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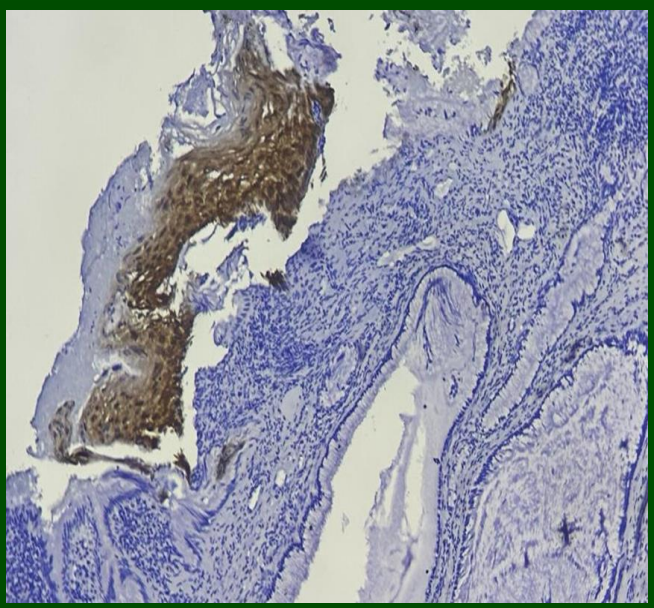

Result of immunohistochemistry of p16 reactivity, showing positive staining in which the expression is beyond of the 2/3 layer.

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CONTENT

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AUTHOR GUIDELINES

Majalah Obstetri & Ginekologi publishes original articles on all aspects of obstetrics and gynecology. Articles can be classified as **original research, case series, review article, systematic review,** and **meta-analysis** that keep the readers informed of current issues, innovative thinking in obstetrics and gynecology. We welcome submissions that contribute to the advancement of knowledge in obstetrics and gynecology. Articles are considered for publication with the condition that they have not been published, submitted, or being under consideration for publication elsewhere. Manuscript must be written in American English with proper grammar. Authors should follow the **Author Guidelines** and the manuscript is arranged according to the **Manuscript Template**. Manuscript must be submitted through online submission by registered users. Authors can register themselves in the journal system. For further question contact us at: mog@journal.unair.ac.id.

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Case series highlights important innovations with wide applicability or previously unpublished complications of new techniques or medications. The authors must confirm in the manuscript that they have obtained **written permission** of those whose case is being presented. The manuscript should be approximately 3500 words. Total number of tables and figures are limited, advisably not more than five, and references are minimally 20 from the last 10 years before the date of submission. The text consists of **Abstract, Introduction, Case Series, Discussion, Conclusion,** and **Disclosures**. The Disclosures consist of **Acknowledgment, Conflict of Interest, Patient Consent for Publication, Funding,** and **Authors Contribution**.

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More than three authors, list the first three authors, followed by et al.

Rose ME, Huerbin MB, Melick J, et al. Regulation of interstitial excitatory amino acid concentrations after cortical contusion injury. Brain Res. 2002;935(1-2):40-6.

2. Books

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Chapter of an edited book

Meltzer PS, Kallioniemi A, Trent JM. Chromosome alterations in human solid tumors. In: Vogelstein B, Kinzler KW, editors. The genetic basis of human cancer. New York: McGraw-Hill; 2002. p. 93-113.

Translated book

Luria AR. The mind of a mnemonist. Solotaroff L, translator. New York: Avon Books; 1969.

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Online proceeding

Muller S, editor. Proceedings of the 10th international conference on head-driven phrase structure grammar [Internet]; 2003 Jul 18-20; East Lansing (MI). Stanford (CA): CSLI Publications; 2003 [cited 2017 Nov 16]. Available from: http://web.stanford.edu/group/ csli publicationsSta/cslipublications/HPSG/2003/toc.shtml

Thesis/dissertation

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Online thesis/dissertation

Pahl KM. Preventing anxiety and promoting social and emotional strength in early childhood: an investigation of risk factors [dissertation on the Internet]. St Lucia, Qld: University of Queensland; 2009 [cited 2017 Nov 22]. Available from: https://espace.library.uq.edu.au/view/UQ:178027

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Diabetes Australia. Gestational diabetes [Internet]. Canberra (ACT): Diabetes Australia; 2015 [updated 2015; cited 2017 Nov 23]. Available from: https://www.diabetesaustralia.com.au/gestational-diabetes

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The family impact of Attention Deficit Hyperactivity Disorder (ADHD) [Internet]. 2009 Nov 1 [updated 2010 Jan 1; cited 2010 Apr 8]. Available from:http://www.virtualmedical centre.com.au/healthandlifestyle.asp?sid=192&title=The-Family-Impact-of-Attention-Deficit-Hyperactivity-Disorder-%28ADHD%29page=2.

CITATION WRITING

As the general rule, the reference numbers:

- should be placed outside full stops and commas
- the citation number can be place next to the author name where emphasis is place on the author eg. Smith[2]
- When multiple references are cited at a given place in the text, use a hyphen to join the first and last numbers that are inclusive. Use commas (without spaces) to separate non-inclusive numbers in a multiple citation e.g. ([2,3,4,5,7,10]) is abbreviated to ([2-5,7,10]).
- Do not use a hyphen if there are no citation numbers in between that support your statement e.g. ([1-2]). Use instead ([1,2])

For example:

Moir and Jessel maintain "that the sexes are interchangeable".[1]
Numerous studies[20-22] have.....
Smith's research .....[21]
Smith and Jones'[22] research .....

Up to 3 authors eg. Smith, Jones and McDonald reported that .....[23]

More than 3 authors eg. Smith et al.[24] reports.

ORIGINAL RESEARCH

Profile of postpartum patients with urinary retention at Koja Regional Hospital, Jakarta, Indonesia

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ABSTRACT

Objective: Postpartum urinary retention (PPUR) is a common voiding disorder, defined as the inability to void spontaneously within 6 hours after delivery with a residual bladder volume exceeding 200 mL. High rates of PPUR in Indonesia indicate a need for greater awareness and intervention. This study aims to assess the incidence and potential contributing factors of PPUR among postpartum patients at Koja Regional Hospital in Jakarta, Indonesia.

Materials and Methods: A descriptive case-control study was conducted, including women who experienced urinary retention following vaginal delivery at Koja Hospital between September and December 2022. Residual urine volume was measured by catheterization 6 hours after delivery. Data analysis, performed using SPSS version 22, included patient demographics and clinical factors such as maternal age, parity, gestational age, neonatal birth weight, and postvoid residual urine volume. These factors were analyzed to determine their association with PPUR.

Results: Out of 300 subjects selected through consecutive random sampling, 63.7% experienced PPUR, while 36.3% had normal urinary function. Patients with a mean age of 26.91 ± 5.02 years ($p = 0.000$), primiparous status (first-time mothers) ($p < 0.001$), and a mean neonatal birth weight of 2980.95 ± 450.52 grams ($p = 0.000$) showed a higher risk of developing PPUR compared to other postpartum patients.

Conclusion: The study indicates a significant association between postpartum urinary retention and maternal factors, including younger age, primiparity, and higher neonatal birth weight. Identifying these high-risk factors can enhance PPUR management, allowing healthcare providers to implement targeted monitoring and preventive measures, potentially improving postpartum outcomes in this patient population. This underscores the importance of monitoring these risk factors to better manage and potentially mitigate the incidence of PPUR.

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Highlights:

1. Maternal age and fetal birth weight are key risk factors for PPUR.
2. Among 300 subjects, 63.7% experienced PPUR, identified using the Suskhan score, with catheterization as a useful tool for prevention and management planning.

INTRODUCTION

Postpartum urinary retention (PPUR) is defined as an inability to spontaneously void 6 hours after delivery, with a residual urine volume exceeding 200 mL. In Indonesia, PPUR incidence stands at 14.8%, with approximately 666,000 women affected annually. However, no comprehensive studies have recently analyzed PPUR in patients post-forceps delivery in Indonesia, though forceps use raises PPUR incidence by 38%.[1] In a study by Suskhan (2015), 13.6% of 500 women developed urinary retention,[2] while in Manado (2019), 10.67% of 365 women post-vaginal delivery experienced PPUR, with 9.27% asymptomatic and 1.4% symptomatic.[3] A Chinese study indicated that 69% of gynecological surgeries caused urinary retention, with vaginal deliveries accounting for 18.6% of cases. Known risk factors for PPUR include physiological changes during pregnancy, regional anesthesia, assisted delivery, perineal trauma, nulliparity, and prolonged labor (over 12 hours). Overstretching of the bladder may impair detrusor muscle function, complicating PPUR management and early detection. Catheterization aids spontaneous voiding but increases urinary tract infection (UTI) risk. Cavkaytar et al. (2014) found that urinary retention is more prevalent in primigravida women than in multigravida women.[4] In primigravida cases involving forceps, the cervical gland's hyperplasia and enlargement increase the likelihood of uterine inversion and bladder compression, hindering urethral drainage.[5] Ultrasound findings by Yang and Huang revealed that cervical displacement led to bladder compression, causing acute urinary retention in some cases.[6] PPUR can lead to postpartum hemorrhage, UTIs, puerperal fatigue, and delayed lactation. PPUR types include symptomatic (overt) and asymptomatic (covert); covert PPUR requires ultrasound or catheter measurement of residual urine, while symptomatic PPUR involves bladder sensation, contractility, and innervation issues, presenting as incomplete urination, frequency, and abdominal fullness.[7-9]

According to Indonesia's Badan Pusat Statistik, 4.8 million deliveries occurred in 2020, with 14.8% experiencing urinary retention, affecting 666,000 women annually. A recent report by RSCM (2022) shows decreased PPUR cases, with 19 inpatient and 58 outpatient cases, attributed to an improved scoring system for PPUR prevention. PPUR treatment at RSCM typically lasts 2–11 days, underscoring the need for further research on the risk factors, diagnostic effectiveness, and treatment outcomes of PPUR. This study aims to advance knowledge on postpartum urinary retention, offering insights that can inform prevention, diagnosis, and management strategies. Understanding the specific characteristics of PPUR patients at Koja Regional Hospital could guide health policy development, ensuring that postpartum care effectively meets patients' needs.

MATERIALS AND METHODS

This research was an analytical case-control study designed to examine the characteristics of postpartum urinary retention (PPUR) in patients from September to December 2022 at Koja Regional General Hospital. The study included all women who underwent vaginal delivery and experienced PPUR, selected through consecutive random sampling. Patients who provided informed consent underwent post-delivery urine volume measurement 6 hours after vaginal birth, with a residual volume of ≥200 mL considered indicative of urinary retention. Data collected included parity, maternal age, gestational age, neonatal weight, and residual urine volume, analyzed using SPSS version 22.

Data normality was assessed using the Kolmogorov-Smirnov test. Continuous variables with normal distribution were reported as mean ± standard deviation, while non-normally distributed data were expressed as median (range). Categorical variables were presented as numbers and percentages. Chi-square tests were used for statistical comparisons, with a logistic regression model applied to evaluate PPUR risk factors. A p-value of < 0.05 was considered statistically significant. The study protocol was approved by the Ethics Committee of the Faculty of Medicine, University of Indonesia (protocol number: 23-11-1916), ensuring ethical standards were met throughout the research process.

RESULTS

During the 3-month recruitment period, 300 subjects were enrolled based on the inclusion criteria. Of these, 191 women (63.7%) experienced postpartum urinary retention, while 109 cases were considered normal, as indicated in Table 1. The demographic details of both cases and controls are presented in Table 2. The mean age was 26.91 ± 5.02 years for women with urinary retention and 30.73 ± 5.83 years for those without urinary retention. Additionally, the mean birth weight of newborns was found to be statistically significantly higher in women with postpartum urinary retention, measuring 2980.95 ± 450.52 g, compared to 2838.59 ± 441.88 g in women without urinary retention. Furthermore, first-time mothers were statistically significantly more prevalent in patients with postpartum urinary retention (62.8%) compared to those without (37.2%). The mean gestational age in women with postpartum urinary retention was 38.50 ± 1.46 weeks, and in women without postpartum urinary retention, it was 38.28 ± 1.86 weeks, with the difference not reaching statistical significance ($p = 0.507$).

Table 1. Incidence of PPUR at Koja General Hospital

Groups	Frequency	Cumulative Index (CI)
PUR	191 (63.7%)	100.0 %
Normal	109 (36.3%)	36.3 %
Total	300 (100%)	

Table 2. Characteristics of patients with or without PPUR

		PPUR (n:191)	Normal (n:109)	P value
Age (years)		26.91 ± 5.02	30.73 ± 5.83	0.000*
Gestational Age (weeks)		38.50 ± 1.46	38.28 ± 1.86	0.507
Birth weight (grams)		2980.95 ± 450.52	2838.59 ± 441.88	0.013*
Parity	Primipara	62.8%	36.7%	<0.001
	Multipara	37.2%	63.4%	

Table 3. Logistic regression analysis of risk factors for PPUR

	P value	OR	95% CI for OR
Age (years)	0.000*	0.862	0.823 – 0.906
Birth weight (grams)	0.000*	1.001	1.001
Parity	<0.001	2.915	1.790-4.478

The results of this study found that age (p = 0.000, OR 0.86, 95% CI 0.82-0.90),birth weight (p = 0.000, OR 1.00, 95% CI 1.00) and parity (< 0.001, OR 2.915, 95% C 1.790-4.478) were found to have an impact on the incidence of postpartum urinary retention (PPUR) (Table 3).

DISCUSSION

This study aimed to evaluate the demographic characteristics of patients at Koja Regional Hospital that play a role in the occurrence of PPUR. In this study, 191 cases (63.7%) of 300 subjects were reported to have urinary retention post vaginal delivery. Outcomes are concerning as it is influenced by various factors, inaccuracies, different diagnostic criteria, and treatment modalities. According to several studies, risk factors in post-partum urinary retention patients include macrosomia births, prolonged labor, assisted delivery, perineal lacerations, induction of labor, assisted delivery, and fundal pressure during contractions. Additional factors include parity, the time of delivery, the use of analgesia during labor, and the period between delivery and the first voiding. A systematic review written by Li et al. (2020), concluded that the etiology resulting in an increased incidence of PPUR was the patients undergoing their first delivery (primigravidae).[7] The exact etiology of these risk factors is unknown. An article written by Nandy et al. (2023) explained that pelvic floor muscle (detrusor muscle) instability and stress urinary incontinence can be long-term complications of post-partum urinary retention. Overactivity of the detrusor muscle can cause involuntary contractions of the smooth muscle during bladder filling. Poor detrusor compliance causes the failure of the bladder to stretch, thus increasing the pressure.[11]

Outcomes have shown a mean age of 26.91 ± 5.02 years in urinary retention, which is significantly different from the control group. In Indonesia, the productive age for pregnancy is between 20 to 40 years. Based on previous studies, this age characteristic corresponds to the highest mean age in post-partum urinary retention, which is approximately 26 to 27 years of age. In this study, age risk analysis resulted in p = 0.000, OR 0.86, 95% CI 0.82-0.90 depicting a significant influence of age and the risk of urinary retention after vaginal delivery.[4,12-14]

Women who have never given birth (nulliparous) have a higher likelihood (OR 2.915) of experiencing postpartum urine retention (PPUR) compared to women who have given birth (multiparous). The increased risk of PUR in nulliparous women may be due to damage to the pelvic nerve plexus during vaginal delivery, resulting from the sudden and pronounced changes in pelvic anatomy. This can further elevate the risk of PUR when combined with changes or injuries that occur due to performing an episiotomy for instrumental deliver.[21]

The study's findings showed a significant relationship between the occurrence of urine retention following vaginal delivery and the newborn's birth weight. Maternities with macrosomic fetuses run the risk of

having a labor that lasts longer than expected because of the pelvic floor muscles' constant contraction, which can cause the pudendal nerve to stretch. The pudendal nerve regulates micturition, according to a number of published studies.[4,16] Changes after vaginal birth include mucosal edema, damage to the pudendal and perineum's innervation during labor, and enlargement of the detrusor muscle.[17] Under acute situations, the compensation cycle—which is characterized by hypoxia and increased blood flow to the serous tissue—is triggered by an overly distended bladder.[18] After vaginal delivery, the bladder's inability to adjust to this situation causes hindered urination, and distended bladder distention causes irreparable harm. Thus, further distention and dysfunction over a prolonged voiding interval can be avoided with an early diagnosis of RUPP. Catheterization can be used to prevent urine retention in cases of prolonged labor.[19]

We found that fetal weight significantly influences the risk of RUPP (p = 0.000, OR 1.00, 95% CI 1.00). This aligns with the findings of Neron et al., who reported that 45% of patients with a birth weight ≥ 4000 grams experienced urinary retention after vaginal delivery compared to those with a birth weight under 4000 grams, with p = 0.230 OR 1.95 (95% CI 0.65-5.84).[16] A birth weight exceeding 4000 grams doubles the risk of urinary retention after vaginal delivery. In this study, the average fetal birth weight in the urinary retention group was 2980.95 ± 450.52 grams, statistically differing from the control group. The urinary retention group with a heavier fetal birth weight showed a statistically significant result compared to the normal group (p = 0.013).

CONCLUSION

The study reveals a high prevalence of urinary retention following vaginal childbirth, underscoring concerns about the various factors, diagnostic variability, and diverse treatment approaches that influence outcomes. Key risk factors such as maternal age, newborn birth weight, and parity significantly impact urinary retention incidence. Further research is essential to explore the underlying mechanisms of yet unidentified risk factors, facilitating the development of more precise and effective interventions.

DISCLOSURES

Acknowledgment

The authors would like to thank all the women who participated in this research study.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Informed Consent

The authors would like to thank all the women who participated in this research study.

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ORIGINAL RESEARCH

p16 expression in patient with Loop Electrosurgical Excision Procedure (LEEP)

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ABSTRACT

Objective: The study aim was to reveal p16 expression with the clinicopathological characteristics of patients who underwent cervical biopsy using the Loop electrosurgical procedure (LEEP) at Udayana University Hospital for the period 2020 – 2023.

Materials and Methods: The research was conducted at Anatomic Pathology Laboratory of Udayana University Hospital, Denpasar, Bali, Indonesia. The samples were selected based on inclusion criteria such as the formalin fixed paraffin embedded (FFPE) sample from positive IVA patient and continue for LEEP procedure. Otherwise, the exclusion criteria were moldy FFPE sample and incomplete clinical data. Then, p16 immunostaining procedure was carried out manually. The interpretation of p16 results was analyzed using SPSS software, version 25 by International Business Machines (IBM) Corporation.

Results: The positive p16 expression was revealed in 12 samples (38.7%), in contrast the negative staining appeared in 19 samples (61.3%). Unfortunately, p16 expression was not significant statistically based on age, parity, and contraceptive history, with p-values of 1.00, 0.45, and 0.65, respectively. Meanwhile, a statistically significant association was found between p16 expression and histopathologic diagnostic (p = 0.02, 95% CI 1.4 – 38.3). In addition, 22.2% of the variation of p16 expression based on multivariate analysis demonstrating a significant correlation (p = 0.01).

Conclusion: p16 expression with histopathology diagnostic characteristic in patient who underwent Loop Electrosurgical Excision Procedure (LEEP) was found statistically significant. Moreover, clinical application of p16 in daily practice should be performed with consideration especially for pre-cancer lesion in LEEP biopsy specimen procedure and clinicopathological approach is essential.

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Highlights:

1. Clinicopathologic characteristic of patient with LEEP procedures dominated by women above 35 years old, with total parity less than 3 and without history of contraceptive usage.
2. The p16 expression analyzed by immunohistochemistry technique. The positive and negative reactivity of p16 ratio was 3:5 on LEEP specimen which microscopically appear as chronic cervicitis, low-grade and high-grade squamous intraepithelial lesion.

INTRODUCTION

In 2020, based on number of new cancer cases both sexes and all age group in Indonesia, cervical cancer (9.2%) ranked third after other cancer type (51.4%) and breast cancer (16.6%). Unfortunately, the age standardized (world) incidence and mortality rate placed the cervical cancer as the second most common cancer that has mortality rate reaches almost a quarter compare to breast cancer.[1] Furthermore, study research that conducted in Bali in the periods of 2017-2019, cervical cancer ranked second based on cancer topography data from private and public hospital in Bali.[2] Meanwhile, the screening program especially for cervical cancer has been purposed to detect it in the early phase, especially squamous intraepithelial lesions (SIL), because the epidemiology of SIL is linked to that of HPV infections, with a peak incidence observed in young women and a declining rate in the ensuing decades.[3] Importantly, HPV DNA is detectable in as many as 80% of women in their early 20s but falls to about 5% among women in their sixth decade of life.[3-4]

In clinical practice, p16 as the one high risk HPV and prognostic marker in cervical cancer, has not been routinely used due to some reason. Firstly, p16 antibodies was not available in daily practice which make it unreliable especially for prognostic marker, in fact challenges in diagnosis of high- or low-grade intraepithelial lesion due to undetermined morphology can be distinguished by p16 expression and the established research of p16 as a specific marker of high-risk oncogenic HPV infection, although some there is challenges of its clinical used especially in daily practice of cervical cancer diagnosis.[5] In other hand, the application of p16 immunohistochemistry is recommended by the Lower Anogenital Squamous Terminology (LAST) standardization project in three specific contexts: to aid in the distinction of HSIL from mimickers of precancer (immature metaplasia, atrophy, reparative changes, or tangential sectioning), to supplement morphological assessment for biopsy specimens interpreted as ≤ LSIL that are at high risk for missed high-grade disease based on the prior Pap or HPV testing result, and to inform the diagnosis of HSIL (CIN 2) versus LSIL in morphologically equivocal cases – block-type staining supports HSIL (CIN 2).6-7 For this reason, this study proposed to reveal the p16 expression with clinicopathologic data of patient who are IVA test positive and underwent LEEP procedure.

MATERIALS AND METHODS

This research with ethical exemption number 1268/UN14.2.2.VII.14/LT/2023 was given by the research ethics committee Faculty of Medicine Udayana University on May 15th, 2023. The study conducted with analytical cross-sectional study design. Furthermore, sample was collected from January 2020 to July 2023, with predefined inclusion criteria like tissue samples are formalin fixed paraffin embedded (FFPE) from patient who are clinically IVA test positive and continued for Loop electrosurgical procedure (LEEP). In contrast, the exclusion criteria were moldy or deteriorated FFPE samples and incomplete clinical data. Moreover, clinical data of these patients were obtained from hospital medical records. Subsequently, the immunohistochemical staining of p16 was performed in eligible FFPE samples. The interpretation of p16 results is defined by positive and negative using scoring criteria.[8-9] The positive expression of P16 divides into three categories and mainly expressed in the nucleus and cytoplasm with brown color. The positive 1 (+) p16 expression is limited to epithelial basal layer; when the p16 expression between 1/3-2/3 layer of the cervix squamous epithelium it is marked as positive 2 (++); In addition, when the expression beyond 2/3 to whole layer of the cervix squamous epithelium then it is marked as positive 3 (+++). On the contrary, if there is no brown color in the cell, or patchy or uneven staining it is marked as negative (-).[4,10-11] Continuously, data were analyzed using statistical IBM software SPSS, version 25.

RESULTS AND DISCUSSION

Squamous intraepithelial lesions (SILs) of the uterine cervix, also known as cervical intraepithelial neoplasia (CIN), are proliferations of squamous cells driven by HPV infection, showing maturation abnormalities and/or viral cytopathic changes that do not extend beyond the basement membrane. They are divided into low-grade SILs (LSILs) and high-grade SILs (HSILs).[4,12] In this study, samples were obtained from patients who are positive with acetic acid application (IVA test) from screening procedure at primary health care and continued for loop electrosurgical excision procedure (LEEP). Most of them are female above 35 years old (71%) and more than half of the patient are private employee. Furthermore, clinically most of the patients did not suffer any signs and symptoms, however some of them suffer from vaginal discharge or vaginal spotting and lower abdominal pain (Table 1).

Theoretically, the epidemiology of SIL is linked to that of HPV infections, with a peak incidence observed in young women and a declining rate in the ensuing decades: HPV DNA is detectable in as many as 80% of women in their early 20s but falls to about 5% among women in their sixth decade of life.[4] In screened

population in high-income countries, LSIL has a cross-sectional prevalence of 5–10% and the prevalence of HSIL is 0.5–1%.[13,14] HSIL typically occurs at an older age than LSIL, although there is broad overlap and HSIL has been demonstrated within a year or two of HPV infection in adolescents. Importantly, the rate of HSIL regression is higher in adolescents and young women than in older populations.[4,12] In this study, based on microscopic findings with routine hematoxylin eosin staining, the histopathological diagnosis of HSIL only 3% compared to LSIL 35% and chronic cervicitis (62%) are become most common findings.

