor, resulting in the G0 / G1 phase stalled. Nanocurcumin makes the cell cycle stop on G1 / S phase by inhibiting the binding of Cdk 4 and Cdk 6, which are G1 / S regulators. Cyclin D1 is inhibited through the Ras / ERK pathway and activation of STAT3.[30]

This study also showed that the administration of MTX + nanocurcumin decreased the BrdU proliferation index greater compared to MTX only in cell culture line of BeWo coriocarcinoma. The administration of nanocurcumin decreases the BrdU proliferation index in various cell cycle phases that have a synergistic effect with methotrexate administration. From these findings, this study showed that the administration of nanocurcumin + MTX could give a better outcome in patients with choriocarcinoma compared with MTX administration alone. The decrease of NF-κB expression could stimulate the apoptosis which its index is used as a prognostic factor in gestational trophoblastic disease.

The limitation of this study is the use of relatively small sample for each group. This study was conducted in vitro using BeWo Choriocarcinoma Cell Line Culture (ATCC CCL-98). However, further study is needed, in primary culture and animal study, to evaluate the correlation of telomerase expression, NF-κB expression with decreasing BrdU proliferation index on nanocurcumin + MTX administration therefore the biomolecular patophysiology could be understood clearly.

CONCLUSIONS

The administration of nanocurcumin + MTX has an effect on decreasing telomerase expression, decreasing NF-κB expression, and decreasing BrdU proliferation index on BeWo coriocarcinoma cell line culture (ATCC CCL-98); and the administration of nanocurcumin + MTX further decreased the expression of NF-κB and further lowered the BrdU proliferation index on BeWo coriocarcinoma cell line culture (ATCC CCL-98) compared with MTX.

Data Availability

The data used to support the findings of this study are available on request.

CONFLICT of INTEREST

The authors declare no conflict of interest.

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Research Article

RELA mRNA Expression in Epithelial Ovarian Cancer: Correlation with rs11820062 Gene Variant

Ekspresi mRNA RELA pada Kanker Ovarium Epitelial : Korelasinya dengan Varian Gen rs11820062

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Abstract

Objective: To examine the distribution of RELA rs11820062 and its correlation to i mRNA expression in low-grade and high-grade EOC's patients from Dr. Cipto Mangunkusumo General hospital, Indonesia.

Methods: This study is cross-sectional with a total of 65 healthy subjects and 80 ovarian biopsies (15 ovarian cysts as expression calibrators, 36 low-grade EOC, and 29 high-grade EOC) were used in this study. The distribution of genotypes and alleles was analyzed using ARMS PCR. The mRNA expressions of RELA were determined by real-time polymerase chain reaction (qPCR) analysis.

Results: There was no significant difference between genotype and allele distributions for RELA rs11820062 in normal and case group. RELA relative mRNA expression was significantly higher in low-grade and high-grade EOC compared to in ovarian cysts ($p<0.01$). RELA rs11820062 CC genotype correlated to higher RELA mRNA relative expression and the TT genotype of RELA rs11820062 correlated with lower RELA mRNA relative expression in low-grade and high-grade EOC.

Conclusion: C allele in rs11820062 caused an increased expression of RELA mRNA, which individuals with CC genotype correlated with higher RELA expression in low-grade and high-grade EOC. In contrast, individuals with the T allele of RELA rs11820062 had a protective effect against EOC risk because the RELA TT genotype tended to have a lower RELA mRNA expression in EOC.

Keywords: epithelial ovarian cancer, NF-kB, RELA, rs11820062.

Abstrak

Tujuan: Mengetahui distribusi RELA rs11820062 dan korelasinya dengan ekspresi mRNA RELA pada pasien EOC low-grade dan high-grade di Rumah Sakit Dr. Cipto Mangunkusumo, Indonesia.

Metode: *Penelitian ini merupakan peneliatan potong lintang terhadap 65 sampel darah perempuan normal dan total 80 biopsi kanker ovarium dengan rincian: 15 kista ovarium sebagai kalibrator ekspresi, 36 EOC low-grade, dan 29 EOC high-grade. Distribusi genotipe dan alel dianalisis menggunakan ARMS PCR dan ekspresi mRNA RELA dikuantifikasi menggunakan teknik qPCR.*

Hasil: *Tidak terdapat perbedaan distribusi genotipe dan alel antara kelompok normal dengan kasus EOC. Ekspresi relatif mRNA RELA meningkat secara signifikan pada kelompok EOC low-grade dan high-grade. Individu dengan genotipe RELA rs11820062 homozigot CC memiliki ekspresi mRNA yang lebih tinggi dibandingkan genotipe lain. Sebaliknya individu dengan genotipe TT memiliki korelasi dengan ekspresi mRNA RELA yang lebih rendah pada tipe low-grade dan high-grade EOC.*

Kesimpulan: *Alel C pada RELA rs11820062 menyebabkan peningkatan ekspresi mRNA RELA pada pasien EO yang dilihat dari individu dengan genotipe CC cenderung memiliki ekspresi mRNA RELA yang lebih tinggi pada tipe EOC low-grade dan high-grade. Sebaliknya, individu dengan alel T RELA rs11820062 diduga memiliki efek protektif terhadap risiko EOC karena adanya korelasi antara genotipe TT dengan ekspresi mRNA RELA yang lebih rendah pada EOC.*

Kata kunci: *kanker ovarium epitelial, NF-kB, RELA, rs11820062.*

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INTRODUCTION

In terms of gynecological cancers, ovarian cancer has the greatest mortality rate. Epithelial Ovarian Cancer (EOC) accounts for more than 90% of all ovarian cancer cases.[1] In 2018, GLOBOCAN recorded 295,414 new cases of ovarian cancer with a mortality rate of 184,799 people.[1] Meanwhile, the incidence of new cases of ovarian cancer in Indonesia in the same year was 14,896, with a total death cases of 9,581.[2] The high mortality from ovarian cancer caused by this cancer is asymptomatic, and no diagnostic method detects the ovaries at an early stage. Therefore, current research on ovarian cancer centers on a new approach or biomarker with diagnostic, prognostic, and predictive potential.[3] Knowledge of the etiology of various histologic types of ovarian cancer might open new pathways in basic research and clinical studies to develop screening and diagnostic methods for ovarian cancer. EOC based on their progressivity, molecular characteristic, and histologic type into low-grade and high-grade EOC. Generally, the low-grade type has a better prognosis characterized by slow progression and a lower proliferation rate than the high-grade type.[4]

