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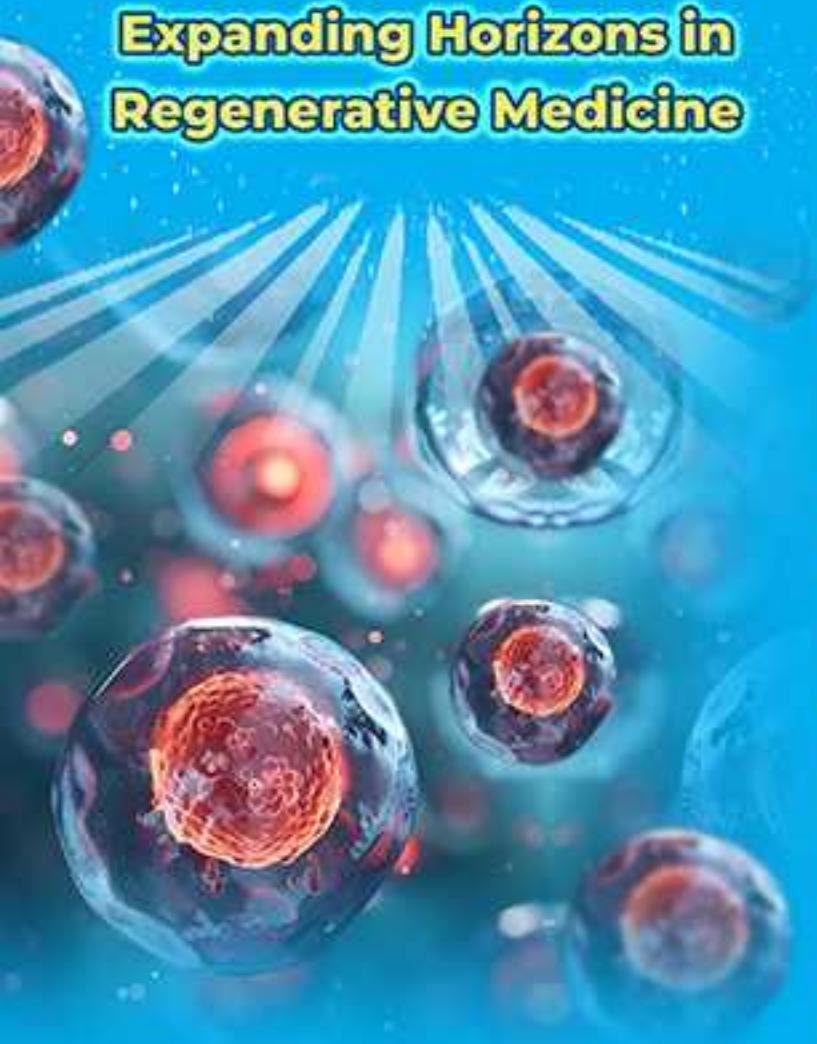

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Editorial

Stem Cell Therapy in Urogynecology – Expanding Horizons in Regenerative Medicine

Budi Iman Santoso

Urogynecology, as a specialized field, continues to evolve in tandem with advancements in medical science.[1] In recent years, stem cell therapy has emerged as a highly promising and sophisticated area of scientific inquiry. The advancement of treatment methodologies has generated significant anticipation.[2] Stem cells, with their unique regenerative abilities, offer transformative potential for a wide range of urogynecological conditions, particularly those related to pelvic floor dysfunction, urinary incontinence, and organ prolapse.[3] This edition of the Indonesian Journal of Obstetrics and Gynecology (INAJOG) focuses on e xploring the integration of stem cell therapy in urogynecology, with a selection of cutting-edge research articles and case studies that underscore the current progress and challenges in this exciting area.

The pelvic floor's intricate structure plays a vital role in maintaining a woman's quality of life.[4] Yet, this complex system is vulnerable to damage due to childbirth, aging, or trauma, which can lead to conditions such as pelvic organ prolapse (POP), stress urinary incontinence (SUI), and fecal incontinence.[5] Traditional treatments like surgical repairs or conservative management, though effective, have their limitations, often leading to recurrent issues or incomplete recovery.[6] Stem cell therapy is an innovative treatment modality that leverages the distinctive characteristics of stem cells, such as self-renewal and differentiation, to regenerate damaged cells and tissues in the human body or to replace them with new, healthy, and fully functional cells through the administration of exogenous cells to a patient.[7]

One article reviews the latest clinical trials on stem cell injections for SUI, highlighting promising preliminary results in restoring sphincter function and strengthening the supportive pelvic tissues.[8] Another study presents findings on the use of stem cells in managing POP, where stem cell applications are investigated not only for tissue regeneration but also for their anti-inflammatory and immunomodulatory effects, reducing the likelihood of recurrent prolapse.[9]

As with any emerging technology, stem cell therapy in urogynecology is not without its challenges. This edition critically examines the barriers to widespread clinical adoption, including issues of cell sourcing, ethical considerations, regulatory approvals, and the cost-effectiveness of treatments. A key focus is on ensuring that these therapies are safe, standardized, and accessible to patients in diverse healthcare settings, particularly in developing regions. Several articles provide insights into the logistical and ethical frameworks needed to integrate stem cell therapies within the current urogynecological practice in Indonesia, laying the groundwork for future clinical protocols.

While much of the research presented in this issue reflects early-stage studies, the trajectory of stem cell therapy in urogynecology is clearly one of growth and potential.[10] As researchers and clinicians, we are on the cusp of a new era where regenerative medicine could revolutionize the treatment of conditions that have, for centuries, caused significant morbidity among women. This volume of INAJOG offers a comprehensive look at the future of urogynecology, positioning stem cell therapy as a key player in the next generation of medical innovations.

We hope that the readers of this issue find inspiration, knowledge, and motivation to continue pushing the boundaries of what is possible in urogynecological care. The integration of regenerative medicine, particularly stem cell therapy, holds the promise of not only repairing the body but also restoring dignity and quality of life for countless women.

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Research Article

Sensitivity and Specificity of the Modified Early Obstetric Warning Score (MEOWS) and Maternal Early Warning Criteria (MEWC) for Predicting Maternal Morbidity: A Retrospective Cohort Study in Pregnant Women with COVID-19

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Abstract

Objective: To compare the sensitivity, specificity, and predictive value of the Modified Early Obstetric Warning Score (MEOWS) and Maternal Early Warning Criteria (MEWC) in predicting morbidity among pregnant patients infected with COVID-19. This research aims to assess the use of these tools as screening methods for determining the appropriate level of care for pregnant COVID-19 patients.

Methods: A retrospective cohort study was conducted on 89 pregnant women with COVID-19 admitted to Bantul Regional General Hospital between January and December 2021.Data analysis was done using the ROC curve to compare sensitivity, specificity, and predictive values of MEOWS and MEWC.

Results: MEWC is better than MEOWS in predicting the morbidity of pregnant patients COVID-19 infection. The MEWC showed better sensitivity (78,3%) and PPV value (78%) compared to MEOWS, though it had a lower specificity (81.8%) and negative predictive value (NPV, 82%). MEOWS had a higher specificity (97.1%) but lower sensitivity. The ROC curve analysis yielded an area under the curve (AUC) of 74.9% for MEOWS and 80% for MEWC.