Subsequently, the monoclonal antibody p16-INK4 clone MX007 was used in this study. As one of the cyclin dependent kinase inhibitors that inhibit cyclin-dependent kinases 4 and 6, p16INK4A is encoded by tumour suppressor gene CDKN2A.[15] The tumour suppressor p16 INK4A plays an important role in cell cycle regulation.[16-17] Beside in cervical cancer, p16 implicated in several cancer such as breast cancer which specifically increased in hormonal estrogen receptor-positive tumor tissues ($p < 0.01$).[14] In contrast, there is no significant correlation was found between the p16 protein expression and the other clinicopathological features. In our study, p16 reactivity through immunohistochemistry procedure was tested on all samples of IVA test positive patient who underwent the LEEP procedure. Totally, negative staining of p16 was 61.3% and only 38.7% were positive. The rise of p16 expression is seen as the organism ages and reduces proliferation of stem cell. In addition, this depletion in the division and production of stem cell protects against cancer while increasing the risk associated with cellular senescence.[18-19]

Unfortunately, mutation in the p16 gene associated with loss or over expression of the protein are associated with increased risk of wide range of cancer and cancer precursor lesion. The immunohistochemical identification of p16 reactivity is shown in Figure 1. Moreover, p16 expression was grouped into two categories positive and negative staining. Positive staining characterized by brown staining of the cell nuclei and cytoplasm of squamous epithelial cells limited in epithelial basal layer (+) or between 1/3-2/3 squamous epithelial layer (++) or beyond 2/3 of the basal cell layer or throughout the thickness of the metaplastic squamous epithelium (+++) (A and B). Meanwhile, negative staining is characterized by negative staining or a patchy appearance or uneven brownish colour on the cervical transitional zone (C and D).

Table 1. Clinicopathological characteristic of patients with LEEP Procedure

Parameters		Frequency	Percentage
Age	≤ 35 y.o	9	29%
	> 35 y.o	22	71%
Education level	Elementary school	4	13%
	Senior high school	17	55%
	Diploma	4	13%
	Bachelor degree	6	19%
Occupation	Health care worker	2	6%
	Private employee	21	68%
	Self-employed	1	3%
	Housewife	7	23%
Marrital status	Married	28	90%
	Single	3	10%
Parity	≥ 3	20	65%
	< 3	11	35%
Clinical symptom	No symptom	12	39%
	Vaginal spot	8	26%
	Vaginal discharge	8	26%
	Lower abdominal pain	3	9%
Contraception history	Yes	6	19%
	None	25	81%
Histopathological diagnosis	Chronic cervicitis	19	62%
	Low grade squamous intraepithelial lesion (L-SIL)	11	35%
	High grade squamous intraepithelial lesion (H-SIL)	1	3%

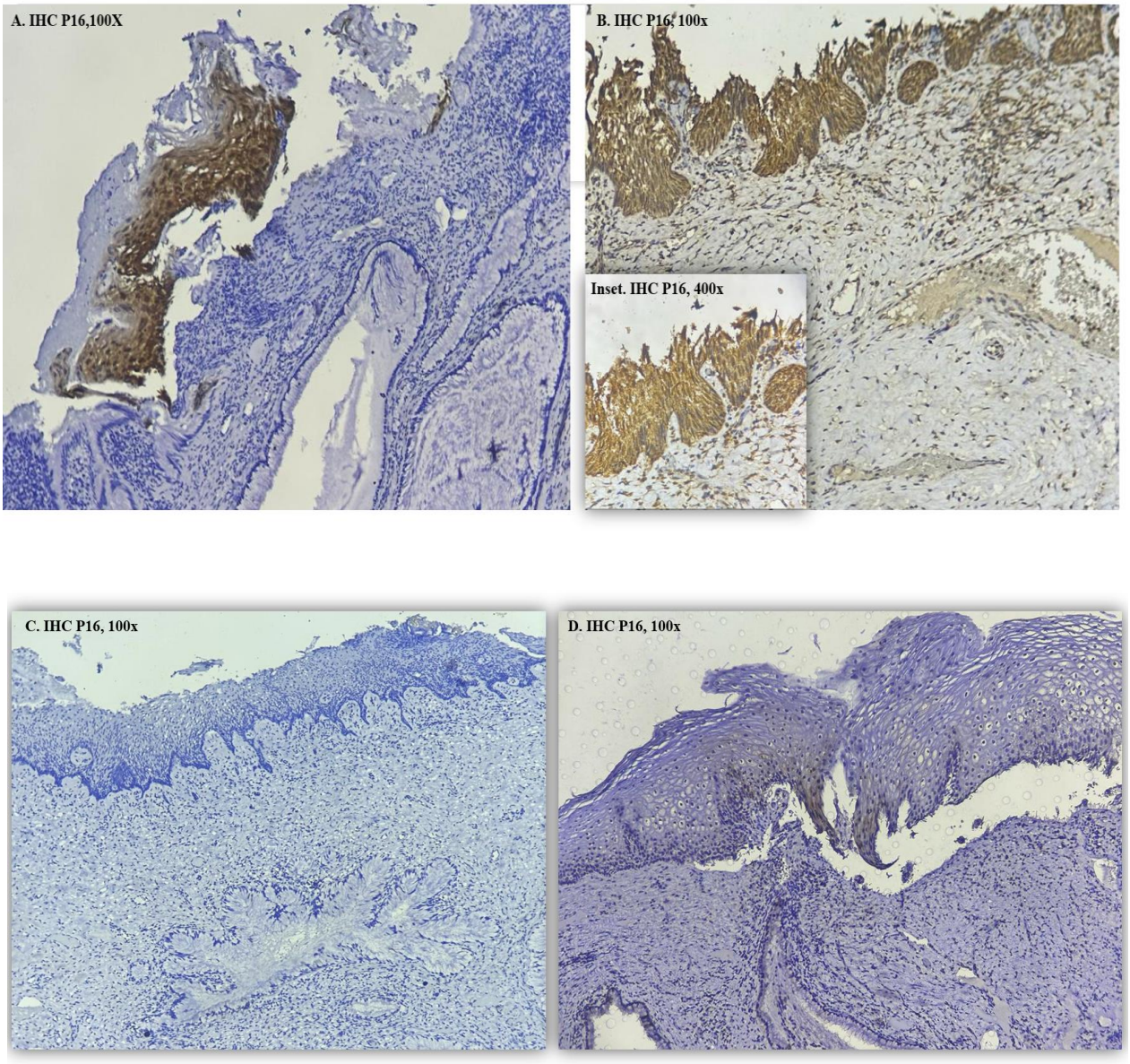

Figure 1. Immunohistochemistry of p16 reactivity result

A-B. Positive staining, the expression beyond 2/3 layer (A) and extends to whole layer (B) of the cervix squamous epithelium (IHC p16, 100x). C-D. Negative staining, there is no brown color in squamous epithelial layer (C), or patchy or uneven staining (D) (IHC p16,100x)

Table 2. Chi-square analysis of p16 expression with clinicopathological characteristic

Parameters	p16 expression		Total	Risk Estimate (CI 95%)	p*	R
	Positive	Negative				
Age						
≤ 35 y.o	4 (25%)	5 (31.6%)	9	0.3-7.0	1.00	0.01
> 35 y.o	14 (68.4%)	8 (75%)	22			
Total parity						
≤ 3	8 (58.3%)	13 (73.7%)	21	0.4-9.3	0.45	
> 3	4 (41.7%)	6 (26.3%)	10			
Contraception history						
Yes	10 (75%)	15 (84.2%)	25	0.3-10.7	0.65	
No	2 (60%)	4 (40%)	6			
Histopathological diagnostic						
Chronic cervicitis	4 (21%)	15 (79%)	19	1.4-38.3	0.02*	
Squamous intraepithelial lesion	8 (67%)	4 (33%)	12			

*Statistically significant if $p < 0.05$

Furthermore, to determine the proportion of p16 expression with clinicopathological parameters, the comparative test Chi-square analysis was performed and presented in Table 2. Based on the result, a statistically significant difference with p value <0.05 can be seen between the p16 expression variable and histo-pathological diagnosis. Unfortunately, the results of other clinicopathological characteristics such as age, number of parities and contraception history were not found to be statistically significant.

Based on the result, p16 expression in this study showed negative staining due to reparative changes of cells which mostly found in chronic cervicitis cases compare to squamous intraepithelial lesion with ratio 5:3. The diagnostic of HSIL in this study only found in 1 LEEP sample, so that the comparative analysis of LSIL and HSIL become one category compared to chronic cervicitis. Some studies have emphasized the significance of using p16 immunostaining as marker for identifying dysplastic and neoplastic lesion caused by high-risk HPV.[13-15] The staining intensity of p16 varies based on amount of dysplasia cells. As the grade of dysplasia increased, the p16 expression increased as well. However, it should be emphasized that other reason for negative expression of p16 in squamous intraepithelial lesion especially LSIL due to a certain percentage in thought to be caused by low-risk HPV types.[19,20] Previous study indicated that viral oncoprotein of low risk HPV such as HPV-6 does not affect p16 because the affinity of HPV-6 E7 protein for cellular pRb is ten-fold lower than that of HPV-16 E7 for pRb.[20]

The main strength of our study lies in the inclusion of a sample of IVA-positive patients undergoing the LEEP procedure, where formalin-fixed, paraffin-embedded biopsy samples were evaluated for p16 reactivity. Additionally, this study is noteworthy as it is the first to analyze p16 in samples from the LEEP procedure, especially in Bali, providing foundational data for future research. However, we acknowledge that invasive carcinoma cases were not included, and the histopathological diagnoses from the LEEP procedure comprised chronic cervicitis, low-grade squamous intraepithelial lesion (L-SIL), and high-grade squamous intraepithelial lesion (H-SIL). Therefore, specimens from other procedures, such as conization and hysterectomy, should be considered. Challenges have also arisen due to LEEP crush artifacts, requiring careful evaluation. Another limitation is the small diagnostic sample size, as no positive cervical cancer cases were identified through the LEEP procedure. Despite these limitations, we believe our findings provide valuable baseline data for future studies and recommendations.

CONCLUSION

The study found that p16 expression, analyzed in relation to the histopathological diagnosis of patients who underwent cervical biopsy using the LEEP procedure, was statistically significant. Our findings contribute to knowledge by highlighting that p16 expression is most frequently observed in cases of chronic cervicitis, likely due to reparative processes, and that the intensity of p16 reactivity varies with the degree of dysplasia. As dysplasia grade increases, so does p16 expression. However, it is important to note that p16 negativity in squamous intraepithelial lesions, particularly LSIL, may be attributed to the presence of

low-risk HPV types. These findings suggest that using p16 as a clinical marker in LEEP biopsy specimens, particularly for pre-cancerous lesions, requires careful consideration, and a combined clinicopathological approach is essential in routine practice.

DISCLOSURES

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Conflict of interest

The authors declare no conflict of interest.

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Author contribution

All authors have contributed to all processes in this research, including preparation, data gathering and analysis, drafting and approval for publication of this manuscript.

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ORIGINAL RESEARCH

Nutritional status, hemoglobin, and albumin levels in predicting platinum resistance in ovarian cancer at Dr. Saiful Anwar Hospital, Malang, Indonesia

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ABSTRACT

Objective: This study aimed to determine whether nutritional status, hemoglobin, and albumin levels could serve as reliable predictors for predicting platinum resistance in patients with ovarian cancer.

Materials and Methods: Conducted as a cross-sectional analysis, this study included 80 ovarian cancer patients who had completed six cycles of platinum-based chemotherapy. Patients were divided into two categories: those with platinum-resistant cancer and those with platinum-sensitive cancer, based on recurrence status following chemotherapy. Nutritional status was assessed through body mass index (BMI), and both hemoglobin and albumin levels were measured pre- and post-chemotherapy to investigate potential differences between the groups.

Results: The analysis revealed no significant difference in BMI between the platinum-sensitive and platinum-resistant groups ($p = 0.743$), suggesting that nutritional status, as measured by BMI, did not correlate with platinum resistance. Hemoglobin levels were similarly non-significant before ($p = 0.072$) and after chemotherapy ($p = 0.055$), indicating no clear association between hemoglobin levels and platinum response. However, hemoglobin levels showed significant increases post-chemotherapy in both the platinum-sensitive ($p = 0.002$) and platinum-resistant ($p = 0.025$) groups, though without affecting resistance outcomes. Pre-chemotherapy albumin levels did not significantly differ between the two groups ($p = 0.218$); but a significant post-chemotherapy difference was observed ($p = 0.027$), with both groups experiencing substantial increases from pre- to post-chemotherapy ($p = 0.000$).

Conclusion: The findings suggest that BMI, hemoglobin, and albumin levels are not reliable predictors of platinum resistance in ovarian cancer patients. Although both hemoglobin and albumin increased significantly after chemotherapy, these changes did not correspond with platinum resistance status.

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Highlights:

1. Nutrition status, hemoglobin, and albumin levels are not predictors for platinum resistance.
2. Hemoglobin and albumin levels increased in both platinum-sensitive or platinum-resistant.

INTRODUCTION

Ovarian cancer is the third malignancy among all types of gynecological cancer after cervical and uterine cancer.[1] Despite its low prevalence, ovarian cancer has a poor prognosis and the second-highest mortality rate among cervical and uterine cancers.[2] Ovarian cancer is more prevalent among Caucasian women, with the median age of diagnosis being 63 years old in the United States.[3] At Saiful Anwar Hospital in the 2017-2019 period, ovarian cancer cases were 37.8% of all gynecological cancer.[4] Ovarian cancer has a lower survival rate than other cancers. It is because this cancer is usually detected at an advanced stage. However, this cancer can have a survival rate of up to 90% if found early and treated immediately.[5] Most types of ovarian cancer are epithelial types (90%) which originate from invagination of the ovarian surface epithelium.[6] Due to its complex varying degrees of heterogeneity, makes ovarian cancer difficult to detect and treat.[7]

The management of ovarian cancer involves performing surgery the first step and followed by systemic chemotherapy, particularly in advanced stages. Patients who exhibit platinum resistance at the beginning of treatment have a lower chance of survival. In addition, a patient's nutritional status can also affect the development of metastases and overall cancer prognosis.[5] However in previous study found that advanced stage, lack of lymphadenectomy, positive lymph nodes and history of breast cancer were increased the risk of platinum-based drug resistance which leading to the poor prognosis.[8]

Body mass index (BMI) and serum albumin levels are used to evaluate the nutritional status of cancer patients. Hypoalbuminemia is caused by malnutrition and cachexia in cancer patients due to the body's response to the tumor and anti-tumor therapy.[9] Platinum chemotherapy binds to protein in plasma which influence their pharmacokinetics significantly.[10] Regarding its effect on resistance to platinum-based chemotherapy, albumin in serum is important to be examined. In addition to the adverse effects on quality of life, the presence of anemia itself is associated with shorter survival times for some types of cancer. Hypoxia may contribute to the malignant behavior of the disease by providing selection pressure for tumor cells with higher mutation rates, ultimately resulting in increased growth of cells, decreased cell response to apoptotic signals, and therapy resistance.[11] Then, this study aims to assess whether nutritional status, hemoglobin levels, and albumin levels can be used as predictors of platinum resistance among ovarian cancer patients after 6 series of chemotherapy at Dr. Saiful Anwar Hospital, Malang, Indonesia.

MATERIALS AND METHODS

This was a cross-sectional study, conducted by collecting data from the medical record of the patients. This study was approved by The Ethics of General Hospital Dr. Saiful Anwar with number 400/228/K.3/102.7/2022. The population in this study included all ovarian cancer patients who have undergone surgery in Dr. Saiful Anwar Hospital, Malang, from July - December 2022.

This study used consecutive sampling and with the inclusion and exclusion criteria. The inclusion criteria were: 1) patients with ovarian carcinoma who have been diagnosed by a clinician in the Oncology of Obstetrics and Gynecology Department who have undergone platinum-based chemotherapy 6 series, 2) patient underwent a laparotomy to confirm the results of the clinical examination, 3) patients who have albumin and hemoglobin examined before and after chemotherapy at the Clinical Pathology Laboratory of Dr. Saiful Anwar Hospital, Malang. The exclusion criteria were: 1) patients with adnexal masses which do not originate from the ovaries, 2) patients suffering from other malignancies, 3) patients with infected adnexal masses.

Patient's data were collected at the Oncology of Obstetrics and Gynecology Department, Dr. Saiful Anwar Hospital, Malang. The data collected included age, type of chemotherapy, BMI, albumin, and hemoglobin levels of patients before and after chemotherapy. The BMI data itself will later be classified into underweight (BMI <17 kg/m^2), normal (BMI 17-23 kg/m2), overweight (BMI 23-27 kg/m^2), and obesity (BMI >27 kg/m^2).[12] Platinum-resistant is defined as the recurrence of cancer under 6 months after the last chemotherapy session.[13] Recurrence in this study means the reappearance of the tumor in the ovary with an increase in Ca125 values and/or the detection of new metastases. Then, the collected data was analyzed using SPSS Software 20 with the Mann-Whitney U test for numerical data and chi-square for categorical data. Results are considered significant if the $p < 0.05$.

RESULTS AND DISCUSSION

Participants divided into two groups, each consisting of 40 participants which then characterized by the age, types of chemotherapy, and BMI (Table 1). Participants in the platinum-resistant group had the mean age of 52.55 ± 11.15 years while in platinum-sensitive had the mean age was 50.75 ± 11.14 years. There was no significant difference between groups regarding age (p = 0.785). Most of the participants were given Carboplatin Docetaxel which 55% in platinum-resistant and 60% in platinum-sensitive. There was no significant difference between groups regarding types of chemotherapy (p = 0.899). BMI showed that most of participants were in normoweight which 60% in platinum-resistant and 55% in platinum-sensitive. There was no significant difference between groups regarding BMI (p = 0.557).

Ovarian cancer typically manifests during the peri-menopausal or post-menopausal period, with 80-90% of cases occurring in individuals above the age of 40, peaking at 60 years old.[14] The disease's 5-year survival rate is low (48%) due to 70% of cases being diagnosed at stages III and IV. This worse prognosis is related to the metastatic process.[15] Beyond the worst prognosis, advanced stages also contribute to a higher recurrence rate within 5 years, with 70% of cases experiencing recurrence.[16] The nutritional status of cancer patients has a significant impact on their quality of life. Research shows that weight loss and decreased appetite are closely linked to decreased quality of life.[17] In addition, studies have found that being overweight or obese increases a person's risk of developing ovarian cancer and experiencing severe symptoms of the disease.[18]

In this study, platinum-resistant patients had mean of BMI is 21.63 ± 3.90 and 21.94 ± 3.83 in platinum-sensitive patients with p is 0.743. Based on the results obtained, there seems to be no significant difference between the BMI and the incidence of platinum resistant among ovarium cancer patients who underwent six rounds of chemotherapy. This finding contradicts previous research that indicated that that obesity can play a role in tumor progression and resistant. Obesity can negatively impact the survival rates of ovarian cancer patients. This effect may be due to changes in the M1/M2 tumor-associated macrophage ratio as well as increased fibrosis, which can reduce chemosensitivity.[19] It is possible that this study comprised a large population of individuals with a normal BMI rather than being overweight or obese, which could account for the contrasting results observed in other studies that suggested obesity could have an impact on pharmacokinetics, metabolic dysregulation, the induction of platinum resistance, or the decision to reduce therapeutic doses to minimize toxicity.

Hemoglobin is a commonly measured parameter in cancer patients, as it can be affected by the cancer itself, its treatment, and patient-related factors.[20] In platinum-resistant (Table 2), shows the measurement of hemoglobin levels before chemotherapy is 10.58 ± 1.72, then changed significantly (p = 0.025) to 11.01 ± 0.94. A significant increase (p =0.002) also occurred in platinum-sensitive patients, with initial hemoglobin from 9.92 ± 1.49 to 10.56 ± 1.16. When comparing hemoglobin levels before and after chemotherapy, there was no significant difference in hemoglobin in the two groups in pre-chemotherapy (p = 0.072) and post-chemotherapy (p = 0.055).

Table 1. Characteristics of study population.

Characteristics	Platinum-resistant (n=40)	Platinum-sensitive (n=40)	p-values
Age (mean ± SD)	52.55 ± 11.15	50.75 ± 11.14	0.785
Types of chemotherapy			
• Carboplatin Brexel	17 (42.5%)	15 (37.5%)	
• Carboplatin Docetaxel	22 (55%)	24 (60%)	0.899
• Carboplatin Paclitaxel	1 (2.5%)	1 (2.5%)	
BMI (mean ± SD)	21.63 ± 3.90	21.94 ± 3.83	
• Underweight	9 (22.5%)	9 (22.5%)	
• Normoweight	24 (60%)	22 (55%)	0.557
• Overweight	6 (15%)	9 (22.5%)	
• Obesity	1 (2.5%)	0 (0%)	

BMI: body mass index

Table 2. Changes in hemoglobin levels

Hemoglobin	Pre-Chemotherapy	Post-Chemotherapy	p-values
Platinum-sensitive	9.92±1.49	10.56±1.16	0.002*
Platinum-resistant	10,58±1.72	11.01±0.94	0.025*
p-values	0.072	0.055	

*p<0.05

Table 3. Changes in albumin levels

Albumin	Pre-Chemotherapy	Post-Chemotherapy	p-values
Platinum-sensitive	3.22± 0.68	3.40± 0.60	0.000*
Platinum-resistant	3.41± 0.67	3.69± 0.54	0.000*
p-values	0.218	0.027	

*p<0.05

This study implies that platinum resistance does not influence hemoglobin levels, and similarly, changes in hemoglobin do not affect platinum resistance. Interestingly, the hemoglobin levels increased significantly in both groups after chemotherapy, which contradicts the conventional theory that states that hemoglobin levels tend to decrease after chemotherapy.[20] However, due to the lack of documentation of oral or transfusion therapy prior to chemotherapy, this could have biased the results. Additionally, low hemoglobin levels can reduce oxygen supply and cause hypoxia, which is linked to resistant to chemotherapy and radiation, increased tumor growth, tissue invasion, metastasis, and poor prognosis.[21]

Albumin plays multiple roles in the body, including functioning as an antioxidant and trapping free radicals.[22] It also serves as an indicator of nutritional status and can help in evaluating the body's response to inflammation. In cases where a tumor leads to hypoalbuminemia, it can reduce the effectiveness of therapy and increase the risk of mortality.[23] As it is known that albumin is a protein in plasma which then bind with platinum chemotherapy and significantly influence their pharmacokinetics, particularly in terms of factors like renal excretion rate. In general, when the leaving group of platinum complexes is not readily replaced by a ligand, their protein-binding ratio tends to be lower, leading to a longer half-life and a higher rate of renal excretion.[10]

In this study, albumin levels before chemotherapy in platinum-resistant patients (Table 3) increased significantly before chemotherapy 3.41 ± 0.67 to 3.69 ± 0.54 with p = 0.000. It also the same in platinum-sensitive patients, which increase from 3.22 ± 0.68 to 3.40 ± 0.60 with p 0.000. For albumin levels before chemotherapy, the p-value = 0.218 which means there was no significant difference in albumin before chemotherapy. However, after chemotherapy, the p-value = 0.027 which showed a significant difference between the two groups where higher in platinum resistance.

Albumin serves as a regulator in acid-base physiology, facilitating the binding and transportation of crucial components in the bloodstream such as hormones, fatty acids, and bilirubin to organs. Additionally, it inhibits platelet function, controls vascular permeability, and helps maintain colloid-osmotic pressure in the body.[24] In chemotherapy, low albumin levels are associated with chemotherapy-induced toxicity.[25] Other study stated that albumin-bound paclitaxel (ABP) blends the hydrophobic paclitaxel with a carrier of human serum albumin. This combination enhances the efficient transportation of paclitaxel to tumor tissues through endocytosis. Additionally, utilizing albumin as the carrier for paclitaxel enables the attainment of higher drug concentrations, increasing the efficacy.[26] This study experienced the opposite, where there was a significant increase in albumin in the platinum-resistant and platinum-sensitive groups. However, it is necessary to pay attention to the history of albumin administration, diet, insulin levels, and history of use of drugs such as corticosteroids, which can influence albumin levels which could have biased the results. This result might be showed that the decrease of albumin was not correlated with the protein-binding but could be the result of the cancer itself.