Chronic infections and the following inflammatory response have been estimated to be responsible for 15% of the world's cancer burden.[5] EOC is frequently associated with a number of disease that causes an inflammatory response, including endometriosis, pelvic inflammatory disease, and polycystic ovary syndrome.[6] In areas of inflammation, epithelial cells are exposed to greater concentrations of inflammatory mediators, such as reactive oxygen species, cytokines, prostaglandins, and growth hormones that hasten cell division and genetic and epigenetic changes.[7] Later, these inflammatory mediators, particularly pro-inflammatory cytokines like TNF, IL-1, and IL-6, would cause a group of transcription factors, called nuclear factor kappa-κB (NF-κB) to become activated. When TNF and IL-1 are secreted, they interact with premalignant cells to activate NF-κB, which then triggers the production of genes that inhibit apoptosis and promote angiogenesis and proliferation to promote malignancy.[8] RelA, RelB, c-Rel, p105/p50 (NF-kB1), and p100/52 are the five members of the NF-κB family (NF-κB2). The NF-κB p65 subunit is regarded to be the family's most potent transcriptional activator.[9]

RelA is a subunit of NF-κB, which has a transcription activation domain (TAD) at the C-terminal end. TAD is a domain that will interact with the basal promoter region of various downstream genes from NF-κB. During inflammation, the classical pathway NF-κB is activated with a dimer in RelA with its p50 subunit pair.[10] These dimer pairs then translocate into the cell nucleus and regulate the expression of various related genes. Therefore, increased expression of the RelA subunit is often associated with multi types of cancer, such as lung, prostate, breast, and other solid cancers, including ovarian cancer.[11] This makes the gene encoding RelA, *RELA* (11q13), a potential proto-oncogene.[12] In many cancer cases, increased *RELA* expression is associated with poor prognosis and low life expectancy.[13] Thus, research on the relationship between *RELA* expression and ovarian cancer is essential.

Numerous disorders, including cancer, have been linked to single nucleotide polymorphisms (SNPs) in the NF-κB signaling pathway genes.[14] Most risk alleles predispose to disease are in the non-coding region, with the mechanism of their function largely unknown. Information regarding the association of variants in this gene with the risk of epithelial ovarian cancer is essential, especially since research in Indonesia has never been done.[15] The variation to be studied in this study is rs11820062 which is in the first intron, almost 5' untranslated region (5' UTR), of *RELA* gene. The position of this variant was predicted to have a transcription factor binding site. In addition, due to its location near the five ′ end of *RELA,* this mutation can alter transcription factors' binding and regulation.[16]. This study aimed to evaluate the correlation of the 5′ most intron variants of *RELA,* rs11820062 C/T, with the susceptibility for EOC with its possible mechanism at the transcriptional level. In addition, the present study aimed to analyze the connection between this genetic variant and the dualistic model of EOC.

METHODS

This cross-sectional study analyzed the relationship between genetic variant and mRNA expression of *RELA* with the risk of low-grade and high-grade EOC. As NF-κB plays major role in cancer development and progression, we hypothesize that *RELA* mRNA expression is increased in both low- and grade- EOC and individuals with genetic variation rs11820062 are at greater risk for EOC.

Primary samples were ovarian cancer biopsies from women that underwent surgery in Dr. Cipto Mangunkusumo Hospital from 2016-2021. Samples were obtained from Biobank Research IMERI-FMUI and Department of Pathological Anatomy FMUI as formalin-fixed paraffin-embedded (FFPE) block. The total ovarian biopsies used in this study were 85 blocks, divided into two groups: 15 ovarian cysts, 36 low-grade EOC, and 29 low-grade EOC. This study also used healthy blood samples from women that underwent blood transfusion at Palang Merah Jakarta Pusat aged 40-70, with no history of ovarian cyst, cancer, endometriosis, or PCOS, and cancer-free in a family for at least three generations. Blood samples from women that match the criteria be a normal group for rs11820062 genotype and allele distribution analysis. The University approved this study by Indonesia's Ethical Committee on Medical Research (No. KET-689/UN2.F1/ETIK/PPM.00.02/2020), and the participants were informed of the study's purpose for normal control. Each participant signed written informed consent forms.

In genotyping analysis, there are two types of DNA sources: whole blood for normal subjects and FFPE biopsies for cases. To isolate DNA from whole blood, the salting-out method was applied, which includes red blood lysis by a red lysis blood solution (RBCs), cell and nuclei lysis by cell lysis solution (Tris HCl 1M, EDTA 0.5M, and 10% SDS) and protein precipitation by ammonium acetate 5M. For DNA and RNA extraction from FFPE ovarian biopsies, the paraffin block had already been cut into 6x5µm and undergone microdissection to separate cancer tissue from other types. Samples were deparaffinized using xylene to dissolve the paraffin, followed by a rehydration process with ethanol. After the deparaffination process, samples followed the gSYNC™ DNA Extraction Kit protocols. The purity of the isolated DNA was measured using NanoDrop (Maestrogen) at a wavelength of 260/280nm. Pure DNA has expected ratios of 1.7–1.9.

RELA Rs11820062 were detected using T-Arms PCR with primers designed using the Primer1 application from http://primer1.soton.ac.uk. The reference sequence used is from NCBI. The primers were then BLAST (NCBI) to determine the specificity of the primers. The primers that were used in this study are listed in Table 1. The expected size of the PCR products is below 200 bp because of the nature of DNA obtained from FFPE, which was heavily degraded. From our experienced, PCR products above 200 could not be amplified (not included).

After the primers were obtained, T-ARMS PCR optimization was performed to get the right PCR settings. The amplification uses three different tubes, each consisting of a pair of primers: forward outer and reverse outer for internal control, forward inner and reverse outer for wild type allele, and forward outer and reverse inner for the alternate allele. The master mix composition (25 uL) consisted of 12.5 µL MyTaq Red Mix 2x, 1 µL of forward and reverse primers, 100 ng of template DNA, and nuclease-free water. Special for amplifying the C allele, 5% DMSO was added into the tube. DNA samples were amplified for up 35 cycles, beginning with 5 minutes pre-denaturation temperature of 94°C, followed by a process of denaturation at 94 °C for 30 seconds, annealing at 57°C for 15 seconds, and elongation at 72°C for 10 seconds. The extension time was extended by 72°C for 7 minutes at the end of the cycle. The amplification results of the PCR products were mixed in 1 tube and visualized using 3% agarose gel electrophoresis with the Hyper Ladder™ 50bp ladder. The primers' sequence and estimated results to be obtained are shown in Table 1.