Discussion: MEWC has a better sensitivity indicating that patients who do not trigger MEWC criteria will have a low risk of experiencing maternal morbidity. Screening tools will prioritize the sensitivity value compared to the specificity value of the instrument used. A screening tool will have a lower positive predictive value if the study population has a lower prevalence of morbidity. Based on the comparison of the predictive value, sensitivity, and specificity of the MEWC and MEOWS instruments, it can be concluded that MEWC is associated with maternal morbidity with a higher sensitivity than MEOWS, although it has a lower specificity. High sensitivity values will result in screening tools with consistent results. The ROC curve can also show that MEWC has a higher sensitivity value by looking at the Y-coordinate, which is higher than the Y-coordinate of MEOWS. MEWC has a better Receiver Operator Curve (ROC) intersection point than MEOWS, where the MEWC intersection point has the furthest point on the upper left of the ROC diagonal line.

Conclusion: MEWC has a higher sensitivity compared to MEOWS, even though it has a lower specificity. High sensitivity values will produce screening tools with consistent results.

Keywords: MEWC, maternal early warning system, maternal morbidity, MEOWS.

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INTRODUCTION

The World Health Organization declared COVID-19, a disease caused by the SARS-CoV-2 virus, a global pandemic on March 11, 2020[1]. This global pandemic has ushered in various impacts on aspects of life, particularly in healthcare systems around the world. One population of significant concern has been pregnant women[2]. In Indonesia, data collected from the Indonesian Obstetrics and Gynecology Association found that 20% of maternal deaths during the first 17 months of the pandemic were among pregnant women who tested positive for COVID-19. This

surge in maternal mortality peaked in July 2021, with death rates tripling compared to earlier in the pandemic[3]. According to data from the Ministry of Health, there were 7,389 maternal deaths in Indonesia in 2021a 56.69% increase compared to 2020, which saw 4,627 deaths. COVID-19 accounted for 2,982 of these deaths. Other significant causes included hemorrhage (1,320 deaths), hypertension during pregnancy (1,077 deaths), and heart disease (335 deaths). In addition to that, there were 207 maternal deaths due to infection; 80 due to metabolic disorders; 65 due to circulatory system disorders; 14 due to abortion; and 1,309 due to other causes[3].

Based on data from The American College of Obstetricians and Gynecologists shows that that pregnant women infected with SARS-CoV-2 are at increased risk of mortality and morbidity due to obstetric complications compared to those not infected with the virus[4]. *The Centers for Disease Control* reported a significant increase in cases of the SARS-COV 2 virus variant, the Delta variant, in late July 2021, with a more contagious character and a worse impact compared to the previous SARS-COV 2 variant. Meanwhile, pregnant women who have received full vaccination as of November 20, 2021, are only around 35%, so data shows that the occurrence of severe maternal morbidity in pregnant women is increasing during the period when the Delta variant attacked the world compared to when the previous SARS-COV 2 variant[5]. Another study found that during the Delta variant period, the risk of pregnant women being admitted to intensive care units increased by 66%, the risk of needing mechanical ventilation rose by 63%, and the risk of death was more than twice as high compared to infections from earlier SARS-CoV-2 variants[6].

The increased risks predominantly due to physiological and immunological changes experienced by pregnant women, particularly when infected with COVID-192. Physiological parameters such as blood pressure, heart rate, respiratory rate, temperature, and mental status can serve as early indicators of critical conditions across various populations, including obstetric patients. These parameters form the foundation of early warning scoring systems for obstetric patients[7]. This early warning system requires periodic monitoring of a patient's vital signs to allow for continuous assessment of their clinical condition. By detecting any deterioration early, timely interventions can be administered, improving the likelihood of positive outcomes[8].

Countries around the world employ various types of Modified Early Warning Systems, including the Modified Early Obstetric Warning Score (MEOWS), Maternal Early Warning Criteria (MEWC), and Maternal Early Warning Trigger (MEWT). The Confidential Enquiry into Maternal and Child Health (CEMACH) recommends the use of MEOWS, though research on its application as an early warning system for obstetric patients remains limited in developing countries, particularly during the ongoing pandemic. A prospective study conducted by Anju Singh and his team in 2016 evaluated the MEOWS scoring system's ability to predict morbidity in obstetric patients. The study found that MEOWS is sensitive in predicting maternal morbidity, with a sensitivity of 86.4% and a specificity of 85.2%. Furthermore, the researchers observed a correlation between the parameters used in MEOWS and obstetric morbidity[10,11]. Anju Singh and his team proved that tool is highly effective for monitoring treatment and predicting obstetric morbidity, and they strongly recommend its implementation in all obstetric units. At the conclusion of the study, Singh also called for further research on the MEOWS instrument in different clinical settings[7].

Another early warning system recommended by the National Partnership for Maternal Safety (NPMS) is the Maternal Early Warning Criteria (MEWC), designed to identify patients at risk of morbidity and mortality. MEWC provides an alert if even a single parameter falls outside the normal range, making it simpler compared to the Modified Early Obstetric Warning Score (MEOWS), which requires the calculation of a severity score based on 11 parameters[12,9]. MEWC, developed through consensus by experts in the United States[13] is recommended for use in all hospitals providing obstetric services by the American College of Obstetricians and Gynecologists (ACOG) District II's Safe Motherhood Initiative. This initiative aims to improve maternal health outcomes in New York, a state with one of the highest maternal mortality rates[14]. Research conducted by David E. Arnolds and his team in Chicago found that there was an association between MEWC and maternal morbidity, showing that MEWC demonstrated high sensitivity and negative predictive values (NPV), but lower specificity and positive predictive values (PPV). While MEWC's high sensitivity aligns with its role as a screening tool, it is recommended because it serves as an early warning system, prompting the need for diagnostic or therapeutic interventions

in women at risk of morbidity. A robust early warning system is believed to be a crucial tool in reducing maternal morbidity and mortality[10].

The purpose of this study is to compare the sensitivity and specificity of MEOWS and MEWC in predicting morbidity in pregnant patients infected with COVID-19. This research could serve as a screening method to help determine the appropriate level of care for pregnant patients with COVID-19.

METHODS

This quantitative research is conducted using a retrospective cohort method. Data collection is performed through the extraction of medical records from Bantul Regional General Hospital. The study was carried out at Panembahan Senopati Bantul Regional General Hospital, with data collected from medical records of pregnant inpatients infected with COVID-19 between November 7, 2022, and December 1, 2022. Based on the results of the medical record analysis, a comparison between the MEOWS and MEWC early warning systems in predicting patient morbidity will be evaluated. The control group consists of pregnant patients with confirmed COVID-19 infection who were monitored using standard care protocols without the implementation of the MEOWS or MEWC early warning systems.