Limitations of this study lie in its small sample size, caused by incomplete data Furthermore, this study did not record a history of transfusions, blood-boosting drugs, administration of extra albumin, or other therapies that could potentially be biased. It was because there were several incomplete pieces of data and documentation in the patients' record. Additionally, this study did not reassess the patient's body mass index after undergoing chemotherapy overlooking potential clinical changes.

CONCLUSION

This study confirms that nutritional status does not play a decisive role in platinum resistance. Additionally, hemoglobin and albumin levels do not directly influence platinum resistance, while in platinum-resistant have been observed to lead to an increase in albumin levels. This research suggests that nutritional status, hemoglobin and albumin were not predictors of platinum resistance, but in patients with good nutritional status, based on their BMI may lead to an increase in hemoglobin and albumin levels. Nevertheless, additional research is necessary to identify other factors that could be contributing to platinum resistance.

DISCLOSURES

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Conflict of interest

The authors declare there is no conflict of interest.

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Author contribution

All authors have contributed to all processes in this research, including preparation, data gathering and analysis, drafting and approval for publication of this manuscript.

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ORIGINAL RESEARCH

Effects of autologous platelet-rich plasma in promoting endometrial thickness on patients with thin endometrium following IVF

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ABSTRACT

Objective: The objective of this research was to investigate the impact of autologous platelet-rich plasma (PRP) in enhancing endometrial thickness among individuals experiencing infertility associated with a thin endometrium.

Materials and Methods: Nine individuals with a thin endometrium who participated in an in vitro fertilization (IVF) program were enrolled in the study. This study occurred in Yasmin Clinic, Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia. Patients underwent a hormone replacement protocol involving the preparation of the endometrium with estradiol valerate. Treatment with PRP was initiated when the endometrial thickness was less than 7 mm. Autologous PRP was infused into the uterine cavity between the 10th and 12th days after administering estradiol valerate, and the assessment of endometrial thickness was conducted using ultrasound 48 hours later. A second administration of PRP was provided in cases where the endometrial thickness was below 7 mm. Frozen-thawed embryo transfer (FET) will be performed if the endometrium reaches adequate thickness (minimum 7 mm).

Results: Seven of nine patients had adequate endometrial thickness followed by FET. Endometrial thickness was improved in 8 from 9 patients (88.8%). Five patients were improved at the first autologous PRP infusion (62.5%) and three patients (37.5%) at the second PRP infusion. The implantation rate was 33.3-100%, clinical pregnancy was 100%, and ongoing pregnancy rate was 83.3%.

Conclusion: The use of autologous platelet-rich plasma (PRP) successfully stimulates endometrial development in individuals with a thin endometrium during frozen-thawed embryo transfer.

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Highlights:

1. It was observed that autologous PRP substantially increased endometrial thickness in individuals experiencing infertility linked to a thin endometrium.
2. The elevated clinical pregnancy rate emphasizes the favorable effectiveness of autologous PRP in addressing issues related to a thin endometrium in IVF programs.

INTRODUCTION

A reduced endometrial thickness, measuring below 7 mm on the day of embryo transfer, is proposed as a factor contributing to diminished pregnancy and live birth rates among patients undergoing IVF. Eftekhar et al.[1] showed that IVF patients with endometrial thickness between 8 to 11 mm on the day of embryo transfer had an optimal pregnancy rate; however, if the endometrial thickness was greater than 14 mm, no pregnancy was achieved.[1,2] The process of angiogenesis is crucial for the development of the endometrium and the proper functioning of the female reproductive system, including folliculogenesis, corpus luteum formation, differentiation, and implantation, as well as in the maintenance of pregnancy.[3] Studies and literatures have demonstrated that VEGF, bFGF, and EGF are several growth factors that play important roles in angiogenesis during follicular development. VEGF is considered the most important growth factor in regulating angiogenesis in the endometrium.[4-6]

VEGF serves as a potent mitogen for endothelial cells and induces vascular permeability. Its expression within the human endometrium plays a regulatory role in endometrial vascularization. It is thought that the thin endometrium is caused by disturbed angiogenesis and reduced blood flow in the endometrium. A thin endometrium is identified by elevated impedance of blood flow in the radial uterine artery, limited epithelial growth, reduced expression of VEGF, and inadequate vascular development.[4,7]

Many studies have attempted to increase endometrial thickness using various therapies, such as pentoxifylline, low-dose aspirin, intrauterine G-CSF infusion, and vaginal sildenafil. However, the results are still inconclusive regarding increasing endometrial thickness or increasing pregnancy rates in patients with thin endometrium.[8-10] Platelet-rich plasma (PRP) refers to the plasma derived from fresh whole blood obtained from peripheral veins, which undergoes centrifugation to concentrate platelets. Upon activation, PRP contains various growth factors, including vascular endothelial growth factor (VEGF), transformation growth factor (TGF), epidermal growth factor (EGF), and platelet-derived growth factor (PDGF). These growth factors are activated after clotting and transformed into the bioactive forms. Because it contains many growth factors, PRP has been widely used in efforts to regenerate tissues, including the endometrium. PRP can be injected directly to the endometrium and specifically activate the cells within it.[1,11]

Research has demonstrated that PRP can enhance cell migration, adhesion, proliferation, and differentiation while facilitating the accumulation of the extracellular matrix. PRP has found extensive applications across diverse medical disciplines, including dermatology, orthopedics, ophthalmology, and surgery. Nevertheless, in the field of gynecology, there has been a scarcity of studies, particularly in exploring the use of PRP to enhance endometrial thickness in individuals facing infertility associated with a thin endometrium.[11,12] The objective of this study is to explore the impact of autologous PRP on the augmentation of endometrial thickness in individuals with thin endometrium undergoing an IVF program at our institution.

MATERIALS AND METHODS

Nine patients with inadequate endometrial thickness (<7 mm) were recruited on the day of embryo transfer. This study was performed at the Yasmin Clinic IVF Center, Dr. Ciptomangunkusumo Kencana Hospital, Jakarta, from February 2018 to September 2019. The study was approved by the Research Ethics Committee of Universitas Indonesia (Reference number: 850/UN2.FI/ETIK/2017). The research adhered to the ethical guidelines outlined in the 2008 revised Declaration of Helsinki. Patients meeting the inclusion criteria provided their informed consent before participating in the project. The data retrieved are collected and analyzed using SPSS ver 27.

A hysteroscopic examination was performed for all the patients to exclude any pathology that might cause thin endometrium, such as intrauterine adhesions or endometritis. Patients with hormonal abnormalities such as hyperprolactinemia and abnormal thyroid hormone levels were also excluded. Patients were evaluated on day 2 or 3 of menstruation via transvaginal ultrasound examination, and an artificial hormone replacement protocol was used before the frozen-thawed embryo transfer (FET) to prepare the endometrium. Estradiol valerate (6 mg/day) was given initially and was increased every three days until the maximum dose was reached (12 mg/day) on days 10th-12th. Endometrial thickness was assessed through transvaginal ultrasound, and intrauterine autologous PRP infusion was administered the next day if the endometrial thickness was below 7 mm. A follow-up transvaginal ultrasound was conducted 48 hours after the PRP infusion to assess post-treatment endometrial thickness.

The second autologous PRP infusion was given if the endometrial thickness did not reach at least 7 mm. However, if the endometrial thickness reached an adequate thickness, 400 mg of micronized progesterone ovule was administered twice daily and 5 mg of dydrogesterone 5 mg was started thrice daily. Frozen-

thawed embryo transfer will be performed four days after administration of progesterone for cleavage (day 3) embryos and/or six days after administration of progesterone for blastocyst (day 5 or 6) embryos. Estradiol valerate and progesterone supplementation were maintained for a duration of 14 days post-frozen embryo transfer (FET). In the event of a positive serum β-hCG concentration, hormone supplementation was sustained until the 10-12th week of gestation. Endometrial thickness was measured via transvaginal ultrasound (Figure 1). Chemical pregnancies were identified by β-hCG serum two weeks after FET, and clinical pregnancies were identified by the presence of fetal heartbeat in 5 weeks after FET.

PRP preparation

A peripheral venous blood sample of 10 ml was drawn and placed in a PRP regent-BCT tube. Subsequently, at a room temperature of 26oC, immediate centrifugation was carried out at 3500 rpm for 5 minutes. The separated blood components formed three layers: cellular plasma in the supernatant, a buffy coat layer in the middle, and red blood cells settling at the bottom. The PRP was acquired by combining platelets with 1 ml of supernatant, resulting in approximately 1–2 ml of PRP. Following this, 1 ml of PRP was introduced into the uterine cavity using an intrauterine insemination (IUI) catheter.

RESULTS AND DISCUSSION

In this study, six patients were diagnosed with PCOS, two patients with tubal factor infertility, and one patient with primary amenorrhea due to WHO class I anovulation, which is hypogonadotropic-hypogonadism. Polyp resection through office hysteroscopy was performed on two patients as part of the intervention. In the remaining seven patients, who also underwent hysteroscopy, no abnormalities were detected. All the participants underwent autologous PRP intrauterine infusion due to suboptimal endometrial thickness. Endometrial thickness was improved and reached ET 8 mm in eight of nine patients (88.8%). Five patients after the first autologous PRP infusion (62.5%) and three patients (37.5%) after the second PRP infusion (Table 1). However, one patient did not reach the ideal endometrial thickness after the second PRP infusion.

Frozen-thawed embryo transfers were performed for seven patients with adequate endometrial thickness, while two patients (5 and 8) did not undergo to FET because the endometrial thickness was still ≤8 mm (Table 2). Patient number nine experienced unsuccessful implantation, whereas six other patients achieved pregnancy, with normal progression observed in five of them. However, one of them had a miscarriage. In this study, the range of implantation rate varied from 33.3% to 100%, the ongoing pregnancy rate was 83.3%, and the clinical pregnancy rate reached 100%.

Table 1. Patients' age, diagnosis and improvement of endometrial thickness

No	Age	Diagnosis	Increase of Endometrial Thickness (mm)
1	39	Tubal factor	0.7/0*
2	32	Primary amenorrhea (WHO class I anovulation)	0.9
3	37	PCOS	0.8
4	38	Tubal factor	0,9/0.5*
5	36	PCOS	-0.78
6	30	PCOS	0.5
7	41	PCOS	0.9
8	41	PCOS	0.2
9	29	PCOS	0.9

*Endometrial thickness: 48h after the first PRP/48h after the second PRP injection

Table 2. IVF outcomes after PRP treatment

Patient number	Amount embryo transferred	Chemical pregnancy	Clinical pregnancy	Ongoing pregnancy	Implantation rate
1	2	yes	yes	singleton pregnancy	50%
2	1	yes	yes	singleton pregnancy	100%
3	3	yes	yes	miscarriage on 8 weeks gestational age	33.3%
4	3	yes	yes	singleton pregnancy	33.3%
5*	-	-	-	-	-
6	3	yes	yes	twin pregnancy	67.7%
7	2	yes	yes	singleton pregnancy	50%
8*	-	-	-		-
9	2	no	no	no	-

*Patients did not undergo FET

Platelet-Rich Plasma (PRP), alternatively termed autologous conditioned plasma, represents a plasma fraction derived from an individual's blood. This plasma is enriched with platelets at concentrations 4-5 times higher than those found in the circulating blood. Platelets serve essential functions within the body, primarily to prevent excessive blood loss and repair vascular walls and adjacent tissues following injuries. By harnessing the regenerative properties of platelets, PRP has garnered attention as a therapeutic modality in various medical fields, aiming to harness the body's natural healing processes for improved tissue repair and regeneration. This autologous approach, utilizing the patient's blood components, aligns with the pursuit of safer and more personalized medical interventions. PRP is known to contain several growth factors, such as VEGF, TGF, PDGF, and EGF, which could regulate cell differentiation, proliferation, migration, and attachment.[11,13,14]

Presently, the application of PRP infusion is on the rise across various medical fields, emerging as a treatment modality for individuals with thin endometrium. PRP is recognized for its favorable attributes, including safety, minimal to no side effects, and ease of accessibility. Despite these advantages, there remains a dearth of conclusive evidence regarding the efficacy of PRP in addressing thin endometrium-related issues, particularly in Indonesia. While the utilization of PRP in medical treatments is expanding, more comprehensive studies specific to the Indonesian population are imperative to establish the effectiveness of PRP in enhancing endometrial thickness and addressing infertility concerns related to thin endometrium. This emphasizes the need for further research and clinical investigations to validate and refine the application of PRP as a therapeutic option for individuals facing challenges associated with thin endometrium in the Indonesian setting.[15]

The role of autologous PRP in thin endometrium was first evaluated by Chang et al., who showed that in five patients undergoing IVF who had poor response to standard hormone replacement therapy after PRP treatment reached their optimum endometrial thickness and all of them were pregnant. Four out of five patients had usually progressing pregnancy, while one patient had a miscarriage.[11] Moreover, a study from Molina et al. stated that endometrial thickness increased to more than 7 mm after the first PRP infusion, and after the second infusion of PRP, endometrial thickness reached 9 mm in all cases. After the PRP treatment, the endometrium thickness of all patients is qualified for embryo transfer. The study reported a 73.7% positive pregnancy test rate, with 26.3% resulting in live births and an additional 26.3% indicating ongoing pregnancies.[16]

Tandulwadkar et al. evaluate the endometrial thickness and also endometrial vascularity after PRP treatment. They included 68 patients in their study, and 64 achieved optimal thickness for the FET cycle. The average endometrial thickness before PRP was 5 mm, and after PRP, it was 7.22 mm. Seventeen patients with sparse to modest vascularity before PRP resulted excellent vascularity after PRP treatment, and the other 47 patients showed improvement from sparse to modest

endometrial vascularity pattern, while four patients remained sparse vascularity after PRP treatment.[17]

Another study conducted by Kim H et al. involved subjects who had experienced failed IVF and had a thin endometrium. These subjects received 2 to 3 intrauterine PRP infusions. Frozen-thawed embryo transfer (FET) was followed, and a follow-up was conducted for up to 20 subjects. The results indicated that 12 patients exhibited an increase in endometrial thickness, while seven experienced a decrease. The ongoing pregnancy rate and live birth rate were 20%, with four out of 20 subjects achieving live births, two experiencing abortion, one having a chemical pregnancy, and 13 not achieving pregnancy.[18]

Several studies were recently published (including 2 randomized clinical trials) regarding the effect of PRP infusion in patients with thin endometrium, and all of them suggest that PRP infusion is significant in increasing the endometrial thickness in patients with thin endometrium.[14,15,18-20] Our study showed that nine patients had poor endometrial thickness, and after PRP infusion, an increase in endometrial thickness was found in eight out of nine patients, which is 88.8%. One patient who got improvement in her endometrial thickness still did not reach optimum endometrial thickness after a second PRP. It is consistent with a meta-analysis that demonstrated patients with an endometrial thickness below 7 mm exhibited a notably reduced likelihood of clinical pregnancy compared to those with an endometrial thickness exceeding 7 mm.[21] Another study used PRP infused into the endometrium to improve endometrial thickness and proceed to ET. It was found that 86% of subjects achieved an endometrial thickness greater than 7 mm, while 14% had a thickness below 7 mm. However, the reasons for the failure to achieve adequate endometrial thickness were not elaborated.[22]

Our study was also in line with previous studies, which resulted in a positive outcome of intrauterine infusion of PRP towards endometrial growth and improvement of pregnancy rate in IVF patients. Out of seven patients who got embryo transfer, six patients were pregnant, and in five of them, the pregnancy went well, but unfortunately, one patient had implantation failure. The implantation rate was 33.3-100%, clinical pregnancy was 100%, and ongoing pregnancy rate was 83.3%.

In the course of our investigation, no adverse effects were discerned after the administration of PRP infusion. Nevertheless, it is noteworthy that one participant encountered a reduction in endometrial thickness, and another experienced a failure in implantation. The precise etiology behind these occurrences remains elusive, necessitating further inquiry. The current body of research needs to be revised to offer comprehensive insights into instances of diminished endometrial thickness after PRP infusion. Furthermore, there are no research or evidence found major side effects of PRP or determined whether it also has a role in implantation failure. This underscores the imperative for additional meticulous studies and systematic exploration to clarify the mechanisms and contributing factors associated with the observed outcomes, contributing to the refinement of our understanding and informing clinical practice in the realm of reproductive medicine. Furthermore, this could necessitate additional studies to investigate the outcomes and potential complications of neonates born to mothers with an improved thin endometrium.

This study has a notable limitation due to including a small number of patients. Consequently, additional research is essential to substantiate the effectiveness and safety of PRP in enhancing endometrial thickness among individuals facing infertility related to a thin endometrium. Furthermore, a comprehensive understanding of the precise mechanisms underlying the intrauterine infusion of PRP is crucial, especially in promoting endometrial growth in patients with a thin endometrium. Unveiling these mechanisms will contribute significantly to advancing our knowledge and improving therapeutic approaches for individuals experiencing challenges related to thin endometrium. Therefore, future investigations with larger sample sizes and in-depth mechanistic exploration are warranted to address these critical aspects and enhance the overall understanding of PRP's role in treating thin endometrium-related infertility, particularly in Indonesia.

CONCLUSION

Addressing the thin endometrium in patients poses a persistent challenge for healthcare providers. Various treatment approaches have been employed, but conclusive and positive outcomes are yet to be achieved. Based on the findings of the current investigation, PRP demonstrates encouraging outcomes in enhancing endometrial thickness in individuals experiencing infertility associated with a thin endometrium. Further studies with larger sample sizes and specific molecular basis data are recommended to enrich our comprehension of PRP treatment in patients with thin endometrium.

DISCLOSURES

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Conflict of interest

The authors affirm no conflict of interest in this study.

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Author contribution

GP collected the data, analyzed the statistics, and wrote the manuscript. MM, AKH, KS, RM, EA, and AMY conceptualized and reviewed the manuscript. All of the authors approved the final version of the manuscript.

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ORIGINAL RESEARCH

The effect of docosahexaenoic acid (DHA) supplementation on total antioxidant capacity (TAC), superoxide dismutase (SOD), and interleukin-6 levels in underweight pregnant women

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ABSTRACT

Objective: Underweight pregnant women face oxidative stress and inflammation, increasing their risk of intrauterine growth restriction (IUGR) and preterm birth. This study investigates the effects of DHA supplementation on Total Antioxidant Capacity (TAC), Superoxide Dismutase (SOD), and Interleukin-6 (IL-6) in underweight pregnant women, along with the correlation between DHA and these markers.

Materials and Methods: This experimental pre-test/post-test study focused on underweight pregnant women in the Made District, Surabaya, Indonesia. Eligible participants were in their second or third trimester, had a BMI below 18.5, and were taking DHA regularly. Exclusion criteria included early pregnancy (gestational age < 14 weeks), BMI above 18.5, irregular DHA intake, and withdrawal from the study. The study ran from July to December 2023, using non-probability sampling to select participants. Blood samples were collected before and after two months of DHA supplementation.

Results: Following the intervention, TAC levels demonstrated a noteworthy increase ($p < 0.05$). SOD levels also exhibited a significant difference ($p < 0.05$), and IL-6 levels showed a significant change ($p < 0.05$). A strong and positive correlation ($r = 0.718$) was observed between the increased TAC and SOD levels. DHA influenced both TAC and IL-6, with a significant relationship between TAC and IL-6 ($p < 0.01$). Furthermore, elevated SOD levels were associated with a decrease in IL-6 levels ($p < 0.01$). The correlation coefficient value of 0.718 between changes in SOD and TAC indicated a robust positive correlation.

Conclusion: The findings suggest that DHA supplementation in underweight pregnant women positively affects oxidative stress and inflammation markers, improving TAC, SOD, and IL-6 levels.

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Highlights:

1. Underweight pregnant women, face an imbalance in energy and protein intake.
2. TAC, SOD, and IL6 with administration of DHA to pregnant women with chronic energy deficiency in the third trimester provide benefits.

INTRODUCTION

Around 3–4% of women in the United Kingdom commence pregnancy with a low body weight. However, globally, the prevalence of malnutrition predominantly affects low- and middle-income nations. For instance, up to 30% of women of childbearing age in many Sub-Saharan African countries are underweight. Various factors such as body image perceptions, genetics, socioeconomic status, and cultural influences contribute to BMI variations, resulting in a diverse population of underweight women. Nevertheless, extensive cohort studies have revealed that underweight pregnant women are more likely to be younger, unmarried, pursuing education, and unemployed. Moreover, there is evidence indicating a higher prevalence of smoking among underweight women.[1]

During the initial evaluation, it is imperative to rule out treatable factors contributing to low BMI before attributing it solely to idiopathic causes. BMI before pregnancy determines neonatal and maternal outcomes. Pregnant women with underweight have risks in pregnancy and childbirth.[2] Several studies in underweight pregnant women found the risk of preterm birth and low birth weight.[3,4] In underweight pregnancies, especially in the second and third trimesters, there is an increase in oxygen pressure, ROS production, and excessive oxidative stress.[5,6] This process of inflammation will trigger a series of effects that will result in IUGR, preterm birth, and stillbirth.[7] Meanwhile, docosahexaenoic acid (DHA) helped to reduce the production of reactive oxygen species (ROS) in astrocyte cells.[7] Several studies have stated DHA supplementation is important during pregnancy.

However, there are no certain indicators of DHA's influence in pregnancy. This study will be the first study that tries to examine the potential benefits of DHA supplementation for underweight pregnant women by finding the relationship between underweight pregnant women and the incidence of oxidative stress through the TAC and SOD pathways and the inflammatory mechanisms through the IL-6 pathway in underweight pregnant women. We hope that this study will contribute to the development of science, give a better understanding for the clinician regarding DHA supplementation in pregnancy, and thus lessen the complications in pregnancy.

MATERIALS AND METHODS

Our study employed an experimental design incorporating a cohort study framework with a quantitative methodology. This approach allowed us to assess the impact of the treatment administered and substantiate the hypotheses formulated. The primary objective of this study was to investigate the influence of DHA supplementation on TAC, SOD, and IL-6 levels in underweight pregnant women. This assessment was conducted over a specified timeframe of 2 months within a predetermined district.

The study population consisted of underweight pregnant women in Made District, Surabaya, East Java, Indonesia. Inclusion criteria encompassed pregnant women registered in Made District, with a gestational age exceeding 14 weeks (trimesters 2-3), and a BMI below 18.5. Exclusion criteria encompassed a gestational age below 14 weeks, a BMI above 18.5, irregular consumption of DHA supplements, and withdrawal from the study. The study was held from July 2023 to December 2023. Sampling utilized non-probability techniques, combining quota and purposive sampling. The experimental group comprised 21 underweight pregnant women receiving DHA supplementation. TAC, SOD, and IL-6 levels were assessed before and after treatment to evaluate the potential impact of DHA supplementation on reducing TAC, SOD, and IL-6 levels in underweight pregnant women.

The research variables included the dependent variable: TAC, SOD, and IL-6; the independent variable: DHA supplementation; and the control variable: gestational age in the second and third trimester, diagnosis of underweight was done with BMI measurement, no other comorbidities (kidney, heart, diabetes mellitus, and chronic hypertension). TAC was measured using the ELISA method, SOD was measured using the ELISA method with the SOD kit, and IL-6 was measured using the quantitative fluorescent immunoassay method.

SPSS 25 was used in the analysis of the data collected in this study. Normality testing was conducted using either the Kolmogorov-Smirnov test or the Shapiro-Wilk test. For pre-posttest comparisons, the paired t-test will be employed if the data follows a normal distribution. Conversely, if the data does not exhibit normality, the Wilcoxon test was utilized. To assess the correlation among the three variables (TAC, SOD, and IL-6), the researcher employed the Pearson correlation test if the data adheres to a normal distribution. In cases where the data is not normally distributed, the Spearman correlation test was used. These statistical methods were chosen to ensure appropriate analyses were conducted based on the normality or non-normality of the data distribution.