Table 1. Primer used in ARMS PCR

Primer	Sequence (5′→3′)	TM (°C)	Amplicon Size (bp)
RELA (rs11820062)			
Forward-Outer	AGAAACACCTGCTTCTTGAGGGA	63	
Reverse-Outer	AACGCATCTGATTCAGTTTCCTCTCTG	63	151
Forward-Inner (T allele)	GGCCTGTTGTACTTTCTTAAGGAACAT	63	81
Reverse-Inner (C allele)	TGGGGCGTGCCCTCCCTAAG	66	115

RNA was extracted from samples who had been deparaffinized before using and following protocol from Quick-RNA Miniprep Plus Kit (ZymoResearch) for FFPE. RNA was and100 ng RNA was made into cDNA using a kit from ReverTra Ace™ qPCR RT Master Mix with gDNA Remover (Toyobo). cDNA template was used as a qPCR template. RELA gene expression was measured relative to GAPDH gene expression. qPCR master mix preparation was done in a cold rack and prepared per manufacturer instruction (SensiFAST SYBR Lo-ROX Mix®, Meridian Bioscience). The primers for RELA were designed via Primer Quest™ Tool (IDT DNA) with the following sequence: F: 5′- AAGAAGAGTCCTTTCAGCGG -3′; R: 5′- GACGTAAAGGGATAGGGCTG -3′ and GAPDH with the following sequence: F: 5′- GAAATCCCATCACCATCTTCCAGG-3′ and R: 5′-GAGCCCCAGCCTTCTCCATG-3′. The amplification was done in a 7500 Fast Real-Time PCR System and conducted twice for each sample. The relative expression level was quantified by the Livak method.

The chi-square was used to assess the connection between genotype and Fisher's exact test for allotype frequency in the EOC group. The difference in *RELA* mRNA relative expression in each group was counted statistically using the nonparametric independent test Kruskal Wallis as the distribution was not normal. The correlation between genotype and mRNA relative expression of *RELA* was done using a Pearson correlation test. All results counted as significant if the p-value <0.05 This statistical study was conducted using version 25 of the SPSS (Statistical Package for the Social Sciences) software.

RESULTS

In this study, the number of samples used was 36 samples for the low-grade epithelial ovarian cancer case group, 29 for the high-grade ovarian cancer case group, and 15 for ovarian cyst tissue as the control group. In addition, blood samples from normal women were also used to see the difference in distribution between the normal group and the EOC cases.

The non-serous histological type predominated in the low-grade EOC group, while in the high-grade EOC, the majority was the HGSC type (Table 2). The average age of subjects at the time of diagnosis for the low-grade EOC group was 49.4 years, and 50 years for the high-grade EOC group. At the same time, the usual group subjects had an average age of 51.1 years.

Table 2. Histological Types of EOCs Subjects

Histological types	N (%)
Low-grade	36 (100)
Endometrioid carcinoma	14 (38.89)
Mucinous carcinoma	9 (25)
Clear Cell Carcinoma	11 (30.56)
Low-grade Serous Carcinoma	2 (5.56)
High grade	29 (100)
High-grade Serous Carcinoma	27 (93.1%)
Carcinosarcoma	2 (6.9%)

Based on the results of the Chi-square test (Table 1), there was no significant difference between the distribution of the genotypes (p = 0.273) and al o types (p = 0.172) *RELA* rs11820062 (Table 3). The most common genotype found in both the control and case groups was CT, while the CC genotype was the genotype with minor frequency in the normal group and TT in the case groups. The alternative allele T has an allele frequency of 53.8% in the control group and 44.6% in the case group. T allele had a protective effect with an odds ratio of 0.690 (0.424-1.125).

Table 3. Genotype and Alleles Frequencies of rs11820062 in the RELA

Rs11820062	Total	Normal	Case	*P-value*
	(n = 130)	(n=65)	(n=65)	
Genotype				
CC	27.7	24.6	30.8	
CT	46.2	43.1	49.2	0.273
TT	26.2	32.3	20.0	
Allele				
C	50.8	46.2	55.4	0.172
T	49.2	53.8	44.6	

Additionally, both the normal and case groups obtained HWE p values > 0.05, indicating that the individuals' alleles were in Hardy-Weinberg equilibrium (HWE) and that their population had little to no random mating and a limited inflow of new genetic material (Table 4).

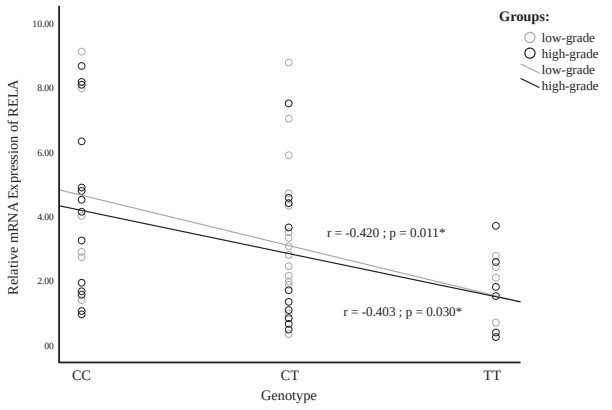
Table 4. HWE Analysis of rs11820062 in the RELA gene

		Normal			Case		
	Genotype	**Expected**	**Observed**	***P-value***	**Expected**	**Observed**	***P-value***
Rs11820062	CC	13.85	16	0.56114	19.94	20	0.99952
	CT	32.31	28		32.12	32	
	TT	18.85	21		12.94	13	

RELA's mRNA expression is significantly higher in EOCs (p=0.002) than in the cyst, but there is no significant difference between expression in low-grade and high-grade EOCs. The ovarian cyst group had a medium of 1.146 (0.330– 2,977), low-grade 2.814 (0.340 – 9.166), and high-grade 2 1.927 (0.274 – 8.724).

The relationship between the rs11820062 RELA genotype and the RELA mRNA expression level can be determined by performing a correlation test. Based on the normality test, it showed that the rs11820062 *RELA* genotype and RELA mRNA expression levels in low-grade and high-grade epithelial ovarian cancer patients were normally distributed (P value> 0.05), so the Pearson correlation statistical test was performed.

The data shown in Figure 2 shows that the relationship between the rs11820062 RELA genotype with the T allele and the level of RELA mRNA expression in both low- and high-grade EOC has a negative relationship. The correlation between the rs11820062 genotype and the *RELA* mRNA relative expression level in both groups falls in the medium criteria (Figure 1).