The research has been carried out in the medical record room of the Bantul Regional General Hospital. The study population consisted of inpatient pregnant patients who were confirmed with COVID-19 infection. Samples are determined through total sampling which aims to obtain a more comprehensive and thorough sample type. Samples were taken through medical records by taking into account the patient's MEOWS sheet, the initial assessment sheet of hospitalization, CPPT (Integrated Patient Development Record) sheet for inpatient care, the patient's entry and exit summary sheet, and the PCR swab laboratory result sheet in the patient's medical record.

Univariate analysis analyses each research variable descriptively by calculating the frequency distribution of the variables and research subjects based on age, gravida status, and gestational age. In this study, univariate analysis was conducted using frequency analysis[15]. Bivariate analysis, or correlation analysis, was used to examine the relationship between two variables: the relationship between MEOWS and MEWC and morbidity and mortality in pregnant patients infected with COVID-19. The statistical technique employed was the Chi-Square test, with a significance level (p-value) of 0.05, as the data is nominally categorical. Receiver Operating Curve (ROC) analysis was also performed to compare the area under the curve (AUC), sensitivity, and specificity of the two maternal early warning systems.

In this study, the population was pregnant women with COVID-19. This study compares MEOWS and MEWC in the early detection of complications in pregnant women with COVID-19 and assesses whether MEWC is better than MEOWS in predicting morbidity in pregnant patients with COVID-19 infection. For this purpose, predictive value analysis, accompanied by sensitivity and specificity value analysis, was carried out to assess the comparison between the two early warning scores. Predictive value, sensitivity, and specificity were analysed with a 95% CI.

RESULTS

The study included a total of 89 pregnant patients infected with COVID-19 who were admitted to Bantul Regional General Hospital. The sample characteristics based on age, gestational age, and gravida status are as follows.

Table 1. Sample Characteristics of Pregnant Patients Infected with COVID-19 in Bantul Regional General Hospital

Sample Characteristics		
	Frequency (n)	**Percentage (%)**
Age		
Reproductive (20-35)	77	86.5
Risk (< 20 and > 35)	12	13.5
Gestational Age (weeks)		
< 37	31	34.8
37-42	57	64.0
> 42	1	1.1
Gravida		
Primigravida	35	39.3
Multigravida	54	60.7

Based on Table 1, it can be observed that the study sample was predominantly composed of pregnant patients infected with COVID-19 within the reproductive age range (20-35 years), accounting for 77 patients (86.5%). Most of the patients had a gestational age of 37-42 weeks, with 57 patients (64.0%), and the majority were multigravida pregnancies, comprising 54 patients (60.0%) of the total study sample, with a 95% Confidence Interval (CI). The following are the

results of the data analysis on the correlation and comparison of MEOWS and MEWC in pregnant patients infected with COVID-19 at Bantul Regional General Hospital. Based on Table 1, it can be observed that the study sample was predominantly composed of pregnant patients infected with COVID-19 within the reproductive age range (20-35 years), accounting for 77 patients (86.5%). Most of the patients had a gestational age of 37-42 weeks, with 57 patients (64.0%), and the majority were multigravida pregnancies, comprising 54 patients (60.0%) of the total study sample, with a 95% Confidence Interval (CI). The following are the results of the data analysis on the correlation and comparison of MEOWS and MEWC in pregnant patients infected with COVID-19 at Bantul Regional General Hospital.

Table 2. Relationship of MEOWS with Morbidity in Pregnant Patients Infected with COVID-19 at Bantul Regional General Hospital

MEOWS	**Morbidity**		***P-value***
	non-morbidity (n)	**morbidity (n)**	
Normal	33 (37.1)	1 (1.1)	21.132
Trigger	26 (29.2)	29 (32.6)	(0.000)
Total	59	30	

Table 2 shows that a sample of pregnant patients infected with COVID-19 with a "normal" category MEOWS score and experienced Non-Morbidity was 33 (37,1%) samples. Based on the calculation results of *Chi-Square*, p-value = 0.000 < α = 0.05 (*p-value value* smaller than α = 0.05 with 95% Confidential Interval (CI)). This means that there is a significant association between the MEOWS and the incidence of morbidity in pregnant patients infected with COVID-19 at the Bantul Regional General Hospital.

Table 3. Relationship of MEWC with Morbidity in Pregnant Patients Infected with COVID-19 at Bantul Regional General Hospital

MEOWS	**Morbidity**		***P-value***
	non-Morbidity (n)	**Morbidity (n)**	
Normal	54 (60.7)	12 (13.5)	24.928
Trigger	5 (5.6)	18 (20.2)	(0.000)
Total	59	30	

Table 3 showed that the majority of the sample of pregnant patients infected with COVID-19 with a normal MEWC score and experienced Non-Morbidity was 54 (60.7%) patients. Based on the calculation results of *Chi-Square*, p-value = 0.000 < α = 0.05 (*p-value value* smaller than α = 0.05 with 95% Confidential Interval (CI)). This means that there is a significant relationship between MEWC and the incidence of morbidity in pregnant patients infected with COVID-19 at Bantul Regional General Hospital.

Table 4. Comparison of Sensitivity and Specificity of MEWC and MEOWS in Pregnant Patients with COVID-19 at Bantul Regional General Hospital

	MEOWS	**MEWC**
AUC	74.9	80
Sensitivity	52.7	78.3
1-Specificity	2.9	18.2
PPV	53	78
NPV	97	82
Odds Ratio	36.808	16.200
Chi Square	21.132	24.928

Table 4 shows that MEWC has a better value sensitivity (78.3%) compared to MEOWS (52.7%), even though MEWC's specification value (81.8%) is lower than MEOWS (97.1%) with 95% Confidential Interval (CI). So the results of the data analysis on MEWC's ability to show truly morbidity individuals from the total morbidity population and the value of the proportion of patients with negative tests who really do not experience morbidity are better than MEOWS' ability. showed that the ability of MEWC to detect an early morbid condition or the possibility of a positive result when performed on a group of subjects at risk of experiencing morbidity is better than the ability of MEOWS. Meanwhile, the ability of MEWC to determine that the subject is not sick or the possibility of a negative result if it is performed on a group of healthy subjects is lower than that of MEOWS.