This study has received authorization from the National Unity and Politics Agency (Badan Kesatuan Bangsa dan Politik) under approval number 070/7145/209/2023. All protocols related to research subjects were executed following the ethical guidelines outlined in the approval granted by the Health Research Ethics Committee of the Faculty of Medicine at Ciputra University (Komisi Etik Penelitian Kesehatan Fakultas Kedokteran Universitas Ciputra), with approval number 089/EC/KEPK-FKUC/X/2023. Before their involvement in this research, all participants have given their written consent.

RESULTS AND DISCUSSION

In this study, an evaluation of various characteristics was conducted. The respondents' ages fell within the reproductive age range of 16 to 36 years, and their gestational age ranged from the second to the third trimesters. All participants were classified as underweight, with a BMI ranging from a minimum of 17.33 to a maximum of 18.40. Systolic blood pressure readings ranged from a minimum of 90.0 to a maximum of 128, while diastolic blood pressure ranged from a minimum of 59 to a maximum of 80 (Table 1). Upon conducting normality tests, it was observed that the data for age, gestational age, mid-upper arm circumference, and systolic blood pressure followed a normal distribution. On the other hand, BMI and diastolic blood pressure exhibited a non-normal distribution. These findings provide valuable insights into the demographic and physiological characteristics of the study participants and inform the choice of appropriate statistical tests based on the normality or non-normality of the data.

Table 1. Research characteristics

No	Characteristics	Mean ± SD
1	Age	27.30 ± 4.45
2	Gestational Age	19.96 ± 10.01
3	Weight	41.96 ± 3.18
4	Height	152.04 ± 3.35
5	BMI	18.10 ± 0.28
6	Mid Upper Arm Circumference (LILA)	24.52 ± 1.67
7	Systolic BP	105.04 ± 10.32
8	Diastolic BP	67.21 ± 7.48

Table 2. Total Anti-Oxidant Analysis (T-test)

No	Characteristics	Mean ± SD	P Value
1	TAC Pre test	0.37 ± 0.13	<0.001
2	TAC Post-test	0.55 ± 0.09	

The evaluation of total antioxidant levels before and after DHA supplementation revealed a significant difference in TAC results between the pre and post-test, indicating a substantial increase in TAC levels ($p < 0.05$) (Table 2). This suggested that DHA supplementation had a notable effect on enhancing total antioxidant levels. Underweight pregnant women, as evidenced by reduced TAC levels, experienced a decline in antioxidant capacity. The inhibitory impact of DHA on NF-kB activation, a key factor in the synthesis of inflammatory cytokines, vascular adhesion molecules, metalloproteinase, and VEGF, contributes to this effect.[8,9] Furthermore, DHA enhances TAC through mechanisms associated with its ability to increase SOD and catalase enzymes. By inhibiting NF-kB and reducing reactive oxygen species (ROS), DHA effectively elevates antioxidant levels in the blood of underweight pregnant women.[10]

Table 3. Superoxide Dismutase Analysis (T-test)

No	Characteristics	Mean ± SD	P Value
1	SOD Pre test	15.55 ± 2.54	0.008
2	SOD Post test	17.44 ± 2.62	

The study identified a significant difference in SOD levels before and after the intervention ($p < 0.05$), with an observed increase in SOD values in underweight pregnant women receiving DHA (Table 3). This indicates that DHA supplementation has a positive impact on enhancing SOD levels. DHA's ability to inhibit oxidative stress and exert anti-inflammatory effects contributes to the improvement of endothelial function. Additionally, DHA plays a crucial role in eliminating ROS-induced DNA damage and reducing H2O2 formation. Through its supplementation, DHA increases the production of SOD, enhancing its scavenging ability in redox signaling. The nuclear factor E2-related factor 2 (Nrf2) emerges as a key player in fighting oxidative stress, and DHA plays a pivotal role in regulating the expression of genes responsible for increasing SOD levels. This regulation by Nrf2, a critical transcription factor, triggers the cellular antioxidant defense system to effectively combat Reactive Oxygen Species (ROS). The findings underscore the multi-faceted impact of DHA on SOD levels and its role in bolstering the cellular defense mechanisms against oxidative stress.[11,12]

Table 4. IL-6 Analysis (Wilcoxon)

No	Characteristics	Mean ± SD	P Value
1	IL 6 Pre test	9.83±17.8	0.005
2	IL 6 Post test	7.28±11.003	

The IL-6 levels in this study exhibited a significant change ($p < 0.05$), demonstrating a notable decrease (Table 4). This indicates that DHA supplementation has a discernible effect on IL-6 levels in underweight pregnant women. DHA, known for its anti-

inflammatory role, operates through competitive inhibition against arachidonic acid (ARA) in phospholipid membranes, resulting in a metabolic shift from pro-inflammatory ARA-derived eicosanoids to DHA-derived lipid mediators. The decrease in ARA is directly linked to the increased DHA levels in the blood, as there is competition between DHA and ARA metabolism involving enzymes such as phospholipase A2, COX, and lipoxygenase. Elevated ARA levels are directly proportional to IL-6 production, indicating an increased inflammatory response, particularly in cases of underweight pregnant women. DHA supplementation effectively lowers ARA levels, thereby reducing the production of inflammatory cytokines, including IL-6. These findings highlight the anti-inflammatory potential of DHA in mitigating inflammatory markers such as IL-6 in underweight pregnant women.[13,14]

Table 5. Pearson's correlation test between TAC, SOD and IL-6

No	Characteristics	P Value	Pearson's correlation
1	TAC and SOD	< 0.05	0,718
2	TAC and IL 6	< 0.05	- 0.600
3	SOD and IL 6	< 0.05	- 0.592

In this study, a significant and robust correlation was observed between TAC and SOD, providing insights into the influence of DHA on TAC and SOD levels with a strong positive correlation coefficient (r = 0.718) (Table 5, Figure 1). This finding suggests that the administration of DHA affects increasing both TAC and SOD activity. The mechanism underlying this effect can be explained through the inhibition of phosphatidylinositol 3-kinase and protein kinase B (PKB), which modulate oxidative stress activation. This modulation subsequently hinders the induction of the NF-kB pathway, as elucidated in the study conducted by Mahdi Sepidarkish.[15] The correlation observed in this study aligns with existing research, supporting the notion that DHA supplementation may contribute to enhanced antioxidant capacity and SOD activity in underweight pregnant women.[15]

The study indicates a relationship between DHA supplementation and alterations in TAC and IL-6 levels. The association between TAC and IL-6 can be explained by the increase in ROS, which triggers elevated expression of inflammatory cytokines, including IL-6, through the activation of NF-kB and activator protein-1 (AP-1). Subsequently, the infiltration of macrophages into adipose tissue is stimulated, leading to increased production of inflammatory cytokines due to enhanced ROS production. These findings align with research conducted by Martinez, who investigated DHA supplementation for 3 months in patients with keratoconus. Martinez found that DHA could elevate TAC levels while concurrently decreasing IL-6 levels. This underscores the potential of DHA in addressing states of chronic inflammation and oxidative stress occurring simultaneously. The study provides valuable insights into the multifaceted impact of DHA on antioxidant capacity and the modulation of inflammatory markers, highlighting its potential therapeutic role in mitigating conditions characterized by inflammation and oxidative stress.[16,17]

The relationship between TAC and IL-6 can be further explained by the activation of the NF-kB pathway initiated by ROS. This activation induces inflammatory processes, triggering the production of pro-inflammatory cytokines, including IL-6. These cytokines, in turn, enhance the synthesis of matrix metalloproteinase (MMPs). The increased presence of MMPs contributes to elevated oxidative stress, causing damage to extracellular constituents, cell membranes, nucleic acids, and protein structures, ultimately resulting in tissue protein damage. DHA, as a source of antioxidants, plays a crucial role in inhibiting tissue damage caused by oxidative stress. By acting as a negative regulator, DHA mitigates the impact of pro-inflammatory cytokines like IL-6. This highlights the potential of DHA in modulating the intricate interplay between oxidative stress, inflammation, and tissue damage, underscoring its significance as a protective factor in such processes.[18]

The correlation test between SOD and IL-6 demonstrated a significant and inversely correlated relationship, where an increase in SOD was associated with a decrease in IL-6 levels. This inverse correlation exhibited moderate strength with a negative correlation coefficient (r = -0.592). The relationship between SOD and IL-6 in underweight pregnant women can be elucidated by examining how decreased SOD levels may contribute to endothelial damage. Endothelial damage triggers an inflammatory process through inflammatory cells, particularly monocytes, which migrate to the sub-endothelium and bind to endothelial adhesive molecules. These monocytes then differentiate into macrophages, and activated macrophages secrete pro-inflammatory cytokines. IL-6 is induced due to the influence of low SOD expression through inflammation mediated by neutrophils. Activated neutrophils, in turn, bind to the endothelium, migrate to the extracellular space, and release reactive oxygen species (ROS), protease enzymes, and chemokines in significant quantities. DHA, recognized as a potent antioxidant, especially in increasing SOD activity, plays a crucial role in mitigating this process.[4,19,20]. This study aligns with the research of Losano, supporting the proposition that DHA supplementation can reduce IL-6 levels,

highlighting the potential of DHA in modulating the intricate relationship between SOD, inflammation, and oxidative stress in underweight pregnant women.[21]

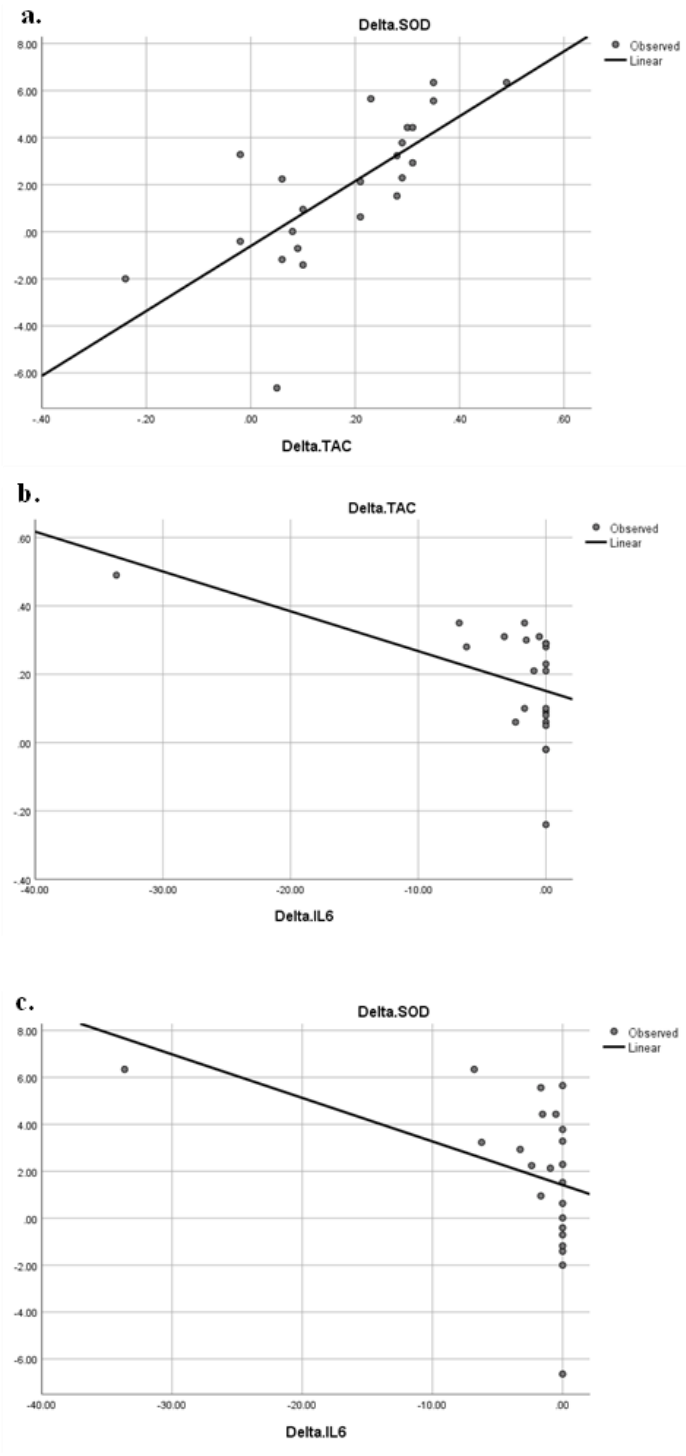

Figure 1. a. Scatter plot of correlation of Delta SOD and Delta TAC; b. Scatter plot of correlation of Delta TAC and Delta IL-6; c. Scatter plot of correlation of Delta SOD and Delta IL-6.

Based on the findings from the three markers in this study, it can be concluded that DHA plays a significant role in underweight pregnant women, particularly in its function against ROS-induced cell damage and death. The production of superoxide radicals (O2-) through the donation of one electron to molecular oxygen (O2) marks the initial step in the formation and dissemination of ROS inside and outside mitochondria. The imbalance between ROS production and enzymatic antioxidant systems increases the susceptibility of tissues and organs to oxidative radical damage. Superoxide radicals can react with nitric oxide (NO) to form peroxynitrite (ONOO-) in a reaction controlled by the production and bioavailability of radicals. Furthermore, in the transition from iron, superoxide radicals and hydrogen peroxide (H2O2) interact with the Haber-Weiss reaction to generate hydroxyl radicals (OH). The high reactivity of peroxynitrite and hydroxyl radicals poses a risk to cell nucleic acids, proteins, or lipids, leading to overall organism damage. Mitigating superoxide radicals can be achieved by increasing mitochondrial superoxide dismutase, subsequently reducing the formation of peroxynitrite and hydroxyl radicals. Hydrogen peroxide can be inhibited by activating glutathione peroxidase (GPX) and catalase (CAT), mechanisms facilitated by the consumption of DHA. This underscores the protective role of DHA against oxidative stress and highlights its potential to preserve cellular integrity in underweight pregnant women.[22]

The results of this study indicate that the pre-test and post-test outcomes for TAC, SOD, and IL-6 were statistically significant, signifying that the administration of DHA brought about changes in these three variables. The administration of DHA increased TAC and SOD levels, while IL-6 levels decreased. Furthermore, in the examination of the correlation test, a significant relationship or correlation was identified between the change (delta) in TAC with both SOD and IL-6. The relationship between TAC and SOD was positive, indicating they moved in the same direction, while the relationship between TAC, SOD, and IL-6 was negative, suggesting an opposite direction based on the correlation test. These findings underscore the impact of DHA on antioxidant capacity, Superoxide dismutase activity, and IL-6 levels, providing valuable insights into the multifaceted effects of DHA supplementation in underweight pregnant women.

The researchers also conducted a demographic test with the change (delta) in TAC, SOD, and IL-6. Based on the results of the demographic test, there was no significant difference observed for the three variables representing changes in TAC, SOD, and IL-6 (Table 6). This implies that demographics were not a confounding variable in this study. The non-significant findings in the demographic test suggest that, regardless of demographic factors, the administration of DHA can be conducted. The study indicates that demographic variables were not influential in the changes observed in SOD, TAC, and IL-6. Therefore, the alterations in these three research markers were attributed purely to the effects of DHA and were not related to demographic factors. This strengthens the inference that the observed changes are indeed a result of DHA supplementation in underweight pregnant women, providing a more robust understanding of the intervention's impact.

Mammalian cells utilize oxygen as the final electron acceptor in energy metabolism, but this process generates unwanted oxygen-based byproducts collectively known as ROS. At low levels, ROS can function in redox signaling and biological processes, but at high levels, they can lead to nonspecific and potentially harmful reactions. The susceptibility of fatty acids to oxidation is believed to depend on the degree of saturation. DHA, despite its high degree of saturation, is susceptible to oxidation, and the relationship between its structure and susceptibility to ROS oxidation is intricate. Both in vitro and in vivo studies have demonstrated that DHA can induce an antioxidant response at the transcriptional or post-transcriptional level. Understanding the molecular mechanisms behind the protective role of DHA is increasingly important due to its potential in preventing chronic diseases. The transcription factor Nrf2, which regulates cellular redox homeostasis, plays a crucial role in activating genes that enhance antioxidant and detoxification capacity. DHA can activate Nrf2 reversibly, initiating a cellular antioxidant response. DHA can undergo enzymatic and non-enzymatic oxidation, resulting in various oxidation metabolites.

Table 6. Correlation Test of Demographic Relationship with Delta TAC, SOD and IL6

No	Characteristics	Delta TAC	Delta SOD	Delta IL-6
1	Age	0.132	0.445	0.117
2	Gestational Age	0.790	0.604	0.445
3	Mid Upper Arm Circumference (LILA)	0.473	0.755	0.744
4	Systolic BP	0.733	0.337	0.709
5	Diastolic BP	0.450	0.586	0.627
6	BMI	0.428	0.416	0.972

These oxidized DHA products can interact with Keap1, a protein involved in Nrf2 degradation, inhibiting this degradation process. As a result, Nrf2 can carry out its activity in regulating the antioxidant response. Understanding these mechanisms provides valuable insights into the role of DHA in cellular antioxidant responses and its potential implications in preventing chronic diseases.[23]

However, this study had some constraints. It focused solely on one district of Surabaya (Made District, Surabaya, East Java, Indonesia). Our study's strength lies in the selection of our sample, as we meticulously monitored their DHA consumption from the outset of the research to ensure proper adherence.

CONCLUSION

In this study, it was found that the administration of DHA can have an impact or change on TAC, SOD, and IL-6 in pregnant women with chronic energy deficiency. In addition, this study also proves that DHA corrected the oxidative status and reduce inflammation.

DISCLOSURES

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Conflict of interest

No conflicts of interest could influence the results or interpretation of this research

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Author contribution

All authors have contributed to all processes in this research, including preparation, data gathering and analysis, drafting, and approval for publication of this manuscript.

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ORIGINAL RESEARCH

Collagen-1 and elastin expression in cervical tissue: A comparison across cervical elongation, pelvic organ prolapse, and combined conditions

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ABSTRACT

Objective: This study aimed to assess differences in the expression of Collagen-1 and Elastin in cervical tissues among patients with Cervical Elongation (CE), Pelvic Organ Prolapse (POP), a combination of CE with POP, and those without either condition.

Materials and Methods: An analytical study with a cross-sectional design was conducted, using immunohistochemistry (IHC) to analyze cervical tissue samples preserved in paraffin blocks. Patient groups included those diagnosed with CE, POP, CE combined with POP, and a control group without CE or POP. All participants underwent surgery between January 2021 and April 2023. IHC was used to measure the expression levels of Collagen-1 and Elastin in each tissue sample. Observations were made under 400x magnification, focusing on five randomly selected visual-field areas in each sample to determine the area fraction. Two experienced pathologists conducted the analyses in a blinded manner to ensure objective evaluation.

Results: Statistical analysis using the Kruskal-Wallis test revealed significant differences in the expression of Collagen-1 across the four groups (CE, POP, CE with POP, and control). Patients with CE showed a higher expression of Collagen-1 than those with CE and POP combined, as well as the control group. However, no significant differences in Elastin expression were observed among the groups.

Conclusion: Collagen-1 expression differs significantly across patients with CE, POP, and CE combined with POP, suggesting a distinct role in cervical tissue remodeling in these conditions. Conversely, Elastin expression was consistent across all groups, indicating that it may not play a differentiating role in these pathologies. These findings highlight Collagen-1's potential involvement in the structural changes associated with CE and POP.

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Highlights:

1. Pathogenesis of cervical elongation is still limited, including the histological and molecular differences between a cervical elongation and a normal cervix.
2. The expression of collagen-1 level in the cervical elongation group was stronger compared to the cervical elongation with POP and control group.

INTRODUCTION

Cervical elongation is the lengthening or hypertrophy of the cervix towards the vaginal introitus with the supporting tissues of the uterus still in good condition.[1] Cervical elongation can occur in both parts of the cervix, the supravaginal part and the vaginal part. Supravaginal CE is found in 18% of patients with POP, while vaginal cervical elongation almost always occurs congenitally.[2] The cervix without adequate fixation from the sacrouterine ligament and pressure from the opposite direction by the pelvic floor muscles could result in cervical elongation. It is supported by the finding of a larger genital hiatus size in the group with CE compared to the POP patients without CE.[3]

The supporting structure of the female pelvic can be divided into three levels. Level 1 is the cardinal-uterosacral ligament complex; levels 2 and 3 consist of the fascia, urogenital diaphragm, and perineal bodies supporting the middle and lower parts of the vagina. Among the three grades, defects in grade 1 result in uterine prolapse. The POP condition is not life-threatening but causes a decrease in women's quality of life.[4] This condition is a common gynecological disorder, with approximately 37% of patients seeking treatment and the risk of surgical procedures being approximately 11-19% of the population.[5] The prevalence of POP in low-income countries such as Tanzania, Ethiopia, and Gambia is 46-64.6%.[6] In Indonesia, in 2007, there were reported 30 cases of grade III-IV prolapse, and there were around 20 operations on cases of grade III-IV uterine prolapse every year.[7] The prevalence of POP was 26.4%, urinary incontinence 15.3%, and fecal incontinence 2.5%.[8]

Not all patients with uterine prolapse experience reduction in the uterine corpus, as occurs in CE. In patients with CE, the supporting structures of level I remain relatively strong compared to uterine descent.[9] Approximately 40% of women with apical component prolapse experience CE, with the age of patients suffering from CE being younger than those suffering from POP. Women with POP were found to have a significantly higher ratio of cervical length to total uterine length when compared with women without POP.[10,11] Knowledge regarding the pathogenesis of CE is still limited, including the histological and molecular differences between a CE and a normal cervix. Most studies carry out examinations to determine the composition of supporting tissues such as the sacrouterine ligament and vagina, but there are still few for cervical tissue. Cervical elongation may have a different pathogenesis, not as a result of weak support of the uterosacral and vaginal ligaments, as has been reported in POP. It is suspected that CE is more of a local process than a systemic disorder, supported by their research, which found that the cervical tissue of patients with CE has higher levels of estrogen and progesterone receptors compared to normal cervix without elongation. The explanation for higher estrogen and progesterone receptor levels is a feedback effect of receptor regulation in response to reduced estrogen and progesterone content in these tissues.[10,12]

Collagen and elastin are important components of the extracellular matrix of cervical tissue. Type 1 collagen is the most abundant form (70%), and type 3 forms 30% of the total collagen. Collagen is an important component of the extracellular matrix that contributes to the biomechanical strength of the cervix. Elastin provides elasticity and stretchability, making up about 1.5% of the cervix of non-pregnant women. Smooth muscle and fibroblasts are cellular parts of the cervix.[13,14] From this study, researchers were interested in knowing the expression of collagen I and elastin in cervical tissue in CE group, POP group, CE with POP group, and group without CE or POP as a control.

MATERIALS AND METHODS

This is an analytical study with a cross-sectional approach. The study participants were cervical tissue patients' specimens from CE, POP, CE with POP, and without CE or POP who had underwent surgery at Dr. Soetomo Hospital during the research period and met the inclusion and exclusion criteria. Samples were determined by consecutive sampling technique. Inclusion criteria include cervical tissue patients' specimens from CE, POP-Q's grade 3 and 4 POP and CE with POP, and normal cervical tissue (without CE and POP). The exclusion criteria include a history of pelvic radiotherapy, hormone replacement therapy, a cervix exhibiting additional pathological characteristics (e.g., congenital abnormalities or precancerous lesions), and incomplete information medical records.

Diagnosis of CE, the length of the cervix and uterine corpus in the specimen are measured in centimeters. Cervical length > 3.38cm or the ratio of the cervix to the uterine corpus > 0.79 is considered as CE. Grade 3 and 4 POP is diagnosed with POP-Q system by measuring the position of the cervix when the patient in valsalva maneuver, and if the cervix descends beyond the hymen as far as between (+1 and TVL-2) then the subject is diagnosed with grade 3 POP and if > (TVL-2), subjects were diagnosed with grade 4 POP.

The Immunohistochemical (IHC) staining was used to determine the expression of Collagen-1 and Elastin level. The expression of Collagen-1 and Elastin in each

sample was viewed using a magnification of 400 times in 5 areas of the visual field were randomly selected to calculate the area fraction.[15] Reading of IHC results was performed by two experienced pathologists, both blinded to the clinical diagnosis of each sample. The collected data was tested for normality using the Shapiro-Wilk test. If it is normally distributed ($p > 0.05$) a Kruskal-Wallis test will be carried out to compare the levels of the dependent variable between the 4 groups. If the distribution is abnormal ($p < 0.05$), a non-parametric test will be carried out with the Mann-Whitney test. Statistical calculations using SPSS 26 software (IBM, Armonk, NY, USA). An ethical clearance letter with number 0653/KEPK/IV/2023 was received from Dr. Soetomo General Academic Hospital on April 17, 2023.