*p-value significant at <0.05 level

Figure 1. Correlation of the RELA rs11820062 genotype with *RELA* mRNA expression levels in the *low*- and *high*-grade EOC.

DISCUSSION

Like other types of cancer, NF-kB plays a huge role in every step of EOC tumorigenesis, from initiation to metastasis. During inflammation, NF-KB is more regulated in a classical manner than the alternative manner by forming RelA and p50 dimers.[17] RelA or p65 subunit of NF-κB, encoded by *RELA,* has a transcription activation domain (TAD), which will later regulate many gene targets, including genes that regulate cell proliferation.[18] *RELA* is a proto-oncogene and is the family's most potent transcriptional activator.[19] Low patient survival is associated with high RelA/p65 expression, which activates the NF-κB pathway. Enhanced p65 phosphorylation and NF-κB activity during tumor growth in a mouse model of OC are a result of increased M2 macrophage infiltration.19

There is no significant difference in rs11820062 genotype distribution between normal subjects and EOC cases. Homozygote CT was the most frequent genotype in both normal (43.1%) and cases subjects (49.2%). Similar to the result in this study, found that the most frequent genotype for both normal and ovarian case groups was CT, with 49.3% for the normal group and 49.7% for cases in the Han-Chinese population. In that study, the rs11820062 genotype alone did not correlate with EOC [20] Failure to detect the correlation of the RELA rs11820062 with epithelial ovarian cancer may also occur because only a single variant was detected in this study. In some cases, the protective or predisposition effect appears when an extreme mRNA expression cannot be detected only by a single genetic marker. [21] In accordance, found a correlation of *RELA* rs11820062 with EOC susceptibility by forming diplotype CC-CC with *RELA* rs7119750. The minor allele in normal groups was the C allele, with a frequency of 46.2%, and the T allele for case groups, with a frequency of 44.6%.20 However, there have been no other studies regarding the distribution of rs11820062 in other populations for epithelial ovarian cancer or other types of cancer. Therefore, information regarding the correlation of this allele with the risk of ovarian cancer is still very limited to support this hypothesis.

In this study, the distribution of *RELA* rs11820062, both in normal and case groups were in the HWE. Allele and genotype frequencies can be calculated using Hardy-Weinberg assumptions. When the ratios of homozygous

and heterozygous genotypes dramatically deviate from what would be expected based on HWE assumptions, it may be a sign of genotyping errors, batch effects, population stratification, or much less frequent association.

In this study, *RELA* relative mRNA expression is significantly higher in EOCs than in ovarian cysts. Rel A/p65 exhibits substantial transactivation potential, as seen by its constitutive activation in various human malignancies.[22] Compared to normal tissues, human tissues from colon adenocarcinoma, gastric carcinoma, lung carcinoma, and pancreatic adenocarcinoma exhibit greater nuclear translocation of RelA and NF-κB-DNA-binding activity.[23] However, there is no significant difference between the expression of RELA mRNA in low-grade and high-grade EOC. Similar results were obtained for other types of cancer where the relative expression of RELA mRNA was known to increase significantly in samples of colorectal tumor tissue compared to normal mucosa as the standard group. In that study, the relative expression of RELA mRNA did not correlate with clinicopathological characteristics, including tumor progression and AJCC staging classification.[24] Moreover, *low-grade* EOCs consist of diverse histological types of EOC, such as mucinous, endometrioid, clear cell carcinoma, and low-grade serous carcinoma, with different gene regulations and mutation characteristics. In high-grade EOC, the sample histological type is more uniform, as it mainly consists of high-grade serous carcinoma. Often the division based on the dualistic model is not correlated with the patient's prognosis and progressivity as it does not count the stage of cancers.[25] In some cases, non-serous low-grade EOC, but in stage III or IV, tend to be more resistant to chemotherapy than high-grade serous carcinoma. [26] The expression of *RELA* mRNA and p65 protein is higher in ovarian cancer cells (A2780CP) resistant to cisplatin than in ovarian cancer cells, which are sensitive to cisplatin and have higher mRNA expression than normal. [26]

This research shows a strong negative correlation between the rs11820062 genotype and RELA-relative mRNA activity, where TT groups had the lowest mRNA expression compared to CT groups, especially CC groups. Rs11820062 is located in first intron, almost to 5′ of *RELA.* As 5′-most intron variation, *RELA* rs1182062 is linked to mRNA expression levels and can initiate transcription from in silico analysis. That silico genotype-gene expression research revealed that this SNP could affect *RELA* mRNA expression and binding to the androgen receptor in immortalized B cells. In that study, the mutant type is the T, shown to be mainly linked to lower *RELA* transcriptional activity.[16] The decrease in RELA mRNA expression in the T allele could explain the odds ratio of the T allele in this study which was below 1 (OR=0.69), indicating that the T allele is protective against epithelial ovarian cancer. [14] In contrast, the C allele may predispose to EOC due to the tendency to high RELA mRNA expression in individuals with the CC genotype. Although correlated with the relative expression of *RELA* mRNA in low-grade and high-grade epithelial ovarian cancer, the Chi-square test shows that rs11820062 has no significant difference in distribution. However, rs11820062 has the potential to be studied further by looking at its interactions with other variants at different loci to be able to produce phenotypes such as epithelial ovarian cancer.

CONCLUSIONS

There was no significant difference between the distribution of RELA rs11820062's genotype and allele in the normal and EOC case groups. *RELA* mRNA relative expression is significantly higher in low-grade and high-grade EOC than in ovarian cysts. However, there is no significant difference between low-grade and high-grade relative mRNA expression of *RELA* allegedly because of the heterogeneity of low-grade EOC's histological subtype and grades. The high *RELA* mRNA expression in EOC due to genetic variation rs11820062 CC genotype. In contrast, individuals with the TT genotype tended to have lower expression of RELA in both low-grade and high-grade of cancer. This result corresponds to the T allele's odds ratio of 0.62 which might indicate the protective effects of T allele from EOC.

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Case Report

Surgical Wound Dehiscence Treatment

Tata Laksana Dehisensi Luka Operasi

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Abstract

Objective: This article describes management of surgical wound dehiscence. in a cesarean section.

Method: Case report.