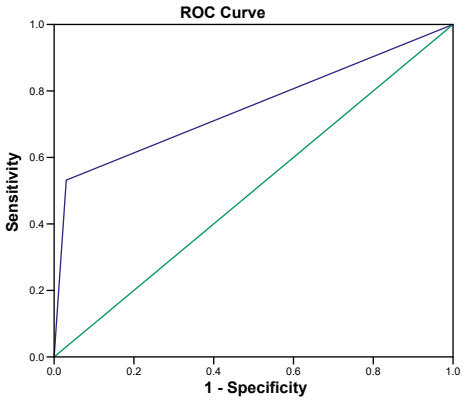

Figure 1. Receiver operator curve MEOWS

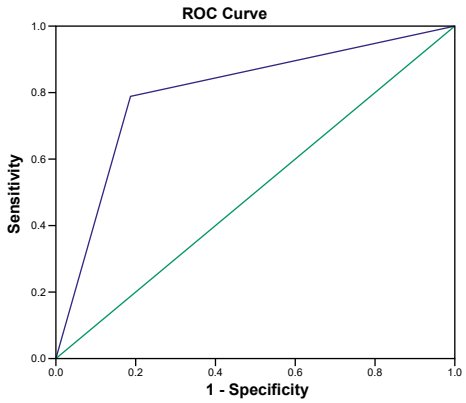

Figure 2. Receiver operator curve MEWC

Figure 1 and 2 shows that MEWC has a bigger area under curve than MEOWS. The results of MEOWS and MEWC data analysis using ROC produce an area under the curve for MEOWS of 0,749 (74,9%) while for MEWC of 0,800 (80%, with 95% Confidential Interval (CI)) so we can see that MEWC has a better ROC intersection point than MEOWS, where the MEWC intersection point has the furthest point on the upper left of the ROC diagonal line. The ROC curve can also show that MEWC has a higher sensitivity value by looking at the Y-coordinate, which is higher than the Y-coordinate of MEOWS.

DISCUSSION

The results of the analysis showed that a large portion of the sample was aged 20-35 years, accounting for 86.5% or 77 patients. This indicates that most pregnant patients infected with COVID-19 at Bantul Regional General Hospital were within the reproductive age group. These findings align with data reported by the CDC, based on the largest sample of pregnant patients in the United States. A Morbidity and Mortality Weekly Report covering the period from January 22 to June 7, 2020, found that 8,207 of the total 326,335 confirmed COVID-19 cases in women of reproductive age were pregnant. The dominance of this age group can be attributed to the fact that women of reproductive age are more likely to conceive, have more frequent interactions with the healthcare system, and exhibit higher mobility compared to other age groups[16].

The data collected in this study found that most of the sample of pregnant women confirmed with COVID-19 was within 37-42 weeks of pregnancy, namely 57 (64.0%) patients. This shows that pregnant women infected with COVID-19 at the Bantul Regional General Hospital are mostly at gestational age in the third trimester. Several studies have found a predominance of pregnant women in the third trimester in pregnant patients with confirmed COVID-19. Although until now the cause is not known for certain, there is a suspicion that there is a possibility of patients undergoing swab tests at the same time as when they come to the hospital with increased obstetric complaints in that trimester[13].

The results of patient data collection in this study found that a large percentage of patients were multigravida, accounting for 60.0% (54) of the total sample. This indicates that, in terms of parity status, most pregnant patients confirmed with COVID-19 at Bantul Regional General Hospital had been pregnant two or more times. Primigravida refers to a woman who is pregnant for the first time, whereas multigravida refers to a woman who has been pregnant more than once. According to findings reported by the CDC and several previous studies, higher parity levels are often associated with older maternal age and larger family size, which may be among the risk factors for the transmission of the SARS-CoV-2 virus[13].

Maternal Early Warning Criteria (MEWC)

Sixty-six or 74.2%, of pregnant patients infected with COVID-19 at the Bantul Regional General Hospital had MEWC assessment results in the "Normal" (non-trigger) category. According to research by David Arnold and his team in Chicago, women who did not trigger criteria (Normal) on the MEWC assessment had a low risk of experiencing morbidity. An increased risk of morbidity can still occur due to physiological and immunological changes experienced by pregnant women, especially when infected with COVID-19[2]. Changes in vital signs such as blood pressure, heart rate, respiratory rate, and body temperature can be used as parameters to predict the onset of morbidity or mortality in various populations, including the obstetric population This forms the basis for the development of maternal early warning systems. This early warning system requires periodic measurement of the patient's vital signs so that the patient's clinical condition can be monitored optimally and any worsening of the patient's condition can be detected earlier so that the intervention given is not too late for better outcomes[7]. The MEWC is a simplified version of the MEOWS early warning system.[9]. The primary difference between the two systems lies

in the number of abnormal parameters needed to activate the warning. In MEWC, an immediate warning is triggered when a single parameter falls outside the normal range. The MEWC system uses parameters based on reviews of various cases of maternal morbidity and mortality, although the effectiveness of the system relies heavily on how well it is implemented by each healthcare provider[11].

Modified Early Obstetric Warning Score (MEOWS)

Table 2 indicates that a significant proportion of pregnant patients with COVID-19 at Bantul Regional General Hospital, assessed using MEOWS, fell into the 'Normal' category, accounting for 38.2% (n=34) of respondents. Previous research indicates that MEOWS can be effectively used to predict obstetric morbidity and serves as a practical bedside screening tool. It meets most of the criteria for ideal screening in pregnant patients, allowing for early recognition of life-threatening situations, which can help reduce maternal morbidity and mortality[7]. However, there has been limited research on the use of MEOWS as a maternal early warning system in developing countries, despite the urgent need for its implementation, especially under current conditions[8]. The escalation protocol used in the MEOWS early warning system is based on parameter score values outside the normal limits, where the warning will be active when the total parameter score is more than equal to 7 or is in the red alert zone (high risk), so that an examination of the patient's condition is needed to determine whether intensive care is needed for the patient[9].

Relationship of Maternal Early Warning Criteria (MEWC) and Modified Early Obstetric Warning Score (MEOWS) with Morbidity Maternal

The chi square continuity correction yielded MEWC and MEOWS scores of 24.928 and 21.132, respectively, with a p-value $<\alpha = 0.05$, indicating a significant relationship between MEWC and MEOWS with the incidence of morbidity in pregnant patients with COVID-19 at Bantul Regional General Hospital. This means that the MEWC and MEOWS scores can predict the incidence of morbidity in pregnant patients infected with COVID-19. The results of this study were supported by the study which showed that the parameters used in MEOWS had a significant correlation with maternal morbidity[7]. In addition, researchers also found MEOWS to be sensitive in predicting maternal morbidity, with a sensitivity value of 86.4% and a specificity value of 85.2%[7]. Research also proves that the MEOWS instrument is very useful as a treatment monitoring tool in predicting obstetric morbidity and highly recommends its use for all maternity care units everywhere7. The results of this who stated that the sensitivity of the maternal early warning system reached a proportion of 89% and a specificity of 85% for predicting morbidity[17]. The results of this study also confirmed a significant relationship between MEWC and maternal morbidity. It was found that MEWC has excellent sensitivity and a high negative predictive value, demonstrating that women who do not trigger criteria in MEWC have a low risk of experiencing maternal morbidity[10]. MEWC's sensitivity is comparable to that of the maternal early warning system recommended by the British Confidential Inquiry into Maternal and Child Health[10]. Its high sensitivity makes it an effective screening tool. MEWC is recommended for its role not only as a screening tool but also as a reminder for the need for early diagnostic or therapeutic intervention in women at risk of morbidity. A strong early warning system is considered a vital tool in reducing maternal morbidity and mortality[10]. The results of this study indirectly support the recommendations issued by the NPMS regarding the use of MEWC to assist health workers in the early identification of obstetric patients who are at risk of experiencing maternal morbidity[10]. The findings of this study are further supported by evidence showing that the MEOWS early warning system, when simplified, becomes more sensitive and useful. The analysis also concluded that simplifying early warning system parameters can reduce the complexity of managing triggered warnings and decrease the overall workload, without diminishing the potential benefits of early warning systems as a patient safety tool. Based on the results of this analysis, it can be concluded that the MEWC is more effective than MEOWS in predicting morbidity in pregnant patients with COVID-19 infection.