RESULTS AND DISCUSSION

Characteristics of research subjects

This research was carried out from May to September 2023. It started with collecting samples, then continuing with IHC examination at the Anatomical Pathology Laboratory, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. Samples were obtained from patient data registered from January 2021-April 2023. The total number of samples was 34 with 8 samples in group 1 (CE), 8 samples in group 2 (CE with POP), 9 samples in group 3 (POP), and 9 samples in group 4 (control). In the four groups, there were variations in subject age, number of parities, menopausal status and other conditions that were risk factors. If the medical record data is incomplete, we contact the subject by phone.

The mean age of the subjects was 51.94 ± 10.02. Meanwhile, for the number of parities, the average was 2.91 ± 1.68. Most of the subjects had a body mass index with overweight status. Most of the subjects did not experience constipation, long-term coughing and no history of pelvic organ surgery. The number of subjects with a history of weight lifting and those without was almost the same between 4 groups.

From statistical tests on the four groups, a significant difference in the mean age of the subjects was obtained ($p < 0.05$). The elongation group had the youngest mean age compared to the other three groups, namely 41.38 ± 3.93. Meanwhile, the POP group had the oldest mean age, 61.33 ± 5.34 (Table 1). These results are in accordance with several previous studies, where POP occurs in almost half of women aged over 50 years with a prevalence reaching 30-50%. POP cases at Dr. Soetomo General Academic Hospital in 2007-2011 showed that the average age of patients was 58.5±10.5 years. Approximately 40% of women with apical component prolapse experience CE, with the age of patients suffering from CE being younger than those suffering from POP.[11]

Table 1. Comparison of the proportion of cervical elongation, cervical elongation with POP, and POP based on patient's characteristics

	Groups				Total	p-values
	CE (n=8)	CE with POP (n=8)	POP (n=9)	Control (n=9)		
Age	8	8	9	9	34	
Mean ± SD	41.38±3.93	59.50±6.70	61.33±5.34	45.22±3.73		0.001
Menopause						
Yes (n%)	0 (0.00)	8 (44.44)	9 (50.00)	1 (5.56)	18	0.775
No (n%)	8 (50.00)	0 (0.00)	0 (0.00)	8 (50.00)	16	
Parities	8	8	9	9	34	0.007
Median (Min – max)	2.5(1 – 3)	3.5(2 – 6)	3 (2 – 9)	2 (0 – 3)		
Baby Birth Weight						
> 3500g (n%)	3 (25.00)	4 (33.33)	4 (33.33)	1 (8.33)	12	0.241
≤ 3500g (n%)	5 (22.73)	4 (18.18)	5 (22.73)	8 (36.36)	22	
Body Mass Index (BMI)						
Obesity (n%)	0 (0.00)	0 (0.00)	1 (100.00)	0 (0.00)	1	
Overweight (n%)	5 (2.73)	7 (31.82)	5 (2.73)	5 (2.73)	22	0.679
Normal (n%)	3 (27.27)	1 (9.09)	3 (27.27)	4 (36.36)	11	
Underweight (n%)	-	-	-	-		

There were no significant differences between the menopausal status of the four groups ($p > 0.05$). However, there were significant differences between the group of CE, CE with POP and the POP. All subjects in the CE group were not yet menopausal, whereas in CE with POP and POP groups, all were menopaused. In accordance with Ibeanu's research,12 the results of research on POP with CE, menopause is not a risk factor. Menopause is the main risk factor for POP, which is associated with a decrease of estrogen level which results in the genital tract atrophy, weakening of the pelvic floor muscles, uterosacral and cardinal ligaments, and a decrease in the ability of the endopelvic fascia to stretch.[7,17]

A case report suggests that a cervix without adequate fixation of the sacrouterine ligament and without pressure from the opposite direction by the pelvic floor muscles can lead to CE. This is supported by the finding of a larger genital hiatus size in the group with CE when compared to the group of POP patients without CE.[18] The symptoms of CE and apical POP that sufferers about are almost the same complain, but it is not yet clear whether CE and POP are different conditions or whether the two conditions always coexist. A study comparing women with and without POP, measuring the uterine corpus and cervix using MRI concluded that the cervix in women with uterine prolapse was 36.4% longer than in women without uterine prolapse ($p < 0.001$).[19,20]

There was a significant difference ($p < 0.05$) in parity in the four subject groups. The lowest average number of parities was owned by the control group (2 (0-3)) and the highest average number of parities was owned by the elongation group with POP (3.5(2-6)). Research by Liu11 provided significantly different results in parity between the elongation group and the POP group. Analysis of birth weight of babies born and BMI showed that there were no significant differences in the four groups ($p > 0.05$). This is in accordance with research by Liu which found that there was no significant difference in BMI between the elongation group and the POP group. [11

Expression of collagen-1 and elastin

After obtaining the data for the research samples in the four groups, a search was carried out for paraffin blocks of cervical tissue in the Anatomical Pathology Laboratory at Dr. Soetomo Hospital. Slides were made and an IHC was carried out for Collagen-1 and Elastin using the indirect immunoenzyme method. The examination used a Collagen-1 antibody kit (ABCLONAL/COL1A1 Rabbit pAb) and an Elastin antibody kit (ABCLONAL/ELN Rabbit pAb) with a dilution of 1:50.

In Figure 1 we can see the results of the CPI examination, viewed with an Olympus cx-31 microscope. Collagen-1 was stained positively in the cytoplasm of collagen fibers with strong intensity at microscopic magnifications of 200 times and 400 times. Measurement of Collagen-1 expression level on each slide was carried out in 5 random fields of view with 400x magnification.

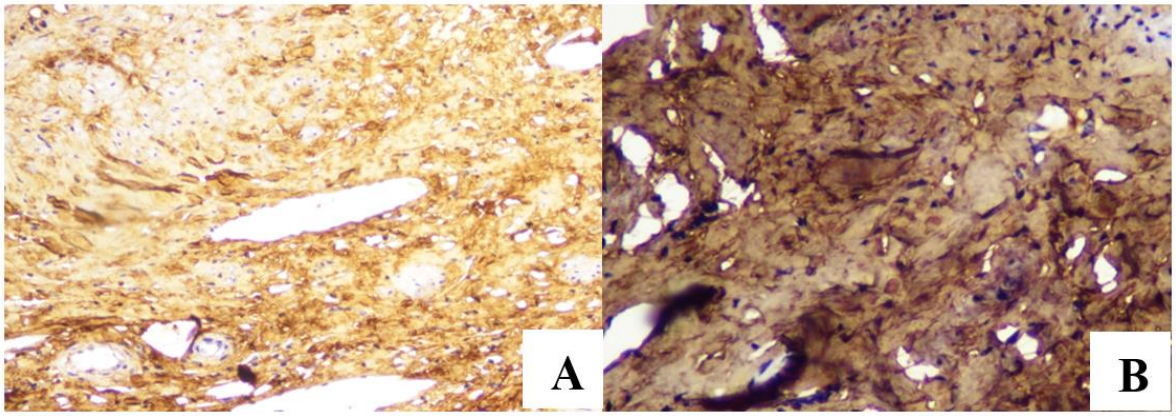

Figure 1. The results of IHC staining with collagen 1 antibodies showed positive staining in the cytoplasm of collagen fibers with strong intensity: A. 200x microscopic magnification, B. 400x microscopic magnification

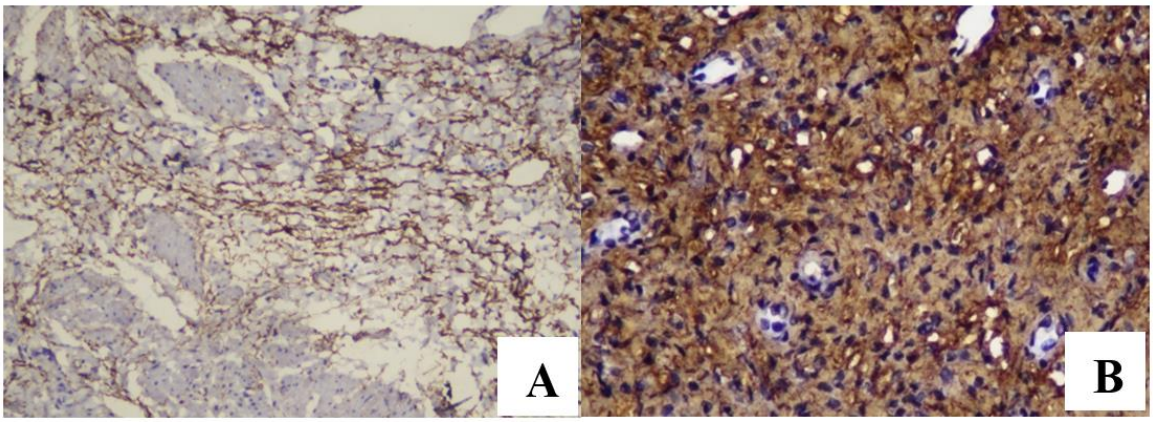

Figure 2. Results of IH staining with elastin antibodies on the cytoplasm of elastin fibers with strong intensity: A. 200x microscopic magnification, B. 400x microscopic magnification.

Table 2. Comparison of collagen-1 and elastin expression between the elongation group, elongation group with POP, POP group, and control group

	Groups				Total	p-values
	CE (n=8)	CE with POP (n=8)	POP (n=9)	Control (n=9)		
Collagen-1 expression						
Negative	-	-	-	-		
Low	-	-	-	-		
Moderate (n%)	0 (0.00)	2 (15.38)	7 (53.85)	4 (30.77)	13	0.010
High (n%)	8 (38.10)	6 (28.57)	2 (9.52)	5 (23.81)	21	
Elastin expression						
Negative(n%)	2 (25.00)	3 (37.50)	2 (25.00)	1 (12.50)	8	
Low (n%)	5 (35.71)	2 (14.29)	2 (14.29)	5 (35.71)	14	0.250
Moderate (n%)	1 (8.33	3 (25.00)	5 (41.67)	3 (25.00)	12	
High (n%)	-	-	-	-	-	

In Figure 2 we can see from the CPI results, Elastin appears to be colored brown. Elastin stained positively in the cytoplasm of Elastin fibers with strong intensity at microscopic magnifications of 200 times and 400 times. Measurement of Elastin expression on each slide was carried out in 5 random fields of view with a magnification of 400 times.

There was a significant difference in collagen-1 expression between the CE, CE with POP, POP, and control group with the results of the Kruskal-Wallis test p = 0.01 (Table 2). There was a significant difference in the proportion of subjects in the 4 study groups between subjects with moderate and strong Collagen-1 expression ($p < 0.05$). Most subjects with moderate Collagen-1 expression (n=7; 53.85%) were subjects with POP. Meanwhile, among 21 subjects with strong collagen 1 expression, 38.10% (n=8) were subjects with elongation and only 9.52% (n=2) were subjects with POP. This shows that the POP group is the group with the most subjects who have lower collagen 1 expression compared to the other 3 groups and the fewest number of subjects who have strong collagen expression. On the other hand, the group with elongation had the highest number of subjects with strong Collagen-1 expression compared to the other 3 groups, and the fewest number of subjects had moderate Collagen-1 expression.

Table 3. Comparison of collagen-1 expression between groups

Groups	CE	CE + POP	POP
CE + POP	1.000	-	-
POP	0.012	0.239	-
Control	0.025	1.000	1.000

The Mann-Whitney post hoc test resulted in a significant difference in Collagen-1 expression in the comparison between CE and POP group (p = 0.012) and between CE and control group (p = 0.025) (Table 3). The number of samples with strong Collagen-1 expression level in CE was greater (n=8; 61.54%)

compared to POP (n=2; 22.2%) and control group (n=5; 38.46%), respectively. Comparison of Collagen-1 expression between other groups did not reveal any significant differences. The results of the Kruskal-Wallis test showed no significant differences in Elastin expression levels between CE, CE with POP, POP group and control group. There was a significant difference in the proportion of subjects in the 4 research groups between subjects with negative, low and moderate elastin expression ($p < 0.05$). The group with overall moderate elastin expression (n = 9) was the control group. Meanwhile, of the 18 subjects with strong elastin expression, 50% (n = 9) were the control group and only 5.56% (n = 1) were the group with elongation. Despite the overall results, this study certainly had limitations. First, samples were surgical specimens from the last 3 years, there is a possibility of changes in tissue properties. Second, there is possibility any bias in diagnosing CE because it is not done by one person.

CONCLUSION

The expression of Collagen-1 level in the CE group was stronger than in control group and CE with POP group, while the expression of Elastin in the CE, CE with POP, and POP did not have a significant differ from control group.

DISCLOSURES

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Conflict of interest

We, all authors have no conflict of interest.

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Author contribution

E Mardiyan Kurniawati, G Hardianto project development, manuscript editing, and approval of the final manuscript. E Hari Kusumastuti, B Iman Santoso, and B Utomo manuscript editing and approval of the final manuscript. A Widyasari project development, data management and analysis, manuscript editing, and approval of the final manuscript.

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SYSTEMATIC REVIEW

Evaluation of anti-Mullerian hormone as parameter of ovarian function in patients with systemic lupus erythematosus: A systematic review and meta-analysis

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ABSTRACT

Objective: The assessment of ovarian function in patients with systemic lupus erythematosus (SLE) holds paramount importance for both clinicians and patients. This systematic review and meta-analysis delves into the role of anti-Mullerian hormone (AMH) as a key marker in evaluating ovarian function among SLE patients. Our study aims to provide valuable insights for clinicians managing ovarian function assessments and to offer practical recommendations for differences in therapy for patient care.

Materials and Methods: Studies comparing serum AMH levels between patients with systemic lupus erythematosus and healthy controls, as well as serum AMH levels between SLE patients, are necessary. PRISMA guidelines were used for this systematic review. Databases like PubMed, SCOPUS, EuropePMC, ProQuest, and Cochrane Central were searched using specific terms ("Anti-Mullerian Hormone" or "Ovarian Function" and "Systemic Lupus Erythematosus") for publications between 2000 and 2023. After removing duplicates, authors screened remaining articles based on abstracts, then reviewed selected abstracts in full-text. Studies meeting criteria were included based on unanimous agreement among investigators, with any disagreements resolved through author consensus.

Results: Data There were 12 eligible studies. In this research, we identified a link between SLE and diminished levels of AMH. Furthermore, it was observed that SLE patients undergoing cyclophosphamide (CYC) treatment also exhibited lowered AMH levels

Conclusion: The systematic review underscores the heightened risk of reduced ovarian reserve in SLE patients. Importantly, CYC treatment emerged as a factor contributing to compromised ovarian reserve. For individuals with systemic lupus erythematosus, particularly women in their reproductive years, assessing serum AMH levels can serve as a pivotal tool to inform therapeutic decisions and preserve ovarian health. Our study contributes to enhanced clinical understanding and patient care within the realm of SLE and reproductive health.

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Highlights:

1. Systemic Lupus Erythematosus (SLE) patients show a strong correlation between Anti-Mullerian Hormone (AMH) levels and ovarian function. Lower AMH levels indicate higher risk of impaired ovarian function and diminished reserve, as revealed by this meta-analysis.
2. The comprehensive synthesis of available data in this study has important clinical implications for the management and counseling of Systemic Lupus Erythematosus (SLE) patients.

INTRODUCTION

The chronic autoimmune disorder called systemic lupus erythematosus (SLE) endures over time. Its future outlook and progression are uncertain, and its manifestations can range from mild to severe. SLE predominantly impacts women of reproductive age, with a much higher occurrence in females compared to males (9:1 ratio). The ability to conceive is unaffected. Nevertheless, the natural decline in fertility occurs as women age. Specifically, the ovarian reserve (OR), which refers to the number and quality of viable eggs in the ovaries, diminishes as women get older.[1–3]

Disease activity and medications used in treatment can lead to alterations in ovarian function for individuals with SLE. The use of drugs like cyclophosphamide (CYC), which have cytotoxic properties, can impact ovarian function in these patients, resulting in disruptions in menstrual cycles, inability to conceive, and primary ovarian insufficiency. The dosage of CYC and the patient's age are factors that contribute to the menstrual problems caused by CYC treatment. Similarly, non-steroidal anti-inflammatory drugs (NSAIDs) and high-dose corticosteroids might also contribute to the irregular menstrual cycles and infertility observed in individuals with SLE. Generally, those undergoing immunosuppressive therapy due to medication are potentially at a significant risk of infections.[4,5]

The identification of anti-ovarian antibodies has revealed a link to the early decline of ovarian function, leading to premature ovarian failure (POF) as indicated by findings. Nonetheless, the primary factor contributing to infertility in individuals with SLE has been the use of CYC therapy. For instance, in a study conducted in Thailand, 11 out of the total of n=91 SLE patients (12%) were diagnosed with early ovarian failure. A retrospective study in Lucknow, India, showed that 17% (n=6) of SLE patients experienced early menopause.[6]

In a study, 15% (n=11) of 71 SLE patients experienced ovarian failure, while 11% (n=9) encountered premature menopause. From Helsinki, Finland, a group of SLE patients reported infertility in 16% (n=33) of cases. Among patients with amenorrhea triggered by CYC, 80% exhibited sustained amenorrhea. Appenzeller et al. also documented persistent amenorrhea in individuals with SLE. Additionally, a prospective study involving 110 SLE patients from Kolkata, India, revealed that 33% (n=22) experienced gonadal insufficiency and 2.7% (n=2) encountered early ovarian failure.[7] This writing aims to provide a systematic review of anti-Mullerian hormone's role in assessing ovarian function in SLE patients, providing valuable insights to clinicians managing ovarian function evaluation and offering practical therapy recommendations.

MATERIALS AND METHODS

The systematic review was conducted using the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (Figure 1). A comprehensive search was carried out across PubMed, SCOPUS, EuropePMC, ProQuest, and Cochrane Central Databases, focusing on articles related to "Anti-Mullerian Hormone" or "Ovarian Function" and "Systemic Lupus Erythematosus" published between 2000 and 2023. Redundant findings were eliminated, and the remaining articles were assessed based on their abstracts by all authors independently to determine relevance. Following this, the complete content of the selected abstracts was meticulously reviewed, and those meeting the established criteria were included in the study. The definitive inclusion of studies was determined through consensus among all investigators, with any disparities resolved through mutual agreement among the authors.

RESULTS AND DISCUSSION

The titles and abstracts of retrieved articles were carefully reviewed to determine their potential relevance and suitability for inclusion in the review. Strict criteria, outlined in Table 1, were applied to select articles for inclusion. The search and inclusion criteria primarily aimed at identifying published studies that presented clinical findings or evaluated the use of anti-Mullerian hormone to monitor ovarian function in SLE patients. Pre-printed and grey literature journals were excluded from the search up to April 1, 2023.

The outcomes of the two independent searches were cross-referenced to identify common results. Any unmatched findings were re-evaluated by the physicians to ensure they met the inclusion criteria. There were no instances of further disagreement between the physicians. If disagreements had arisen, the relevant articles would have been excluded from the analysis. Among the articles meeting the selection criteria, full versions or pre-proofed journals were used for data analysis. Additionally, a secondary search of the listed citations was conducted to confirm the inclusion of all pertinent publications. Only articles written or translated into English were considered for this systematic review. The search period spanned from January 2013 to April 2023.

Table 1. The inclusion and exclusion criteria of article

	Inclusion criteria	Exclusion criteria
Types of studies	Randomized controlled trials that evaluated the sensitivity or specificity of AMH in SLE patients, regardless of their clinical condition, were included. Non-randomized controlled trials reporting effectiveness were considered eligible as long as the focus of the study matched the research scope. All levels of evidence, encompassing safety data, met the criteria for inclusion in the safety analysis.	The research conducted in the study did not make any reference to AMH. The analysis of efficacy excluded materials such as reviews, editorials, opinions, case reports, case series, comments, and letters that lacked primary data. Non-clinical investigations, including experimental, animal, or in vitro studies, were not encompassed. Clinical trials exhibiting substantial concerns regarding quality and a significant risk of bias were not included in the assessment of efficacy. Nevertheless, these trials may be taken into consideration for safety analyses.
Types of participants	Patients diagnosed with SLE, regardless of their age or racial background, who had undergone ovarian function assessments following the use of any medication.	Patients who have not received a confirmed diagnosis of SLE or whose diagnosis relies solely on a presumptive basis.
Types of intervention	Heparin with a low molecular weight or a systemic anticoagulant.	Documentation of the administration of antiplatelet agents. Reports instances of irregular usage of low molecular weight heparin or anticoagulants.
Types of comparators	Healthy individuals as controls or no comparison group.	

Data appraisal and extraction

Information obtained from the located publication comprised: details about study design and results, patient count, intervention duration, intervention specifics, and procedure sensitivity or specificity. This information was organized in a descriptive manner using a dedicated table (Table 2).

Quality assessment

Two authors independently assessed the quality of the studies using the Modified Newcastle-Ottawa Scale (NOS). Each study was assigned a score ranging from 0 to 9, where studies achieving a total score above 7 were categorized as high-quality. Any disparities in the quality assessment were addressed through deliberation with a third author.

The analysis encompassed a total of 12 published works. In these studies, AMH was applied in different assessment methods and for various purposes, all of which were linked to aspects of ovarian function.

In order to examine the influence of AMH serum levels on ovarian function in SLE, a total of 8 studies were incorporated. The data, which encompassed the utilization of anticoagulants with Cyclophosphamide in SLE patients, indicated a diminished AMH in this particular group (odds ratio: 0.33 [0.24, 0.46], $p < 0.0001$; I2: 86%, $p < 0.00001$).

To the best of our knowledge, this represents the most recent systematic review and meta-analysis investigating the connection between SLE and ovarian reserve. In this research, we identified a link between SLE and diminished levels of AMH. Furthermore, it was observed that SLE patients undergoing CYC treatment also exhibited lowered AMH levels.