Case: A 39 years-old woman, P4, presented with reddish pus coming out from open surgical wound on day 4 following a caesarean section. Laboratory findings revealed a condition of Hypoalbuminemia, leukocytosis, and a Staphylococcus aureus was detected on pus from the wound base. Upon the resuturing, the wound was dressed with antimicrobial wound dressings and pad and changed every 12 hours. After 3 days, the wound was dressed with modern antimicrobial wound dressings gel and pad, changed every 3 days and planned for necrotomy afterwards. A presence of dry, granulation tissue was observed before the resuturing.

Conclusion: Selection of dressing regimen should be individualized according to the wounds. Modern antimicrobial wound dressing can be a good therapy option for surgical wound dehiscence after caesarean section.

Keywords: surgical site infection, surgical wound, wound dehiscence.

Abstrak

Tujuan: *Artikel ini melaporkan tata laksana dehisensi luka operasi seksio sesarea.*

Metode: *Laporan kasus.*

Kasus: *Seorang perempuan 39 tahun, P4, datang dengan keluhan nanah kemerahan keluar dari luka operasi terbuka postoperasi seksio sesarea hari ke-4. Temuan laboratorium: Hipoalbuminemia, leukositosis, dan Staphylococcus aureus dari kultur dasar luka. Sebelum operasi penjahitan kembali, luka dibalut dengan pembalut dan bantalan luka antimikroba modern yang diresapi dengan hidrogel, diganti setiap 12 jam. Setelah 3 hari, luka dibalut dengan gel dan pembalut luka antimikroba modern yang diresapi dengan hidrogel, diganti setiap 3 hari dan direncanakan untuk nekrotomi. Sebelum operasi dilakukan kembali, luka tampak kering dengan dasar jaringan granulasi.*

Kesimpulan: *Regimen pembalutan harus disesuaikan dengan kebutuhan masing-masing luka. Pembalut luka antimikroba modern dapat menjadi pilihan terapi yang baik untuk dehisensi luka bedah setelah operasi seksio sesarea.*

Kata kunci: *dehisensi luka, infeksi luka operasi, luka operasi.*

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INTRODUCTION

Surgical wound dehiscence (SWD) has a broad meaning, covering a spectrum of problem that ranges from the separation of a superficial part of the incision, a full depth or to a complete one. Some of SWD synonyms are wound separation, wound disruption and wound opening. The causes of surgical wound dehiscence can be classified as technical issue (e.g. unravelling of the suture knots), disrupted healing (e.g. surgical site infection and comorbidities), and mechanical stress (e.g. coughing).[1]

Surgical wound dehiscence rates for cesarean section is approximately 1.9% - 7.6%.1 Risk factors of SWD are older age, local wound infection, hypoproteinemia, emergency surgery, hypertension, obesity (body mass exceeding 30), chronic obstructive pulmonary disease (COPD), malignancy, pneumonia, smoking, diabetes, malnutrition, radiation and poor perfusion.[2,3] A study shows that patients with more than 5 risk factors may increase the risk of wound dehiscence.[2] Risk factors of surgical wound dehiscence following caesarean section can be classified into patient-related and surgically-related.[4] Patient-related factors include the use of corticosteroid[5], high body mass index at term[5–9], fetal distress[9], chorioamnionitis[7,8], low frequency of antenatal visit[10], fetal macrosomia[8], pre-eclampsia[8], hypertension[6], diabetes mellitus[6], gestational diabetes[6], and prolonged rupture of membrane[10]. Surgically-related factors include hemorrhage[8,11], emergency procedure[6,11], induction of labour[7], absence of antibiotic prophylaxis[6,7,10], and increase duration of cesarean section[10].

Surgical wound dehiscence could affect the patients' social, physical, and mental health. Abdominal SWD occurs in approximately 0.5-3.5% cases[2,12] and causes death (3%-35%) as well as incisional hernia (83%).[1] Surgical wound dehiscence carries a high healthcare cost due to increase of length of hospital stay and increase rates of re-operation.[2] It can also cause severe impact on patient's psychosocial wellbeing.[1] SWD after cesarean section is the cause of resource consumption, prolonged hospital stay, and also mortality and morbidity of the mother and baby. Therefore, comprehensive treatment and preventive measures should be taken into consideration. In this article, we focus on the management surgical wound dehiscence in cesarean section.

METHODS

This article is a case report about surgical wound dehiscence treatment. Permission and written consent were obtained from the patient.

CASE

A 39 years-old woman with P3A1 presented to Emergency Room with chief complaint of reddish pus coming out from an open surgical wound on day 4 following a cesarean section. Cesarean section was done due to transverse lie in labor, obesity grade II, and history of a previous caesarean section. Post-operation, patient complaint pain with score of VAS 3 with no active bleeding. Patient did not have any history of other surgeries nor diseases.

Vital sign recorded a blood pressure of 130/80 mmHg, a pulse rate of 88 beats per minute, a respiratory rate of 19 times per minute and a temperature of 36.3^0C. General physical examination was within normal limit. Localized status found an ellipse-shaped, 10 cm x 6 cm in diameter open surgical wound, along with pus and necrotic tissue. Obstetric examination revealed the uterine fundal to be 2 fingers above the symphysis pubis. From inspection, urethra and vulva were within normal limit, no vaginal bleeding was observed.

Laboratory test of complete blood count showed a Hemoglobin of 10.9 mg/dL, Albumin of 2.87 g/dL and leucocyte of 19.200 per mm^3. Bleeding function and urinalysis test were within normal limit. Pus culture from the wound base was tested positive for Staphylococcus aureus. According to the data collected, patient was diagnosed with superficial incisional surgical wound dehiscence in P3A1 Post CS due to transverse lie in labor, obesity grade II, hypoalbuminemia.

Before resuturing was done, the wound was dressed with a hydrogel impregnated with modern antimicrobial wound dressings and pad and changed every 12 hours. Patient was given ceftriaxone 2 grams/24 hours via intravenous injection. After 3 days, the wound was dressed with the hydrogel impregnated with modern antimicrobial wound dressing gel and pad, changed every 3 days and planned for necrotomy afterwards. A presence of dry, granulation tissue was observed before the resuturing.

The patient was given ceftriaxone and

metronidazole for the empiric antimicrobial until the result of pus culture test came out. The pus culture result revealed that the bacteria were sensitive to ampicillin sulbactam.

During resuturing operation, wound dehiscence 10 cm x 5 cm x 4 cm with wound base subcutis was identified. Excision of necrotic tissue and undermining was performed to achieve tension free and intact fascia. Vertical matrass suture with polypropylene needle no.1 was performed to finish it off. The patient was then given ceftriaxone 2 gram/24 hours IV and metronidazole for 10 days in the ward.