Comparison of Predictive Value, Sensitivity, and Specificity of MEWC and MEOWS

Screening tools will prioritize the sensitivity value compared to the specificity value of the

instrument used. Previous studies have found that MEWC has a very good sensitivity (97%) and negative predictive value (97%), thus indicating that patients who do not trigger MEWC criteria will have a low risk of experiencing maternal morbidity[10]. The findings in this study are in line with Arnold and his team's research, which found a MEWC sensitivity value of 78.3%. Although the specificity value of MEWC (81.8%) when compared to MEOWS (97.1%) does have a lower specificity value, This is because all screening tools, including maternal early warning systems with high sensitivity, tend to sacrifice specificity[10]. The definition of maternal morbidity in each study will also influence the performance of any screening method[18]. The positive and negative predictive values of a screening tool will tend to depend on the prevalence of morbidity, so they are highly individual for each study population. A screening tool will have a lower positive predictive value if the study population has a lower prevalence of morbidity[10]. Based on the comparison of the predictive value, sensitivity, and specificity of the MEWC and MEOWS instruments, it can be concluded that MEWC is associated with maternal morbidity with a higher sensitivity than MEOWS, although it has a lower specificity. High sensitivity values will result in screening tools with consistent results.

Comparison Area under Curve (Receiver Operator Curve) of MEWC and MEOWS

MEWC has a bigger area under curve than MEOWS. Receiver Operator Curve (ROC) is a way of determining the cut-off point of a diagnostic test in the form of a graph that illustrates the trade-off between sensitivity and specificity. Assessment of the ability of a test is done by AUC. AUC values range from 0–1, where the ability of a test is declared good if the AUC is 0.7[19]. Sensitivity is described in the Y-ordinate, while 1-specificity is described in the X abscissa, so that the higher the sensitivity, the lower the specificity, and vice versa. The diagonal line on the ROC shows a line consisting of points with a sensitivity equal to 1, so the closer the ROC curve is to the diagonal line, the worse the result[12]. The best point of intersection is the point farthest to the upper left of the diagonal line. The results of MEOWS and MEWC data analysis using ROC produce an area under the curve for MEOWS of 0,749 (74,9%) while for MEWC of 0,800 (80%) so we can see that MEWC has a better Receiver Operator Curve (ROC) intersection point than MEOWS, where the MEWC intersection point has the furthest point on the upper left of the ROC diagonal line. The ROC curve can also show that MEWC has a higher sensitivity value by looking at the Y-coordinate, which is higher than the Y-coordinate of MEOWS[20].

CONCLUSION

Our study suggests that MEWC outperforms MEOWS in predicting morbidity, due to its superior sensitivity, PPV, and AUC value. While MEWC exhibits a lower specificity and NPV value, the high sensitivity ensures consistent results in identifying patients at risk. It is important to note that this research has limitations because it was conducted quantitatively, so researchers do not yet know in depth the perceptions of health workers regarding the use of MEWC and MEOWS in maternal unit services. Future research could explore ways to deepen this research through qualitative research to reveal the perceptions of health workers so that the barriers and conveniences of each instrument can be identified. Future researchers are also advised to use different methods, such as prospective cohorts. This research is also still being carried out centrally in one hospital, so multicenter research is needed to see more comprehensive results.

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Research Article

Epidemiology of Cervix Uteri Cancer in Saudi Arabia from 2004 to 2017

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Abstract

Objectives: This study investigates the epidemiological pattern of Cervix Uteri cancer (CUC) throughout all administrative regions of Saudi Arabia. It examines the frequency number and percentage of diagnosed cases, the age-specific incidence rate (AIR), the crude incidence rate (CIR), and the age-standardized incidence rate (ASIR) stratified by age group, year of diagnosis, and regions.

Methods: A retrospective descriptive epidemiological investigation of all CUC cases documented in the Saudi Cancer Registry (SCR) between 2004 and 2017 was performed. Statistical Package for the Social Sciences, version 20.0, was utilized to analyze the data using descriptive statistics and the Kruskal-Wallis test (SPSS).

Results: In total, 1,451 CUC-diagnosed cases were reported to the SCR between January 2004 and December 2017. Northern, Eastern, and Tabuk regions had the highest ASIR of CUC among Saudi women (2.2, 2.0, and 2.0 per 100,000 women). In contrast, among Saudi women, Jazan had the lowest overall ASIR of CUC (0.7 per 100,000 women, respectively).

Conclusion: The ASIRs of CUC in Saudi Arabia decreased slightly from 2004 to 2017. The Northern, Eastern, and Tabuk regions of Saudi Arabia had the highest ASIR of CUC among Saudi women, while women in Jazan, Saudi Arabia, were proven to be the least affected by CUC.

Keywords: cancer epidemiology; cervix uteri cancer; incidence rate; oncology; Saudi cancer registry.

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INTRODUCTION

Cervix Uteri carcinoma (CUC) remains a prevalent form of gynecological cancer across the globe, ranking fourth among women and fifteenth among all cancers.[1,2] Annually, over 500,000 new cases of CUC are detected, resulting in roughly 250,000 deaths.[1] In the United States, CUC accounts for roughly 0.7% of newly diagnosed cancer cases annually, with an estimated 14,100 new cases and 4,280 deaths projected by the American Cancer Society in 2022.[3] Typically, CUC is diagnosed in women in their seventies, with an average age of 61. The incidence of CUC is twice as high in Black women compared to White women, and the prognosis for Black women with CUC is worse. In the past two decades, the mortality rates for CUC have risen by more than 100%.[4,5]

The most significant risk factor for CUC is infection with Human Papillomavirus (HPV). HPV is a sexually transmitted virus that can be transmitted through skin-to-skin contact, particularly during sexual activity, hand-to-genital organ touch, and oral sex.[6,7] Women who become infected with HPV are at a higher risk of developing CUC, especially if they have other risk factors such as having many children, smoking tobacco, and using oral contraceptives.[8]

According to the projections made by the International Agency for Cancer Research (IARC), the incidence and mortality rates of CUC among women in Saudi Arabia were estimated to be 1.6 and 2.1 per 100,000 women, respectively, in the year 2020. These rates are notably lower than the rates observed in other Arabian Gulf nations such as Oman, the United Arab Emirates, Qatar, and Bahrain, where the incidence rates were found

to be 6.4, 6.2, 4.1, and 3.9 per 100,000 women, respectively.[9]

The main goals of this study were to evaluate and characterize the prevalence of CUC among women in Saudi Arabia. To achieve these objectives, the study examined the crude incidence rate (CIR) and age-standardized incidence rate (ASIR), which were then categorized based on the year of diagnosis, geographical area, and age group. As a result, we plan to perform observational descriptive epidemiological research on CUC, analyzing the temporal and spatial distribution of cases documented in the Saudi Cancer Registry (SCR) between 2004 and 2017.