AMH, or anti-Müllerian hormone, has become increasingly popular as a diagnostic marker for ovarian function, especially in the quantitative evaluation of ovarian reserve, which is the central focus of this review.[9,10] AMH is expressed by developing follicles before FSH-dependent selection and is detectable in the bloodstream. Ovarian reserve, defined by the quality and quantity of primordial follicles, naturally declines with age. The number of developing follicles derived from the pool of primordial follicles is directly linked to the total count of primordial follicles. Since there isn't a direct serum marker to directly quantify primordial follicles, using a marker that reflects the count of growing follicles currently serves as the most effective surrogate for assessing the quantitative aspect of ovarian reserve.[11] Both serum AMH levels and the count of growing follicles decrease as a person ages, a trend initially observed nearly two decades ago in early research. Based on these initial studies, serum AMH was promptly proposed as an indirect indicator of ovarian reserve, despite our limited understanding of the factors regulating AMH expression in the ovaries and the lack of standardized AMH assays.[6,16,17]

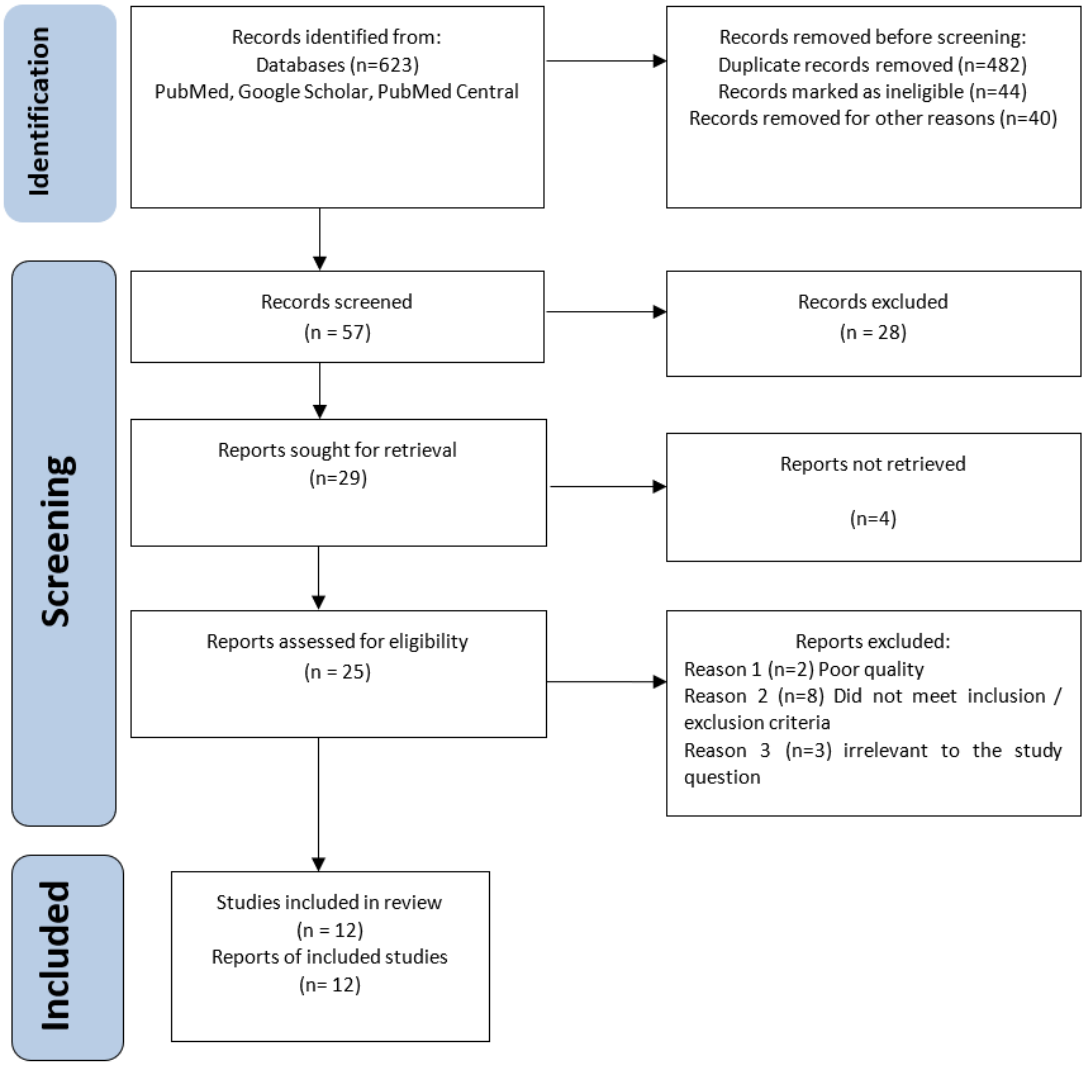

Figure1. PRISMA flow diagram for the included studies.

Table 2. Characteristics of the included studies

Author and year	Study design	Country	Quality score	Characteristics of participants	Age (mean)	SLE	Control	Comments
Sterba et al, 2016. [8]	Prospective cohort	America	7	Adolescent females aged 14 to 19 years, diagnosed with SLE according to ACR criteria, oligoarticular and polyarticular JIA based on ILAR criteria, and healthy controls with a gynecological age (calculated as chronological age minus age at menarche) of at least 2 years.	N/A	16	26	Ovarian reserve, evaluated through AMH levels, seems to be impacted in a minor fraction of pediatric SLE patients when compared to their healthy counterparts.
Mok et al 2013.[9]	Cross-sectional	China	7	Consecutive female patients aged 18 to 52 years, who had experienced menstruation at least once in the past 12 months and met more than four criteria set by the American College of Rheumatology for the diagnosis of SLE.	N/A	216	None	AMH serves as a responsive indicator of ovarian impairment caused by prior CYC exposure in women diagnosed with SLE.
Morel et al, 2013.[10]	Randomized control trial	America	9	Hospitalized COVID-19 patients	N/A	112	N/A	AMH concentrations are diminished in individuals with SLE, and they notably decline with advancing age and exposure to cyclophosphamide. Nevertheless, the likelihood of experiencing difficulties in achieving conception was minimal and was anticipated based on age and cyclophosphamide exposure, rather than AMH levels.
Ma et al, 2013.[11]	Retrospective	China	7	Assessment of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), AMH, and antral follicle count (AFC) is performed to gauge ovarian reserve in SLE patients with consistent menstrual cycles, regardless of any prior alkylating therapy.	30 ± 4,37	42	21	Significantly increased estradiol (E2) levels (P = 0.023), as well as significantly reduced AMH values (P = 0.000) and antral follicle count (AFC) (P = 0.001), were detected in both the SLE and SLE-CTX groups in comparison to the control group. This outcome suggests that even in SLE patients who were not receiving alkylating therapy, experienced regular menstruation, and had a relatively short duration of illness, their ovarian reserve remained compromised.

Gasparin et al, 2015.[7]	Case-control	USA	7	A group of 80 women who had not reached menopause, with 40 meeting the 1997 American College of Rheumatology (ACR) criteria for SLE, and the other 40 being healthy controls, matched based on their use of oral contraceptives.	32,37 ± 8,44	40	40	Females diagnosed with SLE exhibited comparable AMH levels to those of the healthy controls, indicating the maintenance of ovarian reserve.
Isgro et al, 2013.[12]	Retrospective	America	7	Assessment of AMH levels in postmenarcheal adolescent females with pediatric SLE and a background of prior CYC exposure.	N/A	23	23	The presence of CYC exposure in individuals with pediatric SLE is linked to a notable decline in AMH levels. The median AMH concentration among patients with pediatric SLE who had undergone CYC treatment was lower than that observed in patients without CYC exposure and the control group.
Kandil et al, 2022.[13]	Case control	Egypt	8	Evaluate ovarian reserve through the measurement of AMH levels in premenopausal individuals with SLE, explore various factors influencing it, and assess pregnancy outcomes among SLE patients.	N/A	30	30	There were no distinctions in AMH values between individuals with SLE and those without the condition, and the duration or intensity of the disease did not influence its level. Furthermore, the research indicated that immunosuppressive medications such as cyclophosphamide, azathioprine, and mycophenolate mofetil had no impact on fertility among SLE patients.
De Araujo et al, 2014.[6]	Cross-sectional	Brazil	7	The presence of Anti-CoL antibodies was exclusively identified in c-SLE patients (16% vs. 0%, p = 0.103), and this occurrence was not connected with demographic information, ovarian reserve measurements, disease activity or damage, or treatment. A more detailed examination of c-SLE patients subjected to cyclophosphamide treatment unveiled a higher median level of FSH compared to c-SLE patients who did not receive cyclophosphamide and the control group.	N/A	57	21	For the first time, this study revealed that a substantial cumulative dosage of methotrexate might contribute to subclinical ovarian dysfunction in adult patients with c-SLE.
Di Mario et al, 2019.[14]	Cross-sectional	Italy	7	AMH serum levels were evaluated in a consecutive group of 86 female SLE patients with consistent menstrual cycles, and this was compared with a control group of 44 healthy individuals matched for age.	31,1 ± 4,8	86	44	SLE patients exhibited AMH levels similar to those of controls. However, a decline in ovarian reserve was linked to sequential treatment involving CYC and cDMARDs, as well as the severity of the disease.
Gao et al, 20187.[4]	Case-control	China	7	The study investigates alterations in	29,4 ± 6,28	40	40	Reduced AMH levels and a notable occurrence of

				AMH, lymphocyte subsets, menstruation, and other clinical factors in individuals with SLE.				irregular menstruation revealed that the autoimmune processes associated with SLE can negatively affect the ovarian reserve in female patients. Lymphocyte functioning in individuals with SLE exhibited disarray.
Malheiro et al, 2014.[5]	Case-control	Brazil	7	The objective is to evaluate markers of ovarian reserve in females diagnosed with systemic lupus erythematosus (SLE) who experience regular menstrual cycles, and to investigate the associations between these markers, clinical features, and treatment variables.	31 ± 5	27	27	Despite women with SLE having regular menstrual cycles, there is a possibility that their ovarian reserve might be compromised. Nevertheless, it is only through extended monitoring of these individuals until the onset of ovarian insufficiency and menopause that we can ascertain whether these markers serve as dependable predictors of reproductive difficulties.
Morales-Martinez et al, 2021.[15]	Prospective	Mexico	8	The evaluation of ovarian reserve (OR) was conducted by assessing two markers, namely anti-müllerian hormone (AMH) and antral follicle count (AFC), in a group of 64 SLE patients and comparing them with individuals who have normal health.	N/A	64	70	Patients diagnosed with SLE exhibited changes in ovarian reserve (OR), irrespective of whether menstrual cycle irregularities were present. Both the antral follicle count (AFC) and anti-müllerian hormone (AMH) levels were notably lower in SLE patients, regardless of their menstrual status, when compared to the control group.

A, anticoagulant group; B,control group;COVID-19, coronavirus disease 2019; LMWH,low molecular weight heparin; N/A, not available; UFH, unfractionated heparin.

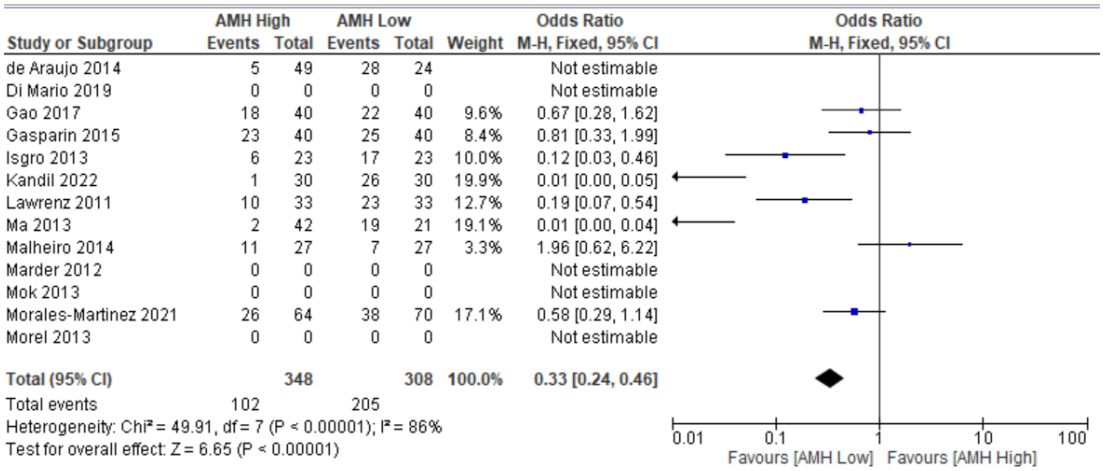

Figure 2. The meta-analysis examines the impact of serum AMH levels by comparing them between SLE patients and control groups.

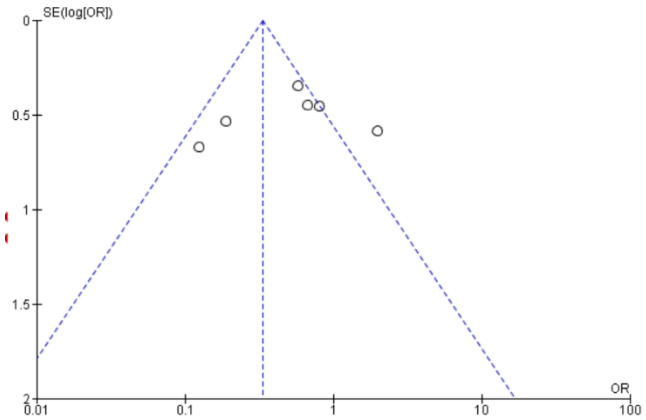

Figure 3. Funnel plot analysis

In adult females, serum AMH levels exhibit an inverse relationship with age. However, research aimed at establishing standard AMH data has also revealed that this correlation depends on the specific age group under investigation.[7,18] AMH levels rise from birth until they stabilize around the age of 25. During this period, which spans up to about 16 years of age, there is a clear positive correlation between AMH levels and age.[19–21] This positive association may be attributed to the increased recruitment rate of primordial follicles observed from birth to roughly 14 years of age. It's only after the age of 25 that a negative correlation between AMH concentrations and age becomes apparent, coinciding with the decline of AMH levels leading to menopause.[21] This pattern appears consistent across various ethnic groups over the years. Nevertheless, research suggests that at any given age, there is significant variability in serum AMH levels. This variability could be influenced by ethnicity, and it should be considered when interpreting AMH values. For instance, while Chinese women exhibit higher peak AMH levels at age 25 compared to European women, their AMH levels decline more significantly with age, resulting in a 28% reduction at age 30 and an 80% reduction at age 45, in contrast to European women.[22,23]

The remaining quantity and quality of eggs in the ovaries, referred to as ovarian reserve, serve as a predictive measure of a woman's fertility potential. To evaluate ovarian reserve, various parameters including

AFC, FSH, and AMH are frequently utilized. AMH stands out for its high sensitivity and specificity in reflecting ovarian response. AMH is produced by granulosa cells within early follicles and is not dependent on gonadotropins. As a result, serum AMH levels remain consistent throughout and between menstrual cycles.[12,24] In a study involving 12 healthy females aged 18 to 24, the highest and lowest recorded serum AMH values were 3.9 ± 1.3 ng/mL and 3.4 ± 1.1 ng/mL, respectively, indicating the stability of serum AMH concentrations throughout the menstrual cycle. Multiple other studies have also confirmed the relative stability of AMH levels throughout the menstrual cycle. Furthermore, the use of contraceptives does not appear to have a discernible impact on serum AMH levels, whether in women with or without polycystic ovary syndrome. Overall, AMH emerges as a dependable and cost-effective marker for assessing ovarian reserve.[26]

One of chronic autoimmune disorder such as SLE, is associated with a range of clinical manifestations. This condition exhibits a distinct predilection for females, and women of childbearing age are disproportionately affected. Among SLE patients, menstrual cycle irregularities have been observed in 54%, and these are linked to the activity of the SLE disease, suggesting that ovarian dysfunction is prevalent in SLE-afflicted women.[9,25] However, our systematic review, which compared serum AMH levels between SLE patients and healthy controls, yielded a pooled SMD of 0.79 (95% CI, 1.41 to 0.18), and as a result, we cannot definitively confirm the aforementioned conclusion. SLE has the potential to induce systemic inflammation, potentially implicating the ovaries, as seen in autoimmune oophoritis, which can lead to a decline in ovarian function. Persistent inflammation can also disrupt the hypothalamic-pituitary-ovarian (HPO) axis. Furthermore, SLE may impact the HPO axis, leading to elevated serum prolactin and FSH levels, as well as reduced progesterone and LH levels. This hormonal imbalance may contribute to ovarian dysfunction, resulting in issues like infertility, menstrual irregularities, and ovarian failure.[15] Nevertheless, current research on the relationship between SLE and serum AMH levels presents conflicting findings, potentially due to uneven sample sizes. Our results, derived from the combined analysis of individual studies, are considered more reliable than previous studies.

Immunosuppressive agents are commonly employed in the treatment of moderate and severe lupus nephritis, central nervous system involvement, and various other conditions.[26] Among these medications, CYC is notably associated with the most detrimental impact on ovarian health. Several investigations have highlighted that ovarian failure frequently accompanies CYC therapy, with factors such as cumulative dosage, prolonged treatment duration, and older age at the commencement of treatment influencing its occurrence. Our research consistently revealed that CYC treatment led to a reduction in AMH levels among SLE patients. In the body, CYC yields two active metabolites: phosphoramide mustard and acrolein. Of these, phosphoramide mustard predominantly instigates follicular damage within the ovaries, inducing ovarian dysfunction by instigating apoptosis in oocytes and somatic granulosa cells.[15,26] In a cohort study, it was observed that 17.5% of SLE patients treated with a dose of 0.75 mg/m2 of CYC experienced sustained amenorrhea, whereas no such amenorrhea was noted among SLE patients administered 0.5 mg/m2 of CYC. This study underscores the significance of cumulative CYC dosage as a substantial risk factor for ovarian failure. Furthermore, other studies have indicated a correlation between AMH levels and CYC dosage, although conflicting findings exist. Notably, Mok et al. reported no discernible link between CYC dose and AMH levels. Resolving the existing controversy regarding the impact of CYC dosage on ovarian reserve in SLE patients remains an important task for future research.[22] The decrease in AMH levels may not be a direct outcome of the therapy but instead could be attributed to the severity of the SLE condition that necessitates the treatment by autoimmune oophoritis itself. In other words, it's suggesting that the decrease in AMH levels might not be due to the therapy, but rather, the underlying severity of the SLE condition being treated which potentially causing premature ovarian failure (POF).[27]

There are some limitations in writing this systematic review, including the use of a single parameter to approximate ovarian function. Nevertheless, AMH still has an advantage in measuring ovarian reserve and does not require invasive tests like the others, such as FSH, E2, LH, and AFH. Lastly, the studies in the research generally have a small sample sized which leads to conflicting or inconsistent result from each individual study. Furthermore, these small sample sizes make it difficult to draw definitive conclusions from the collective research finding.

CONCLUSION

Based on comprehensive systematic review tells that SLE is linked with a raised risk of reduced ovarian function. Moreover, the treatment of CYC can be harmful to ovarian function. Individuals diagnosed with SLE, especially women in their reproductive age should consider having their serum AMH levels assessed to

assist in making treatment decisions and preserving their ovarian health.

DISCLOSURES

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Conflict of interest

No conflicting interests are present.

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Author contribution

Each author has participated in all stages of this research, encompassing preparation, data collection and analysis, manuscript drafting, and granting approval for its publication.

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SYSTEMATIC REVIEW

MicroRNAs obtained from cervical swabs in predicting preterm birth

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ABSTRACT

Objective: Identifying the risk of preterm birth is crucial for early intervention. miRNAs, small noncoding RNAs that regulate gene expression, play a key role in development and tissue maintenance. Under stress conditions, their regulatory functions become significant, linking them to disease states. Using miRNAs from cervical swabs as potential biomarkers could revolutionize preterm birth risk assessment. This systematic review examines current research on the effectiveness of cervical swab miRNAs in predicting and estimating preterm birth risks, aiming to enhance early detection and management strategies for preterm births.

Materials and Methods: Using the PubMed database, 14 articles were obtained using the keywords "microRNA" and "preterm". Reviews and unrelated studies were then excluded from both pooled articles, resulting in 4 articles included in the final review. Risk of bias were examined using the Newcastle Ottawa Scale. Sample characteristics, time of cervical swab collection, and results from each study were extracted for further analysis.

Results: A total of 4 articles were included in this review. Various miRNAs were examined in and were generally successful in predicting preterm birth. miRNA-145, miRNA-199, miRNA-30, miRNA-21, and miRNA-181 family were examined by multiple studies and produced significant results in predicting preterm birth. Based on enrichment analysis, various miRNAs were found to be involved in several biomolecular signaling pathways leading to preterm birth, such as inflammation, chemokine and cytokine signaling pathway, and toll-like receptor signaling.

Conclusion: miRNAs obtained from cervical swabs exhibit statistically significant difference in expression between women with term births and preterm births. Further studies are needed to improve the predicting power and accuracy of miRNAs in preterm births.

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Highlights:

1. Increased expression of certain miRNAs in women experiencing preterm birth could be linked to various molecular pathways which contributes to preterm birth.
2. miRNAs obtained from cervical swabs exhibit statistically significant difference in expression between women with term births and preterm births.

INTRODUCTION

Preterm birth is common and frequently encountered problem in the world. A total of 13.4 million babies were estimated to be born preterm, defined as delivery before 37 weeks of gestation, in 2020.[1,2] Preterm birth is affected by various etiologies, such as individual and environmental factors.[3,4] Some of the known risk factor for preterm birth are adolescent pregnancies, advanced maternal age, low maternal education, and history of previous preterm birth.[5,6] Preterm birth is also accompanied by various related morbidities, such as cerebral palsy, bronchopulmonary dysplasia, and retinopathy of prematurity.[7] The consequences of preterm birth also persist until early adulthood, causing worse neurodevelopmental outcomes, higher rates of hospital admissions, and learning difficulties.[8,9] Identification of possible risk of preterm birth in pregnant woman bears a lot of importance in preparing and taking early action against preterm birth.[10,11]

Clinical studies show that a short cervix is one of the most reliable indicators of preterm delivery.[5,6] However, the molecular equivalent of a sonographic short cervix is still unclear. Indeed, while a sonographic short cervix is thought to be a proxy for "premature cervical remodeling," there is a dearth of information showing what is truly happening (molecularly, biochemically) in cervical tissue at the time a sonographic short cervix is detected. This knowledge is crucial to developing more effective preterm birth prevention techniques. It is well understood that cervix remodeling occurs at any gestational age in order for the fetus to pass through. Investigations on cervical biopsies of women at term showed that gene expression patterns in the cervix differed between laboring and nonlaboring women, as well as between women with and without a ripe cervix. Although these studies shed some light on the process of cervical ripening at term, they use a more intrusive way to determine the molecular phenotype of the pregnant cervix. Notably, no studies have been conducted to far that give information on the molecular changes that occur in the cervix and may be connected with early cervical remodeling and preterm parturition.[12]

miRNAs are tiny, noncoding, single-stranded RNAs that average 22 nucleotides long. miRNAs control gene expression by binding to messenger RNA (mRNA) and repressing it, but they may also trigger translation and regulate transcription.[13] Most species have fewer miRNAs (estimated at around 1000 in the human genome) than mRNAs (roughly 30,000). However, a single miRNA may influence hundreds of mRNAs, resulting in profound and far-reaching impacts on gene expression networks. miRNAs appear to play a fundamental function in controlling development and tissue homeostasis through specialized regulation or "fine-tuning" of gene expression. However, in specific situations (e.g., stress, inflammation, hypoxia), the functions of miRNAs become evident, suggesting that miRNAs play a vital role in disease states.[12] The use of miRNAs obtained from cervical swabs to predict possible risk of preterm birth could be revolutionary in the management of preterm birth. The properties of miRNAs, such as its stability in room temperature and changes in pH, make them ideal biomarkers.[14]

This systematic review aims to summarize the current findings of researches regarding the plausibility and effectivity of miRNAs obtained from cervical swabs to predict and estimate the risk of possible preterm birth.

MATERIALS AND METHODS

Search strategy

The keywords used in the search were “microRNA” and “preterm”. The results following the search were reviewed by the reviewer to determine the eligibility of the study. This study was conducted according to the PRISMA guideline.[15]

Inclusion criteria

The inclusion criteria for the study encompass several key aspects. Firstly, the study must be published in English. Secondly, it should specifically focus on investigating the association between microRNAs extracted from cervical swabs and preterm birth. Thirdly, the publication date of the study must fall within the timeframe from the year 2000 onwards.

Exclusion criteria

The exclusion criteria for the study delineate specific conditions that render studies ineligible for inclusion. Firstly, studies not published in English are excluded from consideration. Secondly, studies conducted in vitro are also excluded from the scope of inclusion. Additionally, studies exploring microRNA obtained from sources other than cervical swabs are excluded. Finally, studies published prior to the year 2000 are not considered within the defined parameters.

Selection process

Utilizing the PubMed database, 14 articles were identified and screened for eligibility. One review article was excluded, leaving 13 articles left for full-text

retrieval. All 13 articles were available for retrieval and were further assessed for eligibility for the final review. A total of 9 articles were excluded from the 13 articles assessed. The reason for exclusion were : studies done in animals (n = 2), study with HIV patients as population (n = 1), miRNAs examined from blood plasm (n = 1), no cervical swabs examined as object of interest (n = 2), studies examining other types of RNA (n = 1), and studies examining other markers (n = 2). The final review included a total of 4 articles that fulfills the criteria described beforehand. Article selection process described in Figure 1.