Figure 1. After CS day 5: wound dehiscence size 10x6 cm, with pus and necrotic tissue

Figure 2. Wound dehiscence size 10x5x4 cm, wound base subcutis (superficial surgical site infection) after wound care

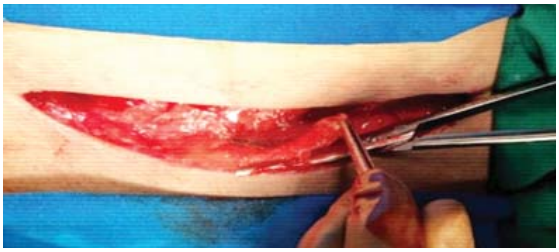

Figure 3. Excision of necrotic tissue (intraoperation)

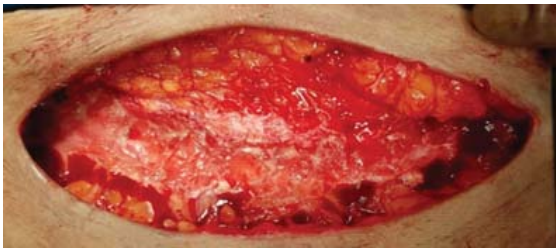

Figure 4. Tension free was achieved with intact fascia (intraoperation)

DISCUSSION

The causes of SWD are similar to the causes of poor wound healing, both in internal and external factors. This patient's superficial SWD can be caused by disrupted healing process due to surgical site infection (SSI) which can be seen from laboratory findings with leukocyte of 19.200 per mm^3 and the presence of *Staphylococcus aureus* from culture of the wound base. This patient also has this following risk factors: obesity, emergency operation, and hypoalbuminemia.

Management of superficial SWD should be done by wound closure, debridement, necrotic tissues removal, appropriate dressing utilization, and management of local or systemic infection.[1] The aim of wound care is allowing wound to heal rapidly without any complications and having the best aesthetic and functional results.

The important initial decision of SWD management is choosing the most appropriate method to achieve closure of the wound. This step depends on the depth of dehiscence, location of incision and timing in relation to the surgery. Secondary closure can be used in superficial SWD with or without infection and also can be used in deeper dehiscence, or where primary closure is not possible. Debridement should be done to remove foreign material and non-viable tissue, reduce biofilm, bioburden and inflammatory stimulus. Autolytic debridement is frequently used for superficial dehiscence and surgical debridement is often used for deeper dehiscence.[1]

The choice of dressings should be based on their ability to facilitate autolytic debridement, maintain a moist wound environment, and protect the wound from external contamination. The performance of each dressing is based on the type, constituent and construction of material. It is difficult to generalize the exudate handling capacity and absorbency of different dressing.[1] Selection of dressing regimen should be individualized according to the characteristic of the wounds. Consideration in choosing the suitable dressings is the characteristics of the dressings, the patient's primary disease, and physiological mechanism of the wounds.[3]

Table 1. Type of Modern Dressings Suitable for Each Type of Wounds[13]

Variety	Description	Characteristics	Suitable Conditions
Hydrogel	Three-dimensional network of hydrophilic polymers	Moisturizing, removal of necrotic tissue, and monitoring of the wound	Pressure ulcers, surgical wounds, burns, radiation dermatitis
Hydrocolloid	Hydrogel mixed with synthetic rubber and sticky materials	Excellent exudate absorotion properties	Severe exudative wound
Alginate	Consists of polvsaccharides derived from brown seaweed	Excellent exudate absorption properties, hemostasis	Infected and non-infected wounds with a large amount of exudate
Foam	Consists of polyurethane or is silicone-based	Semipermeability, thermal insulation, antimicrobial activity	Infected wounds
Film	Consists of adhesive, porous, and thin transparent polyurethane	Autolytic debridement properties, impermeable to liquias and bacteria	Epithelializing wounds and superficial wounds with limited exudate

For this patient, we used hydrogel impregnated with modern antimicrobial wound dressing. This type of dressing can be used for any types of wounds, including contaminated, colonized to infected wounds, and also lightly to highly exuding wounds. Modern antimicrobial wound dressing uses physical mode of action by using hydrophobic coating that was made from diakylcarbamoylcgloride (DACC). This helps to reduce the bacterial load in the wound. The mechanism of this dressing is different from the traditional antimicrobial dressing, which contains pharmacologically or chemically active substances to reduce bacterial load in a wound. DACC encourages the natural hydrophobic interaction where hydrophobic organisms are attracted and held together by surrounding water molecules.[14]

A multicenter European surveillance study on 116 subjects assessed the efficacy of this type of modern antimicrobial wound dressing and found that 21% of the patients' wounds healed in this study and 72% of the patients showed good improvement in the healing process.[15] Other prospective descriptive study of 27 patients with partial thickness burn wounds showed that this modern antimicrobial wound dressing was a good alternative therapy option resulting in about 27% wounds appeared to have healed well and was associated with no subjective noticeable pain.[15]

Modern dressing showed better outcome compared with traditional dressing; a hydrogel dressing with gauze soaked in povidone-iodine solution in prospective study on 49 pressure ulcers. The percentage of healed wounds was 84% in hydrogel dressing group and 54% in gauze group, and the result was statistically significant ($p < 0.04$).[16] In a study which compared modern dressings and traditional dressing in a prospective study with 25 subjects showed modern dressing was as cost-effectiveness as traditional dressing with better satisfactory outcome in terms of wound healing and comfort.[17]

Modern antimicrobial wound dressing used in this patient showed a desireable result in healing process. After 4 days of using the modern antimicrobial wound dressing, the wound looked dry with base of granulation tissues. Modern antimicrobial wound dressing can be a good therapy option for surgical wound dehiscence after cesarean section.

Microbial culture for diagnosing surgical site infection remains controversial. Some of the reasons are because the deep surgical wounds, superficial sampling, particularly swabbing, can only detect superficial bacteria and not deeper tissues.[3] In this patient, microbial culture was done, and *Staphylococcus aureus* was found in the culture of the wound base.