METHODS

The present study aims to perform a comprehensive descriptive epidemiological analysis of all cases of CUC reported in Saudi Arabia between 2004 and 2017. The study includes data from all regions of the country and seeks to identify trends and patterns in the incidence rates of CUC during the specified time frame. The incidence rates of CUC in Saudi Arabia are readily available through the Saudi Cancer Registry (SCR) reports, which are published annually by the Ministry of Health. Therefore, ethical approval was not required for this retrospective observational study as the data was obtained from a publicly accessible source. The SCR was established in 1992 and serves as a population-based cancer registry, collecting data on cancer cases diagnosed and treated in Saudi Arabia. It is worth noting that the first report on cancer in Saudi Arabia was published in 2001, with subsequent reports published annually. However, the most accurate report on cancer in Saudi Arabia was published at the beginning of 2004, providing a reliable baseline for the present study. Furthermore, the most recent data available through the SCR was collected in 2017, providing a comprehensive view of the epidemiological status of CUC in Saudi Arabia during the study period.

The Saudi Cancer Registry (SCR) has been publishing reports on the epidemiological patterns of cancer in Saudi Arabia since 2001. These reports provide information on the frequency and percentage of cancer cases, as well as the age-standardized and crude incidence rates (ASIR and CIR), stratified by Saudi Arabian provinces, age group, and years of diagnosis. Full reports covering the period from 2004 to 2017 are currently available for 13 administrative regions. These reports cover a period of fourteen years. In order to provide a descriptive epidemiology of CUC in Saudi women, these reports were used to undertake critical data collection on all of the information extracted from the SCR.

Data analysis was carried out using version 20.0 of the Statistical Package for the Social Sciences (SPSS). The descriptive epidemiological analysis involved computing the overall percentage, age-specific incidence rate (ASR), crude incidence rate (CIR), and age-standardized incidence rate (ASIR) by age group, geographical region, and year of diagnosis. The Kruskal-Wallis test was employed. to investigate the variation in CIR and ASIR of CUC across different regions in Saudi Arabia.

RESULTS

Between January 2004 and December 2017, the SCR reported 1451 cases of CUC, accounting for an average percentage of 2.0% of all cancer cases. The analysis revealed a slight increase in cases over the 14-year period. The age group 45-49 years showed the highest number of cases with 210 (14.5% of all cases), followed by the age group 50-54 years with 198 cases (13.6% of all cases), while the age group 40-44 years also had a considerable number of cases, with 187 cases (12.9% of all cases). The incidence of CUC was low among women under the age of 30, with only 30 cases (5% of all cases). It can also be noted that among Saudi women, the overall number and percentage of CUC cases between 2004 and 2017 was 104 (2.0% of all cancer cases).

The overall age-specific incidence rate of CUC from 2004 to 2017, expressed per 100,000 female population, was calculated from the SCR. The data reveals that the overall age-specific incidence rate of CUC was very low among women aged 0-24 years, with rates ranging from 0 to 0.2 per 100,000 women. The overall age-specific incidence rate increased steadily from the age of 30 years, with the highest rates observed in women aged 70 years and above, with rates ranging from 6.5 to 6.7 per 100,000 women. The highest rate was observed in the age group 70-74 years, with a rate of 6.6 per 100,000 women.

According to Figure 1, the CIR of CUC in Saudi Arabia was found to fluctuate over the study period from 2004 to 2017. The peak incidence rate was observed in the year 2012, with 1.4 cases per 100,000 women, while the lowest rate was observed in 2006 and 2010, with 0.9 cases

per 100,000 women. The mean and median CIR were calculated to be 1.1 cases per 100,000 women, indicating a relatively stable incidence rate of CUC over the study period. The standard deviation (SD) was 0.14, indicating a relatively low degree of variability in the data. However, the overall CIR of CUC among Saudi women was 1.1 (95% CI: 1.0 to 1.2) per 100,000 women between 2004 and 2017.

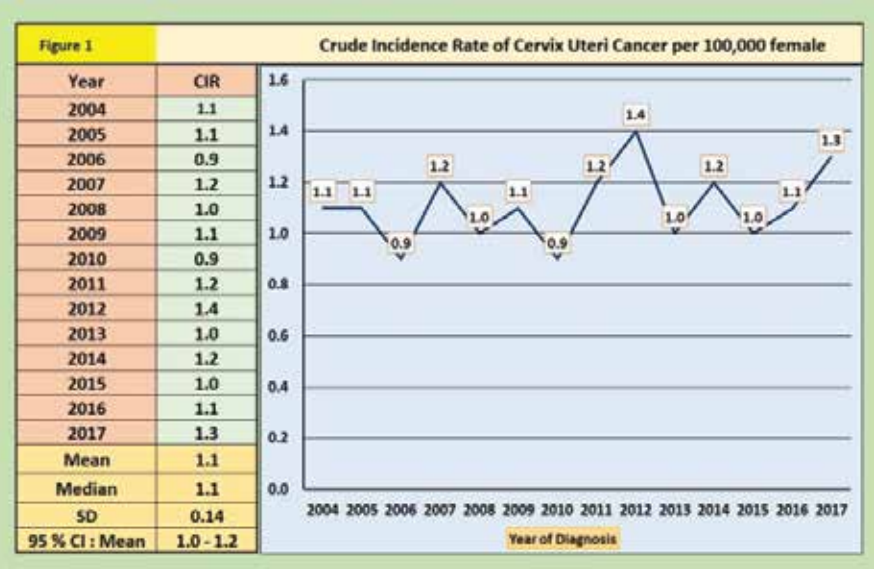

Figure 1. Incidence rate (Crude) of Cervix Uteri Cancer cases among Saudi women from 2004 to 2017

The overall CIR of CUC per 100,000 women in different regions of Saudi Arabia from 2004 to 2017 in shown in Figure 2. The incidence rate varied between regions, with the highest rate in the Eastern region at 1.5 cases per 100,000 women, followed by Tabuk and the Northern region, with rates of 1.3 cases per 100,000 women. The Kruskal-Wallis test for non-normally distributed data revealed statistically significant differences between these locations and other regions of Saudi Arabia, $X^2(12,N=181) = 48.705$, $P < 0.001$). In contrast, the lowest incidence rate was in Jazan, with only 0.5 cases per 100,000 women.