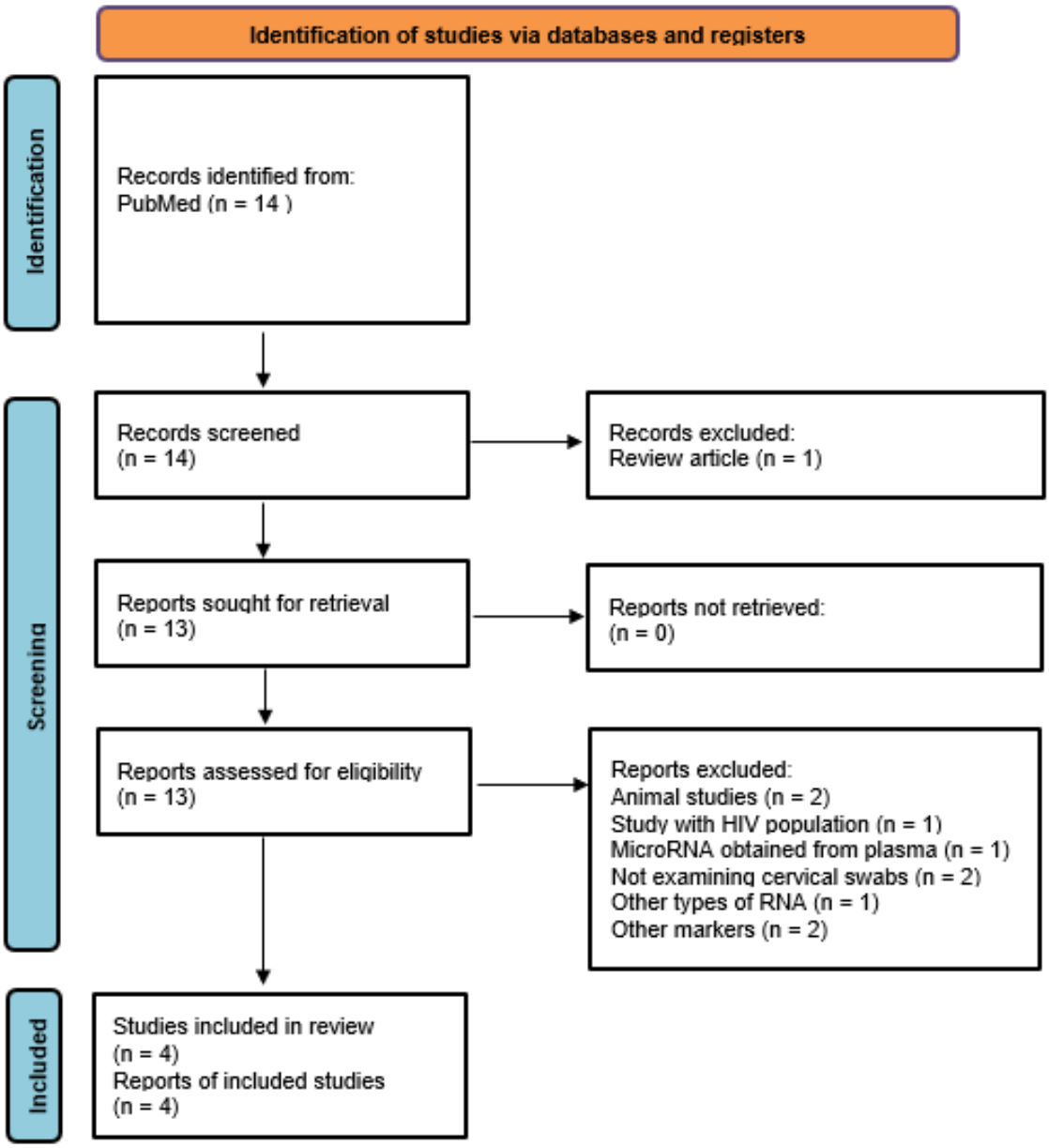

Figure 1. Article selection process according to PRISMA flowchart.[15]

Risk of bias assessment

Risk of bias assessment in animal studies were done using the Newcastle Ottawa Scale for case-control and cohort studies. The Newcastle Ottawa Scale examines three components: selection, comparability, and outcome. The selection components were examined using 4 indicators, those indicators being: representativeness of the exposed cohort, selection of the non-exposed cohort, ascertainment of exposure, and demonstration that outcome of interest was not present at start of study. The comparability component was examined by reviewing the comparability of cohorts on the basis of the design and analysis. Lastly, the outcome component was judged by using 3 indicators, which are: assessment of outcome, whether the follow-up was long enough for outcomes to occur, and adequacy of follow up of cohorts.[16] Risk of bias assessment were described in Table 1.

Table 1. Risk of bias assessment

	Selection	Comparability	Outcome
Burris et al., 2023	****	**	***
Sanders et al., 2015	****	*	***
Peng et al., 2020	****	*	***
Elovitz et al., 2014	****	*	****

Data Extraction

The following data were extracted from the studies included: author name, year of publication, study participants, time of cervical swab collection, and results. Extracted data were described in Table 2.

Table 2. Study characteristics

First Author	Year	Design	Participants	Collection of cervical swab	Results
Burris	2023	Case-control	sPTB : 25, control : 49, subjects were matched by age, parity, and race	Mean gestational age of 17.1 weeks (4.8 months) of gestation	Cervical microRNA expression was significantly higher in the sPTB group compared to control group. A total of 95 individual microRNAs were significantly upregulated (>2-fold change) in the sPTB group compared to control group.
Sanders	2015	Prospective cohort	53 pregnant Mexican women	Between 16 and 19 weeks of gestation	A total of 6 miRNAs were significantly associated with gestational age at the time of delivery (miR-21, miR-30e, miR-142, miR-148b, miR-29b, miR-223). Gestations were 0.9 days shorter on average for each doubling in miR-21 expression and by 1-1.6 days shorter on average for each doubling of miR-30e, miR-142, miR-148b, miR-29b, miR-223.
Peng	2020	Case-control	PTB : 52, PPROM : 60, term birth : 70	Within 30 minutes of delivery	A significant decrease in miR-199a-3p in both the PTB and PPROM groups compared to term birth group.
Elovitz	2014	Case-control	PTB : 10, term birth : 10	Between 20 weeks to 23 weeks 6 days gestation and between 24 weeks to 27 weeks 6 days gestation	A total of 99 miRNAs differed between the PTB and term birth group and 24 miRNAs had a >2-fold change between the PTB and term birth group.

Table 3. Significant miRNAs analyzed in the studies

	First Author			
	Burris	Elovitz	Sanders	Peng
Significant miRNAs mentioned by multiple studies	145	145	-	-
	199b	199a-3p / 199b-3p 199b-5p	-	199a-3p
	30a-5p 30d 30e.3p	30e	30e-5p	-
	29a	-	29b-3p	-
	21	-	21	-
	181c	181a-1 / 181a-2	-	-
Significant miRNAs mentioned by only one study	28.3p	140-5p	142-3p	-
	23b	338-5p	148b-3p	-
	542.3p	19a	223-3p	-
	29b	148b	-	-
	130b	106b	-	-
	28	128-1 / 128-2	-	-
	425.5p	3176	-	-
	9	374b	-	-
	342-3p	.24-02	-	-
	1291	15a	-	-
	7	25	-	-
	942	1260	-	-
	-	143	-	-

RESULTS AND DISCUSSION

A total of 4 articles were included in the final review.[12,13,17,18] Risk of bias assessment utilizing the Newcastle Ottawa Scale resulted in no significant risk of bias in the articles included. A summary of study characteristics could be found in Table 1 and 2.

Studies authored by Burris et al. and Elovitz et al. both produced significant results for miR-145. Burris et al. reported a fold change of 3.2 (OR : 1.71, 95% CI : 1.21-2.40, p = 0.0021) and Elovitz et al. reported a fold change of 11.5 (p = 0.0009).[12,13] The miR-199 family was found to be significant by Burris et al. (miR-199b, fold change : 6.7, OR : 1.48, 95% CI : 1.09-2.00, p = 0.012), Elovitz et al. (miR-199a-3p/miR-199b-3p, fold change : 7.2, p = 0.0048 and miR-199b-5p, fold change : 2.4, p = 0.0041), and Peng et al. (p < 0.001).[12,13,17] The miR-30 family was found to be significant by Burris et al. (miR-30a-5p, fold change : 2.2, OR : 1.9, 95% CI : 1.25-2.88, p = 0.0027, miR-30d, fold change : 2.1, OR : 1.95, 95% CI : 1.25-3.05, p = 0.0035, and miR-30e.3p, fold change : 2.6, OR : 1.47, 95% CI : 1.11-1.95, p = 0.0078), Elovitz et al. (miR-30e, fold change : 2.9, p = 0.0047), and Sanders et al. (miR-30e-5p, p = 0.016).[12,13,18] The miR-29 family was found to be significant by Burris et al. (miR-29a, fold change : 3.9, OR : 1.62, 95% CI : 1.17-2.24, p = 0.0039) and Sanders et al. (miR-29b-3p, p = 0.045).[13,18] miR-21 was found to be significant by Burris et al. (fold change : 2.6, OR : 1.48, 95% CI : 1.10-1.98, p = 0.0091) and Sanders et al. (p = 0.009).[13,18] The miR-181 family was found to be significant by Burris et al. (miR-181c, fold change : 3.1, OR : 1.37, 95% CI : 1.07-1.74, p = 0.0115) and Elovitz et al. (miR-181a-1/ miR-181a-2, fold change : 3.81, p = 0.0048).[12,13] A summary of the miRNAs mentioned in the studies could be found in Table 3.

Utilizing the data obtained in the study, Burris et al. performed an AUROC calculation, resulting in a value of 0.71, successfully predicting sPTB 71% of the time.[13] miRNA enrichment analysis also shown several pathways that could contribute in PTB (inflammation, chemokine and cytokine signaling pathway (IL-4 and IL-1), and Toll-like receptor signaling).[13,19,20] Peng et al. in the investigation of miR-199a-3p, discovered a link between miR-199a-3p and the HMGB1 signaling in the inflammation pathway.[17] miR-199a-3p inhibits the expression of HMGB1, which activates TLR4, leading to NF-kB activation, inducing the production of pro-inflammatory cytokines (IL-1 beta, TNF alpha, and others).[17]

Results from multiple studies suggests that the increased expression of certain miRNAs in women experiencing preterm birth could be linked to various molecular pathways which contributes to preterm birth. The miRNAs found to be significant in multiple studies could be utilized as a ground for further research regarding those specific miRNAs. An AUROC value of 71% obtained by Burris et al. suggests the potential of miRNAs obtained from cervical swabs in predicting and estimating the risk of preterm birth in pregnant woman.[13] Further studies with a larger sample size,

performed in more genetically diverse participants, and implementing AUROC calculations could pave the road for future research and implementations on this topic.

The strength of this systematic review lies in its topic novelty. Research in the use of miRNAs obtained from cervical swabs in predicting preterm birth is a rather new and novel concept, being only examined by a select few studies. Our summarized findings from multiple studies could lead to a development of further studies examining the applications of miRNAs obtained from cervical samples to predict future preterm birth with emphasis on miRNAs found to be significant and appearing in multiple studies such as the miRNA-145, miRNA-199, miRNA-30, miRNA-21, and miRNA-181 family. Future research could draw inspiration from this finding and focus on combining these miRNA families into a more definitive and comprehensive sampling and examination, resulting in a more detailed and powerful predictive value. The limitations of this study are its lack of available material, again, due to its novelty in topic. Further research is highly encouraged in this topic due to its proven significance, proved by multiple studies. Further studies down the line could discover many more miRNAs that could possibly contribute in the development of preterm birth. The studies included in this systematic review are mostly case controls, with only one cohort study identified in the literature searching process. Generally, case control studies suffer from multiple limitations, such as recall bias and selection bias. The use of the Newcastle Ottawa Scale risk of bias assessment tool helped to mitigate any possible potential for bias, but cannot eliminate completely the disadvantages of a study design. A cohort study design is more preferable due to its strength of establishing the causality between events, in this case between miRNAs and the event of preterm birth. However, due to the nature of cohort designs requiring more time and resources, this study design is harder to execute in actual research setting. Another limitation of the study is the various time point where cervical swabs are collected, complicating the comparison process between studies and its results. A future study could be done in collecting cervical swabs in multiple time points in one pregnant woman in order to discover the optimal point of cervical swabs sampling with emphasis on the fold change expressed by various miRNAs in various time points. Such study could be done in conjunction with the antenatal care service provided by obstetricians in a regular clinical setting. The sample characteristics displayed in multiple studies also makes it rather difficult to apply the findings to the general population, necessitating a need for research in detecting which miRNAs are most prominent in each population base.

CONCLUSION

miRNAs obtained from cervical swabs exhibit statistically significant difference in expression between women with term births and preterm births. Further studies are needed to improve the predicting power and accuracy of miRNAs in preterm births.

DISCLOSURES

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Conflict of interest

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Author contribution

All authors have contributed to all processes in this research, including preparation, data gathering and analysis, drafting and approval for publication of this manuscript.

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REVIEW ARTICLE

Current preeclampsia prediction model and biomarker

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ABSTRACT

Preeclampsia (PE) is a serious hypertensive disorder that occurs during pregnancy and is often accompanied by proteinuria (excessive protein in the urine), posing significant risks to both maternal and neonatal health worldwide. PE is a leading cause of maternal and neonatal morbidity and mortality and is notably challenging to predict due to its unpredictable nature and steadily rising incidence rates globally. As a result, substantial efforts have been directed toward developing predictive models and identifying biomarkers to assess the risk and progression of PE. However, existing models vary widely in their design, methodologies, and efficacy. Current prediction models recommended by notable organizations, including the National Institute for Health and Care Excellence (NICE), the American College of Obstetricians and Gynecologists (ACOG), the Fetal Medicine Foundation (FMF), and the World Health Organization (WHO), generally involve screening based on maternal characteristics and known risk factors. These include parameters such as maternal age, body mass index (BMI), number of pregnancies and births, blood pressure, and uterine arterial pulse index (UtA-PI). Additionally, biomarkers like mean arterial pressure (MAP), UtA-PI, and the ratio of soluble fms-like tyrosine kinase-1 to placental growth factor (sFlt-1/PlGF) are employed to improve predictive accuracy. Despite the diversity of predictive models and biomarkers, there is no consensus on the optimal model for PE prediction, largely due to the limitations in comparative studies and the challenges involved in cross-study comparisons. However, literature suggests that the FMF model demonstrates superior detection capacity compared to other predictive models.

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Highlights:

1. Most studies report that FMF predictive models involving a combination of maternal factor screening and biomarkers have significantly better detection capacity than risk factor screening alone.
2. All predictive models generally use maternal factors as the basis for calculations and algorithms.
3. Several biomarkers that have been reported in studies to act as elements of prediction models include MAP, UtA-PI, and the ratio of sFlt-1/PlGF level.

INTRODUCTION

The mortality and morbidity among pregnant are mostly caused by hypertension worldwide. It affects 1 in 10 women worldwide, with 20% pre-existing (chronic) and 80% de novo defined as gestational hypertension or preeclampsia.[1] Almost 15% of mortality among pregnant women is caused by preeclampsia or eclampsia. It has also ranked second or third in global rankings of maternal causes of morbidity and mortality, particularly in low to middle-income countries.[2–4] Hypertension in pregnancy includes gestational hypertension, preeclampsia, and eclampsia, characterized by enhanced blood pressure and multiorgan dysfunction. The World Health Organization (WHO) also reported that hypertension is a major leading for maternal death, which accounted for 14% of cases.[3]

During pregnancy, PE could be one of the complications among pregnant women that can impact the condition of the mother and the baby. It even leads to morbidity and mortality globally each year.[1] Preeclampsia happens when there is new-onset hypertension with positive proteinuria in ≥20 weeks of pregnancy, along with any of the following characteristics: defective placentation, the occurrence of placental ischemia, abnormal spiral artery remodeling conditions, increased oxidative stress at the maternal-fetal interface, and an imbalance between angiogenic in the maternal circulation against subsequent endothelial and end-organ damage. This condition can also lead to maternal complications such as placental abruption, kidney disease, eclamptic seizures, and HELLP syndrome. Moreover, fetal growth disturbance and respiratory distress syndrome in neonates could be complications in a fetus with a history of preeclampsia during pregnancy.[5]

Over the past 50 years, there has been a trend toward an increase in the incidence of these exacerbations in low and middle-income countries.[6,7] Previous studies found that most PE-related deaths were due to inadequate treatment. Hence, the maternal and fetal condition in PE cases was associated with broad access to services, quality care, and management of complications, which leads to improved perinatal outcomes. Moreover, better outcomes for both the mother and the baby could arise by precising and early identification and treatment of preeclampsia patients.[8]

Unfortunately, the onset and disease severity are still unpredictable despite the high quality of hospital equipment.[9] Preeclampsia remains one of the major pathological manifestations of preterm birth, approximately 15% of all preterm births10–12, and increases the length of stay of the mother or newborn in the ICU. This condition leads to the enhancement of health care costs.[13] To date, several predictive models have been developed to investigate the risk and development of PE and even the development of biomarkers that can be evaluated in early pregnancy. However, these predictive models have different characteristics and capacities. Some recent studies have shown different findings regarding the ability and capacity of existing predictive models. Therefore, this review aims to identify and assess the current prediction models for PE.

The literature search was carried out from January 2022 to January 2023 through three online scientific journal databases, namely ScienceDirect, PubMed and Google Scholar. The keywords used in the search were "preeclampsia", "biomarker", "prediction", "model", and "diagnosis" accompanied by the use of boolean operators such as "AND" and "OR". The inclusion criteria used in this literature review are (1) publications in the last 10 years; (2) studies use English or Indonesian. After a literature study was carried out, a study screening was then carried out so that all articles that were suitable as the main reference and had been reviewed in terms of validity. importance, and applicability.

PREECLAMPSIA

Definition

Preeclampsia is a condition that only affects pregnant women with hallmarks, including hypertension (high blood pressure) and endothelial dysfunction, which cause extensive end-organ damage. This end-organ damage usually can be evaluated in the liver, blood, brain, placenta, and especially kidneys, represented in proteinuria. Preeclampsia is a systolic blood pressure ≥140 mmHg and/or ≥90 mmHg with positive protein in the urine.[14–16]

Preeclampsia after twenty weeks of pregnancy is diagnosed by the occurrence of new hypertension that is accompanied by one of the following conditions1, 1proteinuria (24-hour proteinuria above 300 mg/24 hours, a urine dipstick test with a result of ≥1+, or enhancement of the proteinuria/creatinine ratio (>0.3 mg/mg); 2), there is maternal organ dysfunction, such as renal impairment (creatinine level > 1.02 mg/dL), liver impairment (transaminases levels arise twice above normal or there is a pain in the right hypochondrium), and persistent neurological symptoms such as scotoma or cephalgia which is followed by hyperreflexia or mental state disturbance);3) dysfunction of utero-

placental which can be assessed from the existence of fetal growth limitation.[16,17]

Risk Factors

Preeclampsia in prior pregnancies, nulliparity, extreme pregnancy age (<20 years old or >40 years old), and African American descent are major risk factors for PE. Body mass index (BMI) of more than 35 kg/m2 and use of contraceptives,[18,19] pre-existing chronic medical history such as chronic hypertension, diabetes mellitus, renal disease, antiphospholipid antibody syndrome, and chronic hypertension[17,19] were also identified as risk factors for PE. Furthermore, previous studies showed that PE rates are also influenced by multiple pregnancy rates and the mean age of women at first pregnancy.[20–22]

The National Institute for Health and Clinical Excellence (NICE) also identified risk factors for PE, which are divided into two categories, namely "moderate risk" and "high risk." This classification aims to provide a tool that can be used to determine which group needs to adopt preventive actions immediately. The conditions are considered "high risk," including any history of hypertensive disorders during prior pregnancies, autoimmune (antiphospholipid antibody syndrome or SLE), diabetes, chronic kidney disease, and persistently high blood pressure. The following conditions are deemed to be "moderate risk," including age above 40 years old, 10 years or more duration between pregnancies, BMI above 35 kg/m2, family history, multiple pregnancies, and primiparous women.[8]

Classification

The classification of PE also affects the timing of applying the appropriate condition. There are several classifications for the disease severity by measuring blood pressure, such as mild PE and severe PE. Mild PE occurs when the blood pressure is 140/90 or more in two inspection times; each inspection is at least 6 hours apart and without organ damage. Severe PE occurs when the blood pressure is 160/110 or more with target organ damage that is represented by proteinuria, pulmonary edema, oliguria, headache, epigastric pain, or oligohydramnios. In addition, there is a classification based on the onset of events, namely early onset (<34 weeks of gestation) and late onset (>34 weeks of gestation).[23]

The classification based on the onset of events is generally based on the differences in etiology and pathogenesis. In early onset, the development of PE commonly involves abnormal placentation under hypoxic conditions. One of the initial examinations that can be performed is Uterine Doppler. Several studies have reported high accuracy in analyzing patients who will subsequently develop early-onset PE. On uterine doppler examination, there will be signs of abnormally high impedance in uterine blood flow, which causes failure of the spiral arteries to form. Early-onset PE generally has a poorer prognosis due to early developmental delay than late-onset PE.[16] In late-onset (also called maternal PE), the development of PE is generally involved in interactions between placental tissue and abnormal maternal factors, such as endothelial dysfunction and oxidative stress. However, the two types of PE are generally indistinguishable clinically because of similar manifestations, especially in the presence of abnormal placentation in both types of PE.[16]

SCREENING AND PREDICTION MODEL

Clinical guidelines for PE prediction models generally involve screening for risk factors, including the number of pregnancies and births, BMI, blood pressure, maternal age, and uterine arterial pulse index (UtA-PI). A recent systematic review involving 70 studies with 425,125 participants also reported that the most commonly used predictive models were patient characteristics and risk factors.[24] Based on findings in literature searches, preeclampsia prediction models generally use clinical findings and maternal history in overall risk assessment. A number of studies also report the use of supporting examinations in the form of blood biomarker examinations to increase predictive value and make more appropriate treatment decisions, such as placental protein which will be discussed later. Four models have been developed by the most commonly used organizations, including guidelines by The National Institute for Health and Care Excellence (NICE), the American College of Obstetricians and Gynecologists (ACOG), and Fetal Medicine Foundation (FMF), and the World Health Organization (WHO) models. Based on previous studies, for gestational age <32 weeks, the ACOG model detected 94% of PE, NICE detected 41% of PE, and FMF detected 100% of PE.[25] Both NICE and ACOG have been reported for use in the entire population of pregnant women without the need for additional testing. However, the FMF model uses additional biomarker tests that aim to increase sensitivity compared to NICE or ACOG.[26,27] All prediction model discussed in this review is described in Table 1.