The systemic antibiotic can be used for patients with systemic surgical site infection or erythema more than 5 cm from the incision along with induration or necrosis. The choice of antibiotic should be made based on antibiotic policy and microbial resistance patterns, location of the incisions, and the results of microbial analysis. For local management of infection, sutures and

clips remaining should be removed. The topical antimicrobial can be used for management of local infection and prevention of infection in surgical wound dehiscence patients who are at increased risk of infection. A wide range of topical antimicrobials is available for wounds, including silver, iodine, and polyhexamethylene biguanide (PHMB).[3]

Before the result of microbial culture was obtained, the patient was given ceftriaxone and metronidazole as the empiric antimicrobials. After the culture and resistance test was done, the patient was given ampicillin sulbactam in the ward. The wound was also dressed with the hydrogel impregnated with modern antimicrobial wound dressings and pad, which also acted as a topical antimicrobial.

CONCLUSION

Dressing regimens should be individualized to meet the needs of each wound. Modern antimicrobial wound dressing can be a good therapy option for surgical wound dehiscence after cesarean section. Modern antimicrobial wound dressings and pad, changed every 12 hours, can be used for caesarean section SWD. If the wound showed good healing process, dressing can be changed to modern antimicrobial wound dressings gel and pad and changed every 3 days.

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Literature Review

Role of Platelet-Rich Plasma Application on Mesh-Tissue Integration

Peran Aplikasi Platelet-Rich Plasma pada Integrasi Mesh dengan Jaringan

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Abstract

Objective: To review the advantage of PRP use on mesh-augmented surgery.

Methods: Literature review of PRP application of mesh.

Results: The application of PRP on mesh shows potential promising outcome.

Conclusion: PRP may improve the mesh-tissue integration.

Keywords: mesh-augmented surgery, pelvic organ prolapse, platelet-rich plasma, wound healing.

Abstrak

Tujuan: *Untuk menganalisa keuntungan penggunaan PRP pada pembedahan rekonstruktif dengan mesh.*

Metode: *Kajian pustaka dari penggunaan PRP pada pembedahan rekonstruktif dengan mesh.*

Hasil: *Aplikasi PRP pada mesh menunjukkan hasil yang positif.*

Kesimpulan: *Aplikasi PRP dapat meningkatkan integrasi mesh dengan jaringan*

Kata kunci: *pembedahan rekonstruksi dengan mesh, penyembuhan luka, platelet-rich plasma, prolaps organ panggul.*

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INTRODUCTION

The prevalence of POP globally ranges from 3% - 50%, with a higher prevalence among menopausal women. In Indonesia, based on research conducted at Dr. Cipto Mangunkusumo Hospital in 2016, the prevalence of POP was 26.4%.[1-3] Mesh-augmented surgery, previously known as a procedure, to treat hernia, has now been widely used to treat pelvic organ prolapse. It gives satisfactory results with an effectiveness and recurrence rate of 76–96% and 7.4% respectively compared to the short-term success rate of anterior colporrhaphy of 35-72% and recurrence of 30%.[8] Nevertheless, mesh-related complications negatively impact women's quality of life in the form of abnormal vaginal discharge, infection, chronic pain, and extrusion. Mesh extrusion is the most common complications reported (55%) and believed to occur due to the dysfunctional wound healing process and tissue integration into the mesh.[14] Therefore, the procedure has become a focus in pelvic organ prolapse studies, one of those is the application of PRP.

Reconstructive Surgery in Pelvic Organ Prolapse

Most patients with POP have no symptoms. However, as the protrusion of the organ into the vaginal introitus progresses, the symptoms will also become more disturbing. The symptoms vary from vaginal bulging, urinary and defection

problem, sexual disorder, infection, and uterine/vaginal ulcer.[4]

POP management usually requires reconstructive surgery to restore the pelvic organs' anatomical structures. Reconstructive surgery for POP started using the native-tissue-repair (NTR) technique, utilizing only pelvic organ support tissues. Mesh-augmented surgery then employed to treat POP which offers a more durable result. However, mesh augmentation is troubled by the high rate of complications due to the dysfunctional mesh-tissue integration process.[5,6]

The mesh-augmented repair technique was first used as a treatment for inguinal hernias. The meshes used to treat hernias are generally rigid because the implantation site is on the abdominal wall tissue. This is not the case in pelvic organ prolapse, in which the mesh is placed on a highly innervated and vascularized thin vaginal mucosa.[7] There is a higher possibility of frictions in vaginal meshes due to intestinal peristalsis and sexual activity. Moreover, during the treatment of POP, the anatomical appearance may not become the most crucial outcome but rather the restoration of normal urinary, defecatory, and sexual function are. With this in mind, improving the integration of mesh for POP is essential to avoid complications such as erosion or extrusion.[8]

In general, mesh products are divided into synthetic and biological mesh.[9,10] Biological mesh can be derived from human, bovine, or porcine tissue, which has been decellularized to release the collagen matrix.[9,10] This biologically derived material is also more prone to degradation and has a weaker structure, increasing the risk of recurrence.[12] Several types of biological mesh include allograft, xenograft, and autograft.[12,13] There are two synthetic mesh types used in urogynecology, namely absorbable and non-absorbable materials. The absorbable mesh was developed to reduce long-term complications and help postoperative fibroblast activity; however, this mesh showed weaker scar tissue formation, causing high recurrence rates. Non-absorbable mesh is reported to have suitable mechanical properties and long-term stability, making it easy to modify intraoperatively. Polypropylene (PP) mesh is a synthetic mesh commonly used in urogynecology to repair POP and stress urinary incontinence (SUI).[13] This mesh has varying flexibilities, diverse inflammatory responses, is easy to manipulate, and relatively inexpensive. In order to improve the quality of the mesh, polypropylene mesh has been currently designed with a lighter material that can increase the inflammatory response, reduce mesh contraction, and improve tissue integration.[14] This mesh appears to be more durable, non-toxic, and has low antigenicity.[14]

Wound Healing Process in Mesh Implantation

Wound healing is a physiological process involving three concurrent phases: hemostasis/inflammation, proliferation, and remodeling. The inflammatory phase begins with hemostasis and chemotaxis; proinflammatory cytokines will activate the tissue's neutrophils and other immune cells. The processes of angiogenesis, re-epithelialization, collagen formation, and wound contraction occur in the proliferative phase.[15-17]

Implantation of the mesh will trigger a foreign body reaction associated with the wound healing process. The reaction plays an essential role in the tissue-mesh integration, which is characterized by one of the three stereotypical reactions; tolerance, destruction, and rejection or removal of the implanted material.[11,16] Foreign body reaction is a complex defense mechanism that will trigger infiltration of various foreign-body giant cells, macrophages, fibroblasts, and angiogenesis in the mesh-implanted tissue.[17] The infiltration of fibroblast will cause new collagen deposition. Moreover, angiogenesis will allow tissue remodeling thus scar tissue will not replace the mesh. Subsequently, mesh-host tissue integration will take place along with the deposition of new collagen until resorption of the mesh begins. The integrated mesh will last for several years after implantation, whereas non-integrated mesh will be degraded within 2-3 months. However, exaggerated inflammation response can lead to excessive scarring, mesh encapsulation, and degradation.[10,12]