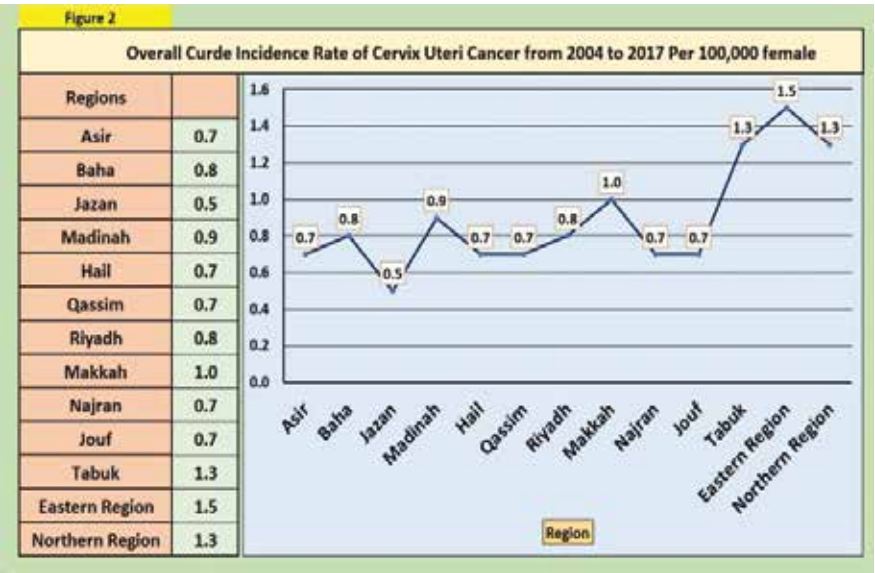

Figure 2. Overall incidence rate (Crude) of Cervix Uteri Cancer cases among Saudi women by region from 2004 to 2017

The ASIR of CUC cases among female Saudis, stratified by year of diagnosis from 2004 to 2017 per 100,000 people, was observed from the SCR (Figure 3). The Age Standardized Incidence Rate (ASIR) of Cervix Uteri Cancer per 100,000 women in Saudi Arabia from 2004 to 2017. Similarly, the ASIR has fluctuated over the years, with a peak of 2.1 cases per 100,000 women in 2012 and a low of 1.3 cases per 100,000 women in 2015. The mean and median ASIR were 1.7 cases per 100,000 women, indicating a relatively stable incidence rate of CUC over the study period after adjusting for age differences among populations. The standard deviation was 0.23, suggesting that the ASIR values were distributed around the mean. However, the overall ASIR of CUC among Saudi women was 1.7 (95% CI: 1.5 to 1.8) per 100,000 women between 2004 and 2017.

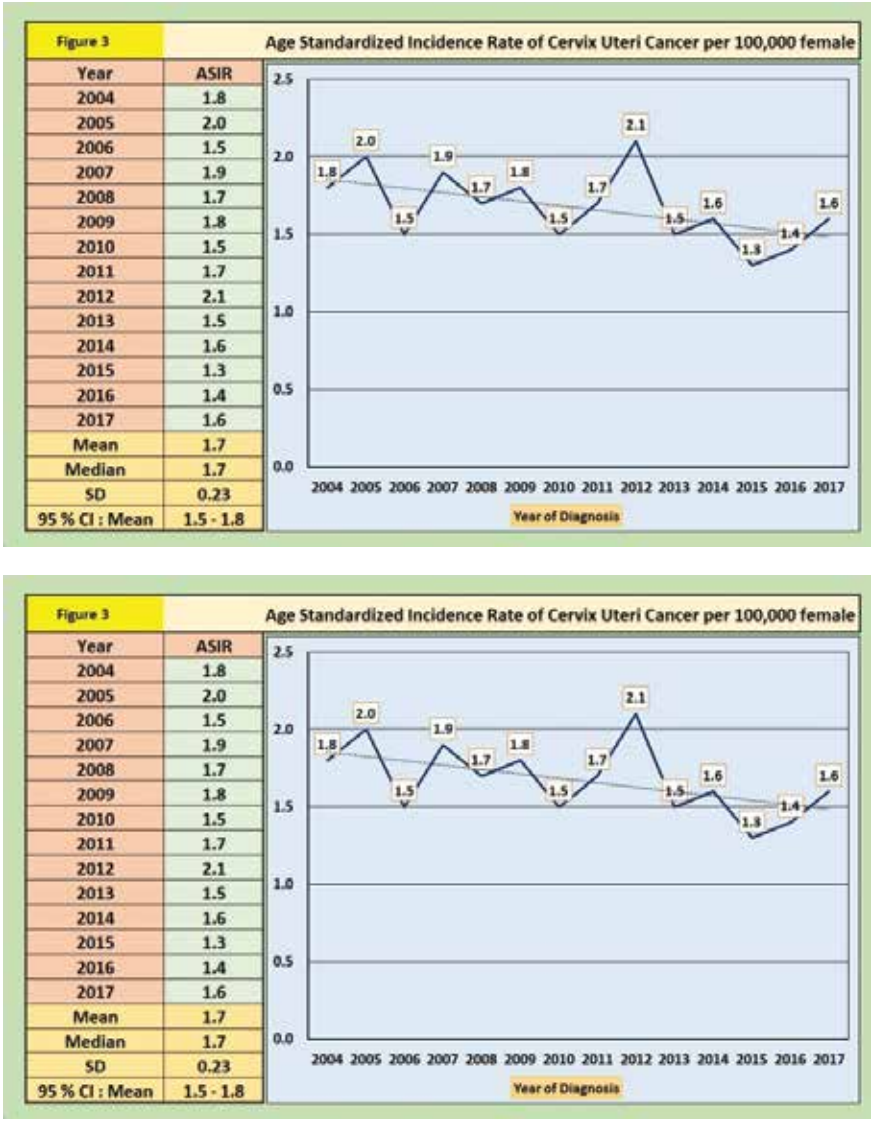

Figure 3
Age Standardized Incidence Rate of Cervix Uteri Cancer per 100,000 female
Year
ASIR
2004 1.8
2005 2.0
2006 1.5
2007 1.9
2008 1.7
2009 1.8
2010 1.5
2011 1.7
2012 2.1
2013 1.5
2014 1.6
2015 1.3
2016 1.4
2017 1.6
Mean 1.7
Median 1.7
SD 0.23
95 % CI : Mean 1.5 - 1.8
2.5
2.0
1.5
1.0
0.5
0.0
2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017
Year of Diagnosis

Figure 3. Incidence rate (age-standardised) of Cervix Uteri Cancer cases among Saudi women from 2004 to 2017

Figure 4 represents the overall ASIR of CUC per 100,000 women in different regions of Saudi Arabia from 2004 to 2017. The ASIR was calculated to adjust for the effect of age distribution on the incidence rate of CUC in different regions. The highest ASIR was observed in the Northern region and the Eastern region, with rates of 2.2 and 2.0 cases per 100,000 women, respectively. Similarly, the region of Tabuk had a relatively high ASIR of 2.0 cases per 100,000 women. On the other hand, the lowest ASIR was found in Jazan, with only 0.7 cases per 100,000 women. The mean and median ASIR were 1.4 and 1.3 cases per 100,000 women, respectively. The Kruskal-Wallis test indicated statistically significant differences in the ASIR of CUC between the regions, $X^2(12,N=180) =41.927$, $P < 0.001$).