National Institute for Health and Care Excellence (NICE)

Following the NICE recommendation, a person is determined to have a high risk of experiencing PE if you

have more than one moderate risk factor such as nulliparity, age over 40, BMI more than 35 kg/m2, a family history of PE, or an interval between pregnancies that is more than 10 years) or one of the risk factors tall. The high-risk factors include hypertension in previous pregnancies, chronic kidney disease, diabetes mellitus, or autoimmune diseases. Thus, NICE guidelines also recommended taking an aspirin of 75 to 150 mg daily starting at 12 weeks of pregnancy and continuing until labor.[1,25,27]

American College of Obstetricians and Gynecologists (ACOG)

The ACOG guidelines are quite similar to the NICE guidelines. Still, additional factors, such as a history of small gestational age and sociodemographic characteristics, including African American race or low socioeconomic status, are classified as moderate risk factors.[28,29] Previous study that evaluated ACOG 2013 guidelines showed that only 5% (95%CI: 2%–14% and 2% (95%CI: 0.3%–5%) of preterm and term PE are detected in screenings, with a 0.2% false-positive rate (FPR). In contrast, screening based on the NICE recommendation achieves detection rates for preterm and term PE of 39% (95% CI: 27%-53%) and 34% (95% CI: 27%-41%), respectively, with 10% FPR.[25]

Fetal Medicine Foundation (FMF)

The FMF guideline uses a comprehensive combination of biomarkers and maternal history. Mean arterial pressure (MAP), uterine artery PI (UTPI), and serum PLGF are usually used as biomarkers at 11–14 weeks (or PAPP-A when PLGF is not available) of pregnancy. These combined maternal characteristics with MAP, UtA-PI, and PlGF levels have been shown to improve the prediction of PE in the first trimester of pregnancy. The screening program for preeclampsia (SPREE) study has found that this combined approach has a better detection rate than the NICE method (82.4% vs. 40.8%).[27] As a pooled analysis showed that the combined approach detected 90% of early PE in <32 weeks of gestational age. Therefore, this combined approach is generally recommended for early-onset PE. Furthermore, with a 10% FPR, the overall FMF combined test provides detection rates of 90% for early PE and 75% for preterm PE, respectively. Therefore, it can be concluded that a screening based on the FMF recommendation should be carried out whenever possible.[27,30]

Table 1. PE prediction models based on international organizations guidelines

Prediction model (years)	Country	Description	Evidence
NICE (2019)	United Kingdom	Utilized into two categories of risk factors, namely high risk with a history of pregnancy before with PE, persistent hypertension, autoimmune, diabetes mellitus, chronic kidney disease, antiphospholipid syndrome) and moderate-risk (nulliparous woman, advanced age more than 40 years old, gestational interval more than >10 years, BMI more than 35, family history, and multifetal gestation.	Applicable and feasible to access for all women and can be used to give prophylactic aspirin, but with modest detection capacity
ACOG (2018)	United States of America	Similar to NICE. However, multifetal gestation is categorized as a high-risk factor, and the addition of socio-demographics is moderate-risk.	Applicable and feasible to access for all women and can be used to give prophylactic aspirin, but with modest detection capacity
FMF (2021)	Global	Combination of maternal risk and several biomarkers such as MAP, UTPI, and serum PLGF. When the PLFG is not available, the PAPP-A could be used.	Archives have a higher detection capacity even in early pregnancy, but with additional cost, thus can not be applied in all screening settings.
WHO (2021)	Global	Traditional risk factors of PE, including a history of PE before, diabetes, persistent hypertension, renal disease, autoimmune disease, and multiple gestations	Applicable and feasible to access for all women and can be used to give prophylactic aspirin, but with the lowest detection capacity

World Health Organization (WHO)

In WHO guidelines, examination and assessment are only focused on assessing risk factors, including previous PE in earliest pregnancy, diabetes, chronic hypertension, renal disease, autoimmune disease, and multifetal pregnancy. However, WHO guidelines are more commonly used globally because the previous model used only a certain country's population base. In addition, WHO recently issued additional guidelines regarding drug management and preventive therapy for women at risk of PE, including calcium supplementation and antiplatelet therapy if needed, such as aspirin.[31,32]

In general, all prediction models use the evaluation of maternal risk factors as the main basis. However, each guideline or recommendation's systematic calculations and algorithms can cause very different detection rates. So far, the test, combined with other tests and biomarkers, such as FMF, is reported to have the best detection capacity.[33] In addition, the administration of aspirin with adjusted doses has also been reported to have good benefits in all types of prediction models.[33,34] However, the application of the prediction model is also strongly influenced by the target population. The detection capability and validity of the prediction model can vary widely in different countries. This is due to the different maternal characteristics between countries; for example, women of Asian race have a lower average BMI than Caucasian.[31,32] This causes the need to test the validity and adaptation of the model, such as the application of the Bayes-theorem in the FMF model in Asian populations.[35]

In addition, there is a new model that also requires attention, namely the Full Preeclampsia Integrated Estimate of Risk (FullPIERS) that utilizes and assesses not only the signs and symptoms of the mother but also so the laboratory finding. It can be used to predict maternal side effects and perinatal outcomes in patients with PE. The FullPIERS can also be used in planning delivery times and preventing complications for both mother and child. The factors or elements used are the age of the pregnancy, chest pain or dyspnea, oxygen saturation, platelets, creatinine, and AST/ALT.[1]

Apart from applying prediction models through a number of guidelines, weaknesses and limitations of prediction models can be resolved by applying biomarker examinations during pregnancy. Based on a number of recent studies, there are a number of biomarkers that can be used and are being developed in the diagnosis and treatment of preeclampsia. This group of biomarkers was developed based on the pathophysiology and mechanisms involved in the development of preeclampsia, namely damage to placental tissue, vasculature and organ involvement which can later be detected in the patient's body fluids, including urine and blood. The biomarker products that are currently being developed to complement currently available predictive models include RNA, DNA, protein and tissue metabolite products. The placenta is generally the main tissue involved in the pathophysiology of preeclampsia, especially during early pregnancy (gestational age >11 weeks), such as placenta-enriched RNAs, adrenomedullin (Adm) which decreases during the second trimester of pregnancy in preeclampsia patients. In addition, microRNA (miRNA) is also reported to be able to predict the occurrence of preeclampsia, such as miRNA on chromosome 19 and exosomal miRNA. Furthermore, RNA examination also supports the examination of placenta-related proteins, including placental protein 13 (PP13), Pregnancy-associated plasma protein A (PAPP-A), Growth Differentiation Factor 15 (GDF-15), and alpha fetoprotein (AFP) which have been reported to have significant predictive value. The inflammatory process found in preeclampsia also causes vascular involvement, which supports the examination of vascular-related biomarkers, such as endothelin and nitric oxide (NO) proteins and their related substrates which are significantly increased in preeclampsia patients.[36–40]

BIOMARKER TESTING IN PREDICTION MODEL

Another predictive factor that can be used in the PE prediction model is the biomarkers during pregnancy. In clinical practice, biomarker tests are commonly associated with other pregnancy conditions and can also monitor fetal development. In cases of PE, biomarker examination can be performed at the beginning of pregnancy (early first trimester) and late pregnancy (second and third trimesters). Screening tests are generally used when there is some risk of PE accompanied by other risks of pregnancy disease, such as persistently high blood pressure without signs of PE or non-specific general symptoms such as persistent nausea and dizziness.[36–40]

Several key biomarkers used in cases of PE include MAP, UtA-PI, and the ratio of sFlt-1/PIGF. Previous studies have shown that the combination of PE risk assessment with biomarkers produces a better detection capacity than maternal risk analysis alone. So far, only FMF recommendations have integrated analysis of biomarker results and underwent extensive internal and external validation.[37,38,40]

MAP and UtA-PI values have been integrated with FMF algorithms and other predictive models. However, the sFlt-1/PlGF ratio has yet to be further developed. Several factors expressed in the placenta such as sFlt-1, have a role as anti-angiogenic factors and PIGF as pro-angiogenic factors. Those factors are associated with placental dysfunction. In the 20– 34 weeks of pregnancy, the sFlt-1/PlGF ratio is useful and rules out PE risk based on NICE and the European Society of Cardiology assessment.[41,42] Moreover, the sFlt-1/PlGF ratio could be detected in >37 weeks of pregnancy. Previous studies have shown that the sFlt-1/PlGF ratio has a negative predictive value (NPV) of 99.3% with a cut-off ratio of <38. However, within the next four weeks, a lower positive predictive value (36.7%) was also reported with a cut-off ratio of ≥85. Therefore, the current recommendation states that women with a 38 – 85 sFlt1/PlGF ratio require enhanced monitoring followed by a retest after 1–2 weeks or immediately if there are changes in the clinical situation. Furthermore, with a value of less than 100 pg/ml, the measurement of PlGF alone can also be employed in pregnant women suspected of having PE. This method has a sensitivity and negative predictive value (NPV) of 96% and 98%, respectively. This accuracy was claimed to be higher than common clinical tests, including blood pressure, ALT, and proteinuria.[43]

Most of the blood biomarkers studied in preeclampsia studies show high predictive value and diagnostic capacity, such as sFlt1 and PlGF with sensitivity and specificity values reaching >90% at gestational age <35 weeks. However, there are limitations to its application, including the capacity to predict positive cases ("rule in") preeclampsia and limitations in predicting preeclampsia at a gestation period of >37 weeks. In direct clinical application, the results of biomarker examinations can be changed and adapted to the conditions and characteristics of patients in certain populations.[44] The conversion method can use multiples of the median (MoM) equivalent and Bayes-theorem. These conversions are influenced by characteristics such as age, weight, and ethnicity. The MoM value is determined by dividing the actual value by the predicted value of the biomarker. In the multivariate analysis, the predicted value is determined using a formula that integrates all parameters identified as independent predictors of the biomarker.[45,46] It is also necessary to standardize and routinely monitor biomarker inspection procedures because one of the biggest obstacles in biomarker examination is unstandardized protocols for biomarker measurements and low-quality assessment, which causes inaccurate measurement results.[40]

CHALLENGE AND BARRIER

The main obstacles to applying the prediction model for PE in clinical practice are limited examination facilities and delays in evaluation or screening at primary health centers. Most prediction models are carried out in the first trimester of pregnancy. However, some pregnant women generally find clinical changes related to PE in the second and third trimesters, so cases of PE often cannot be detected and treated properly. Examinations are also generally limited to screening maternal factors and many available examination parameters. In addition, the limitations of studies that comprehensively assess each model's internal and external validity in the global population need to be revised in choosing the most suitable predictive model. Patient characteristics between studies were considerable. Several studies have evaluated the external validation of PE prediction models. In addition, no prospective study has compared the accuracy of single risk factor screening with risk prediction by one or more specified algorithms.

Comparative studies similar to systematic reviews and meta-analyses also need help comparing several studies with different models due to the high heterogeneity of studies, so there is a high risk of bias during the analysis process, especially for models with multifactor algorithms. However, in several studies, high detection capability and more effective prevention through the application of models with additional examinations and multifactor algorithms, such as FMF, show good potential in clinical applications. Apart from this, only a few studies have reported evidence and clinical utility in specific populations, especially in Asia and Indonesia. Further evaluation can also be carried out to compare different models in specific populations, especially in Asian countries, so that the number of examinations can be fewer and more specific, especially by only relying on basic tools in primary health centers.[47]

CONCLUSION

In summary, several predictive models and biomarkers have been developed to investigate the risk and development of PE. Currently, all prediction models, such as NICE, ACOG, FMF, and WHO, generally involve maternal characteristics and risk factors screening. Several biomarkers are also used, including MAP, UtA-PI, and sFlt-1/PlGF ratio. However, there is yet to be a conclusion regarding the best predictive model due to the limitations of comparative studies and some barriers to comparing studies. Based on the current literature, FMF recommendation has the best detection capacity compared to other predictive models.

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Conflict of interest

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CASE REPORT

Acute fatty liver of pregnancy: An atypical case report

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ABSTRACT

Objective: To present an atypical case of acute fatty liver of pregnancy.
Case Report: A 33-year-old woman was referred to the Emergency department of Dr. Soedarso Tertiary Referral Hospital, Pontianak, Indonesia, previously from the district hospital over 580 KM with diagnosis of multigravida (gravida 3 para 2) at 31 weeks gestation, suspected acute fatty liver disease (AFLP), severe oligohydramnios, and intrauterine growth restriction (IUGR). The patient had a history of nausea, vomiting, epigastric pain, polydipsia and polyuria, and seizure for less than 5 minutes with loss of consciousness. The patient originally did not know she was pregnant and using a contraceptive implant. She missed her period in the last six months ago, which was the first onset of epigastric pain. Further clinical examination resulted in pitting oedema but jaundice as a cardinal sign was not present. Laboratory data showed leukocytosis, normal haemoglobin level, normal blood glucose, hyperuricemia, increased function liver test with high transaminase and bilirubin, normal coagulation profile. Urinalysis showed proteinuria. The viral hepatitis and HIV tests were negative. Transabdominal ultrasound demonstrated a single intrauterine pregnancy with no echogenic liver features. Cardiotocography (CTG) showed category 2. This patient was diagnosed with AFLP based on Swansea Criteria (7 out of 10) and terminated pregnancy two days after diagnosis. A baby boy was born with birth weight 1.100 gr, birth length 34 cm, apgar score (AS) 9 and 10 at 1 and 5 minutes. The mother had a good prognosis, while the baby died on day 23 of life.
Conclusion: Acute Fatty Liver during Pregnancy is an uncommon but life-threatening obstetric emergency. Early screening, diagnosis, timely handling delivery, and intensive supportive care are essential to decrease morbidity and mortality for both mother and fetus. Multidisciplinary opinion needed for the best management of this case.

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Highlights:

1. Acute Fatty Liver of Pregnancy (AFLP) is an uncommon condition but life-threatening obstetric emergency, that increases the risk of fetal death due to preterm birth.
2. AFLP can be challenging for primary health workers (especially for general practitioners (GP) and midwives) since it mimics preeclampsia, viral hepatitis, intrahepatic cholestasis of pregnancy, and HELLPS syndrome, so more concern is needed in this case.

INTRODUCTION

Acute Fatty Liver of Pregnancy (AFLP) is a life-threatening, uncommon condition in obstetric emergencies. It is estimated to affect 1:7000 – 1:16.000 pregnancies worldwide and is most typically present during the third trimester or early postpartum period.[1] Sheehan first described it in 1940 and was once referred to as "acute yellow atrophy of the liver." In the past, the maternal mortality rate due to AFLP was reported to be quite high, around 75%. Recent studies report a reduction in maternal mortality by an average of 18% with early diagnosis and prompt and appropriate treatment.[2] This is usually due to complications such as DIC, sepsis, shock, and kidney failure. Meng Z et al reported in their study, that in the last 10 years there were 9.4% (10 out of 106) of mothers who died from AFLP, the most maternal complication was acute kidney injury.[3] Chen et al reported that the maternal mortality rate due to AFLP was 36.8%, where the prognosis for AFLP was worsened by complications of hepatic encephalopathy (HE), and the maternal mortality rate due to AFLP related to HE was around 87.5%.[4] A study conducted at one of Indonesia's tertiary hospitals found that maternal mortality due to AFLP was quite high, approximately 66.7%.[5]

Early detection and diagnosis are crucial to decrease the risk of fatal complications. However, it can be challenging for primary health workers since it mimics preeclampsia, viral hepatitis, intrahepatic cholestasis of pregnancy, and HELLPS syndrome.[6] Despite that, the diagnosis can be made based on Swansea criteria, a combination of clinical, laboratory, and imaging features. The cornerstone of management of AFLP is prompt termination of pregnancy and intensive supportive care with a multidisciplinary approach.[7,8]

Therefore, this study aimed to report an atypical case of AFLP with good maternal prognosis, while the baby was born preterm and died. AFLP is uncommon but life threatening so that requires more concern for rapid assessment, diagnosis, and comprehensive management to avoid complications and death.

CASE REPORT

A 33-year-old woman was referred to the Emergency department of Dr. Soedarso Tertiary Referral Hospital, Pontianak, Indonesia, previously from the district hospital over 580 KM with a diagnosis of multigravida (gravida 3 para 2) at 31 weeks gestation, suspected acute fatty liver disease (AFLP), severe oligohydramnios, and intrauterine growth restriction (IUGR). She had a history of nausea, vomiting, epigastric pain, polydipsia and polyuria, and seizure for less than 5 minutes with loss of consciousness. The patient did not know she was pregnant before and using a contraceptive implant. She missed her period in the last six months ago, which was the first onset of epigastric pain. On admission, the vitals were stable, but her blood pressure was 165/105 mmHg, and her heart rate was 100 beats per minute—further clinical examination resulted in pitting edema in both hand and foot with no jaundice.

Laboratory data in our hospital showed leukocytosis (11.33 x 103/ µL), average hemoglobin level (13,6 mg/dL), and thrombocytopenia (26.000/ µL). The biochemical markers showed the following: total bilirubin 5.13 mg/dL, direct bilirubin 1.92 mg/dL, aspartate transaminase (AST) 102.9 IU/L, alanine transaminase (ALT) 127.1 IU/L, albumin 3.60 g/dL, serum urea level 70.1 mg/dL, serum creatinine level 0.89 mg/dL, total cholesterol 249 mg/dL, blood glucose level 121 mg/dL, hyperuricemia 10.5 mg/dL, hyponatremia 131.88 mmol/L, and hypocalcemia 0.91 mg/dL. The coagulation profile was average: prothrombin time (PT) 11.3 sec, activated partial thromboplastin (APTT) 30.9 sec, and INR 0,77. A D-dimer test was also done and revealed 836.17 ng/mL FEU. Urinalysis showed proteinuria. The viral hepatitis and HIV tests were negative. Transabdominal ultrasound demonstrated a single intrauterine pregnancy with no echogenic liver features. Cardiotocography (CTG) showed category 2. The diagnosis of AFLP is based on Swansea criteria, at least having 6 of 14 combinations of clinical and laboratory features. This patient has seven as follows: nausea and vomiting, abdominal pain, polydipsia/polyuria, bilirubin >0.8 mg/dL, WBC >11x109/L, AST or ALT >24 units/L, and urate >340 µmol/L or >5.7 mg/dL.

For this case, we held a conference to obtain a multidisciplinary opinion for the best management of this patient. The pregnancy would be terminated with cesarean section after receiving 12 units of thrombocyte concentrates and reaching the target of platelets>50.000/L, would be in an intensive care unit pre-and post-surgery, echocardiography examination would be carried out and showed normal, and the baby would be treated in the neonatal intensive care unit (NICU) because of very preterm and meager birth weight. After the multi-department conference, the patient underwent an elective cesarean section on the day-2 admission. The outcome was a live male baby with a 9/10 Apgar score, birth weight of 1.100 gr, and birth length of 34 cm. On day two after surgery, the surgical wound was good, and there was no hemorrhage postpartum and no wound dehiscence. The mother had a good prognosis, while the baby died on day 23 of life.

DISCUSSION

The sex hormones estrogen and progesterone progressively increase in pregnancy, leading to physiological changes that affect hepatic metabolic, synthesis, and excretory functions. These physiological changes promote fetal growth and development. Hepatic disorders in pregnancy often occur in approximately 3% of pregnancies in developing countries.[9] Reports of AFLP cases in Indonesia are scarce, but it is reported increasing 2-3 issues per year at Dr. Soetomo General Academic Hospital Surabaya, Indonesia. AFLP incidence at dr Hasan Sadikin General Hospital Bandung-Indonesia from 2010-2013 is ten pregnancies of 10.766 (1: 1538 or 0.065%), and at Dr Soetomo General Hospital Surabaya-Indonesia from 2011-2015 is 18 pregnancies.

Several liver diseases during pregnancy often have similar features, which makes it challenging to identify.[10] Herein, we report on a case of atypical acute fatty liver of pregnancy. Our patient presented at 31 weeks of pregnancy – the third trimester with gastrointestinal symptoms as the patient's main complaint. A retrospective study showed the average gestational age for AFLP is 36 weeks (32-38 weeks)11, and a survey from Dr. Soetomo General Academic Hospital showed the average is 33 weeks (20-45 weeks). However, a case occurred at 19 weeks gestation.[5]

The initial symptoms of AFLP are non-specified, with frequent gastrointestinal symptoms such as nausea, vomiting, abdominal pain/epigastric pain. Several studies revealed jaundice as a cardinal sign of AFLP.[11] Nausea and vomiting occur several days or 1-2 weeks before jaundice, commonly present lately.[12] A study at Parkland Hospital showed patients with nausea, vomiting 57%, abdominal pain 53%, and jaundice 33% on admission.[13] Based on studies in Indonesia, the presentation of jaundice is relatively high, 100% at Dr. Soetomo General Academic Hospital and 70% at Dr Hasan Sadikin General Hospital.[5,14] Shi Y et al. showed jaundice (69%), fatigue (62%), nausea and vomiting (46%) being the most common signs and symptoms of AFLP.[15]

Recently, diagnosis of AFLP was made by a minimum of 6 of 14 Swansea Criteria. Our patient was diagnosed with Swansea criteria for AFLP, which met the required minimum features, 7 out of 14 parts. Her blood glucose level, hemostatic test (PT or APTT), and renal function remained normal, and she was not jaundiced and encephalopathic in perpetuum. No biopsy was performed, considering an invasive procedure despite being a gold standard diagnostic for AFLP.

Swansea criteria were used for presumptive diagnosis of AFLP with a high negative predictive value (100%) in diagnosing microvesicular steatosis, so it is not precise for early diagnosis. Several signs and symptoms will appear in line with AFLP progressions, such as encephalopathy, coagulopathy, or ascites. Likewise, laboratory and radiology findings such as bright liver on ultrasonography or profound hypoglycemia that occurs if liver function gets worse.

A study showed that aminotransferase, liver function, and coagulation tests are essential for diagnosing AFLP. Patients with GI symptoms, abnormal liver function tests, and coagulopathy without liver disease history had a high risk for AFLP. This study demonstrated several diagnostic criteria and levels of sensitivity and specificity. GI symptoms, elevated transaminase, elevated bilirubin, and prolongation of PT/APTT have a sensitivity of 97.6% and specificity of 97.1% for AFLP.[16] Dwivedi et al., AFLP most often occurs with jaundice, elevated bilirubin, and moderately elevated transaminase.[11]

Our patient did not meet the cardinal criteria, such as jaundice, like some previous studies that reported atypical AFLP. Onwuagbu et al. reported an atypical presentation of AFLP, which did not meet the required minimum Swansea Criteria, at 21 weeks gestation with no jaundice, developed anasarca, blood glucose, and renal function remained regular, unstable liver function tests, thrombocytopenia, and there was elevated international normalized ratio of 3.72 on day 12 admission. In this study, AFLP was confirmed by core liver biopsy that showed the presence of microvesicular steatosis (ballooning and feathery degeneration of hepatocytes) on day 17 admission.[17] In our patient, a liver biopsy was not carried out because of hemorrhage risk and limited time. Our patient also had a seizure pre-hospital. Although seizures are more common in preeclampsia, they are also reported to occur in around 15% of AFLP cases at Dr Hasan Sadikin Bandung-Indonesia.[14] Moreover, experiencing preeclampsia had a 50% risk for AFLP.[11]

Hypertension, average blood glucose, regular coagulation test, and no encephalopathy presence in this patient made it more challenging to differentiate from other liver diseases in pregnancy. Hypertension occurs most often in HELLPS syndrome and can differentiate between these two, but several studies report the incidence of hypertension in approximately 57% of AFLP cases.[5,13,16] Laboratory tests in AFLP usually demonstrate hypoglycemia in about 12% of patients with baseline <72 mg/dl. Hypoglycemia in a non-diabetes mellitus/gestational diabetes pregnant woman

and abnormal liver dysfunction and thrombocytopenia give a clue for AFLP. Profound hypoglycemia occurs in line with liver function progression. Hypoglycemia can also occur in HELLP syndrome, but in a small number, likewise coagulopathy.[11,16,18] Several signs and symptoms present at the late phase, such as encephalopathy, ascites, and bright liver on ultrasonography. Hyperuricemia and thrombocytopenia can be additional signs in cases of AFLP.

Differential diagnoses resembling AFLP are preeclampsia, HELLP (Hemolysis, Elevated Liver enzymes, and Low Platelets) syndrome, viral hepatitis, and intrahepatic cholestasis.[17,19] Preeclampsia presents in the second/third trimester with hypertension occurring after 20 weeks, edema and proteinuria, and neurological deficits can be found (headaches, seizures, coma).[19] HELLPS syndrome commonly presents in the third trimester between 28 and 36 weeks with mildly elevated transaminase, earlier elevated blood pressure, coagulation function, and hypoglycemia rarely changes. The symptoms of viral hepatitis are almost the same as AFLP, but the patient must have a history of previous liver disease, especially hepatitis B. In Intrahepatic cholestasis, occurring in the second and third trimester with commonly elevated bile acid, jaundice <25% of patients, pruritus of palms and soles, and mild liver dysfunction, but the coagulation test was rarely changed besides severe hypertension or elevated bile acid.[16,18–20]

AFLP had no specific signs and symptoms, making diagnosing it challenging. CSOG MFM Committee Guideline and previous studies recommend prenatal AFLP screening for outpatients at 34-37 of pregnancy. First-grade screening indications are gastrointestinal symptoms, complete blood count, liver function test, and coagulant test. Then, continue with second-grade screening, which includes renal function test, blood sugar, and ultrasonography.[16,21] Zhong et al. made a diagnostic procedure for AFLP; pregnant women with gastrointestinal symptoms in the third trimester should be suspected of AFLP, and an assessment of liver disease history, blood pressure, liver function, and coagulation function is carried out.[16] When the diagnosis of AFLP has been established, the definitive treatment is delivery.[22] Studies reported that if delivery is carried out within a week of the onset of diagnosis, the survival rate can be up to 100%. In comparison, 30% of cases had poor prognosis if delivery was carried out beyond two weeks after onset.[21,23]

Uncommon but life-threatening cases should concern health workers, especially in the first line, general practitioners (GP) and midwives. AFLP should be suspected in third-trimester pregnancy with gastrointestinal symptoms, abnormal liver function, and coagulopathy without liver disease history. Education to primary health workers must also be carried out that hyperemesis gravidarum with gastrointestinal main complaints is only limited to 14-15 weeks of pregnancy; if more than 14-15 weeks, then AFLP is suspected.[5,16]

CONCLUSION

Acute Fatty Liver during Pregnancy is an uncommon but life-threatening obstetric emergency. Early screening, diagnosis, timely handling delivery, and intensive supportive care are essential to decrease morbidity and mortality for both mother and fetus.

DISCLOSURES

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Conflict of interest

The authors declare that they have no conflict of interest.

Patient consent for publication

The patient has signed the informed consent form and agreed that her case is to be published in a case report.

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Author contribution

All authors have contributed to all processes in this research and approval for publication of this manuscript.

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