Dysfunctional tissue reaction is more profound in menopausal women due to the low estrogen level. Estrogen favors wound healing process and has a vital role in both stages of hemostasis/inflammatory, proliferation, and remodeling by reducing wound size, increasing collagen deposition by controlling the amount of collagen I and III in the remodeling phase, and strengthening the tissue. Estrogen could also increase angiogenesis and re-epithelialization-epithelialization by increasing the mitotic rate of epidermal cells. Low estrogen levels in animal models of rabbits in a study showed inhibition of

the wound healing process.[12,14]

This phenomenon was observed in experiments performing bilateral oophorectomy on menopausal rabbits. Their results showed that in menopausal tissue, the wound healing process occurred slower than that in premenopausal tissue. In addition to that, histological findings revealed more significant neovascularization, accumulation and maturation of granulation tissue, collagen deposition, and re-epithelialization processes during the first 35 days following the surgery. They also found an increased in acute and chronic inflammatory response. This phenomenon seemed to happen due to increased response of neutrophil elastase and fibronectin degradation, which resulted in delayed wound healing and scar contraction.[12,16]

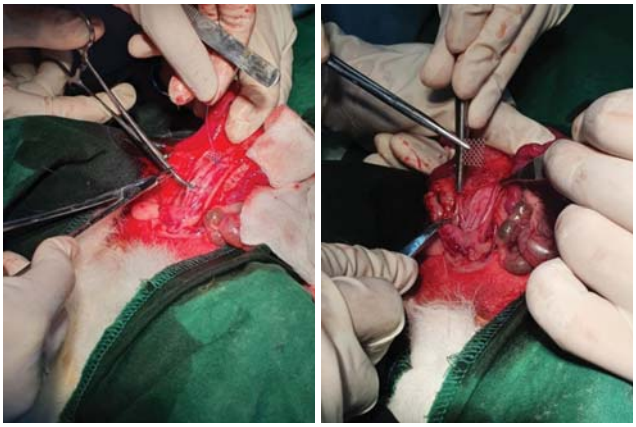

Figure 1. Mesh implantation in rabbits' vagina[25]

Many factors could affect the mesh-host tissue integration. These factors will work together during each phases to determine the degree of integration between the mesh and tissue. In previous studies, these factors were inflammatory response, fibroblast proliferation, angiogenesis, and infection. Moreover, the integration of implanted meshes and healing process depend on the intrinsic mesh characteristics such as the primary material, filament structure, and pore size.[17]

Various mesh materials have been used to repair disorders of the pelvic floor. Based previous studies, it can be concluded that polypropylene (PP) mesh has an appropriate biomechanical property and is adequate to repair pelvic floor abnormalities.[18] Polypropylene mesh is also the gold standard material for treating urogynecology cases. It poses a lower risk of inflammation than other materials.[12,17] Previous studies found that PP mesh could induce milder foreign body reactions than other synthetic materials, while polyester mesh showed a more significant foreign body reaction.[12,17]

Platelet-Rich Plasma Roles in Mesh-Host Tissue Integration

Platelet-Rich Plasma (PRP) is known to contain several growth factors such as Interleukin 6, Interleukin 8 (IL-6, IL-8), and vascular endothelial cell growth factor (VEGF), all of which could accelerate the wound healing process by lowering inflammatory response while also increasing angiogenesis and collagen deposition.[19] The exact mechanism of PRP's role in the wound healing process, especially in postmenopausal/ hypoestrogenic conditions, has not been fully understood yet.

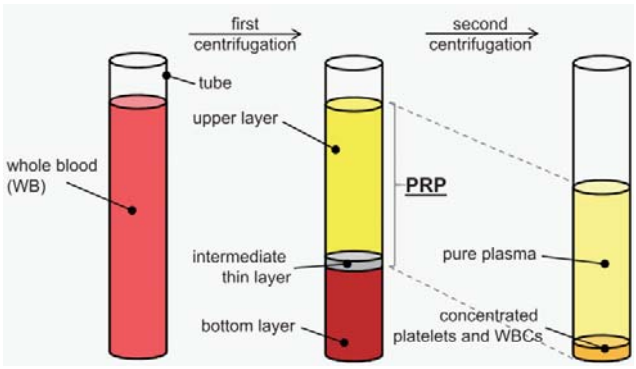

Figure 2. Platelet-rich plasma (PRP) preparation[20]

Studies on the effect of PRP on mesh-host tissue integration are scarce, and each has its limitations. Several studies showed a significant decrease in the expression of proinflammatory cytokines IL-17 and IL-1β with increased CD-31 expression and collagen deposition in the meshes implanted with PRP compared to those without PRP. The addition of PRP to the surface of the mesh implanted in adult rabbits decreased the number of inflammatory cells on the 30th-day post-implantation. However, this study was using non-hypoestrogenic adult rabbits, even though POP is more prevalent in menopausal women.[21] Another study showed that PP meshes coated with PRP had lower inflammatory infiltration within 30 days after mesh implantation than those without PRP. There was also an increase in collagen III deposition at 90 days after implantation.[22] Another in vitro study was conducted on 7 different types of mesh combined with PRP. The result showed that after 6 weeks of mesh implantation combined with PRP, reduced adhesion was observed in all meshes, and biocompatibility of

the mesh had improved. Moreover, mesh coated with PRP showed less severe adhesion, reduced hernia recurrence, increased angiogenesis, increased neovascularization, and improved mesh integrity.[23]

The results of a study conducted by Dimitri et al. on autologous plasma utilization in treating POP and SUI showed that this procedure was relatively safe and did not increase perioperative complications.[24] They also performed a separate analysis on the presence of growth factors in PRP and showed a significant increase in PDGF, VEGF, TGF-b1, and EGF compared to the concentrations in whole blood. Platelet-derived growth factors play an essential role in the early phase of wound healing. The application of PRP to damaged tissues plays a role in USL regeneration by modulating tissue healing. These studies have shown that PRP can improve tendon and ligament healing. Therefore, PRP application has a promising role to improve mesh-tissue integration and further research is needed.[25]

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