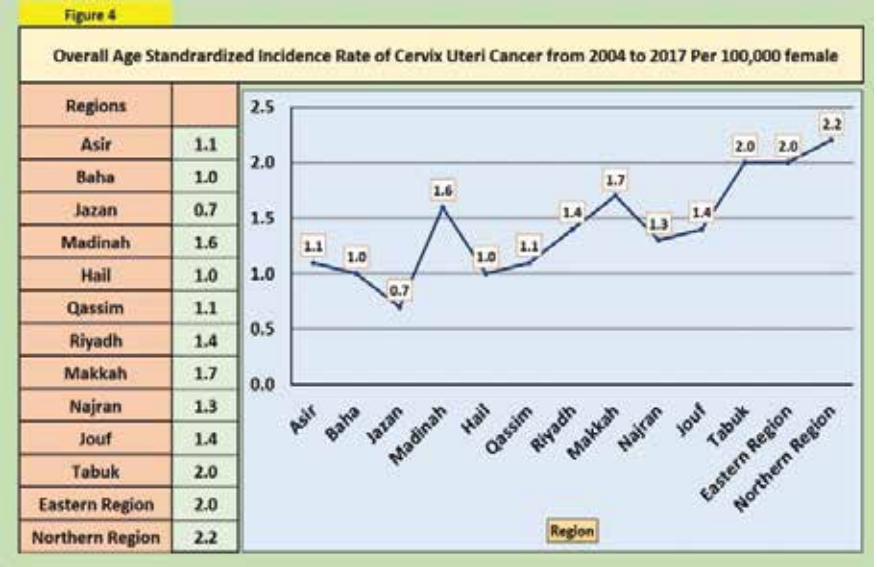

Figure 4. Incidence rate (age-standardised) of Cervix Uteri Cancer cases among Saudi women by region from 2004 to 2017

DISCUSSION

The present study had the objective of investigating the CIR and ASIR of CUC in Saudi Arabia from 2004 to 2017. To the best of our knowledge, this is the first descriptive epidemiological research that analysed the spatial and temporal distribution of CUC among women in all administrative regions of Saudi Arabia, based on the PubMed database. This study aimed to provide an overview of the CUC trend in the country and contribute to the knowledge of the relevance of the disease in Saudi Arabia.

This study sheds light on the incidence of CUC among Saudi women between 2004 and 2017, with an average of 104 cases diagnosed during this period. The findings indicate that while CUC is not a common cancer in Saudi Arabia, it disproportionately affects women aged 45-49 and older, who account for the majority of cases and have a higher incidence rate compared to younger women. This finding is consistent with earlier research that found the highest prevalence of CUC among women aged >65, with rates being much higher among older black women.[10] However, a study reported an unexpected increase in the incidence of CUC among 20–24-year-old women in England, with rates jumping from 2.7 per 100,000 in 2012 to 4.6 per 100,000 in 2014. This abrupt increase has raised concerns that it may be related to the cessation of cervical screening among 20–24-year-old women.[11]

The incidence rates of CUC have been declining in many countries, including Saudi Arabia and the United States. In Saudi Arabia, the ASIR of CUC among Saudi women has dropped steadily from 2004 to 2017. Similarly, in the United States, the incidence rates of CUC have decreased gradually from 2004 to 2019.[12] While the decline in CUC incidence rates is encouraging, there is still a need for effective screening programs that can help detect and treat cases of pre-cancer before they progress to cancer. The screening program should aim to screen the largest possible proportion of women targeted by the national program and ensure appropriate management for all those who have a positive or abnormal test result. This approach can potentially decrease the incidence of CUC by detecting and treating pre-cancerous lesions, ultimately reducing the burden of CUC. Additionally, screening can detect CUC in women at an early stage when the cancer can still be successfully treated.[13]

Our results reveal that between 2004 and 2017, Saudi women in the Northern, Eastern, and Tabuk regions had the highest overall ASIRs for CUC. This shows that Saudi women residing in these places may be highly exposed to HPV, the leading risk factor for CUC. Therefore, the Saudi Arabian government, represented by the Ministry of Health, may concentrate its screening efforts on these regions. In contrast, Saudi women in Jazan were least affected by CUC, indicating that they were more exposed to CUC protective factor than women in other regions. Nonetheless, a case-control study should be done in the Northern, Eastern, and Tabuk regions to determine the probable risk factors that contribute to the rise in

ASIR of CUC among Saudi women. Additionally, it is necessary to explore the protective factors of CUC in the Jazan region that contributed to the disease's decreased prevalence.

CUC is a significant public health issue in many countries, including those in the Arabian Gulf. Our study shows that in Saudi Arabia, the estimated ASIR for CUC among Saudi women in 2020 was 2.8% per 100,000 women, which is lower than in other countries in the region. This is encouraging news, indicating that preventive measures may have a positive impact on reducing the incidence of CUC in the country. Interestingly, our study also found that Oman and the United Arab Emirates had the highest ASIR rate of CUC among women in the Arabian Gulf, at 6.4 and 6.2 per 100,000 women, respectively. This is almost 2.3 times greater than the rate in Saudi Arabia.

Similarly, these two countries also had the greatest ASMR of CUC among women in the region, at 4.2 and 3.9 per 100,000 women, accounting for approximately 2.5 times greater than Saudi Arabia. These findings highlight the need for targeted efforts to prevent and control CUC in these countries.

The findings of this study also highlight the significantly lower burden of CUC among Saudi women compared to other African countries, such as Eswatini, Malawi, Zambia, and Tanzania. These countries had ASIRs of 84.6, 67.9, 65.5, and 62.5 per 100,000 women, respectively, which were 22 to 30 times greater than the overall ASIR of CUC in Saudi Arabia.9 While the reasons for these differences are not entirely clear, they may be attributed to differences in the prevalence of risk factors such as HPV infection, screening practices, and access to healthcare. It is important for policymakers and healthcare providers in Saudi Arabia to continue efforts to improve CUC screening and prevention programs, particularly in high-risk populations. Further research is also needed to better understand the factors contributing to the relatively low burden of CUC in Saudi Arabia compared to other countries in the region and globally.

Finally, this study provides valuable insights into the incidence of CUC in various regions of Saudi Arabia and offers a foundation for future research to investigate the potential risk and protective factors associated with CUC among Saudi women. However, it is important to note that descriptive epidemiological studies like this one have inherent limitations, such as the lack of a control group and the inability to establish causal relationships between risk factors and the incidence of CUC. Additionally, the study was unable to calculate the overall mortality rates of CUC due to missing data on CUC-related deaths in the SCR. Future studies that address these limitations and employ more robust study designs are needed to provide a more comprehensive understanding of the epidemiology of CUC in Saudi Arabia.

CONCLUSION

This study revealed a slight decrease in the ASIRs of CUC among Saudi women. The regions of Northern, Eastern, and Tabuk had the highest CUC ASIRs, while Jazan had the lowest rates. Compared to other countries in the Arabian Gulf, Saudi Arabia has the lowest CUC ASIR. This suggests that the Ministry of Health in Saudi Arabia could focus its screening efforts on these high-risk regions. However, to gain a more comprehensive understanding of the potential risk and protective factors associated with CUC in Saudi Arabia, further epidemiological research is required. These findings can serve as a foundation for future studies aimed at reducing the incidence and mortality rates of CUC in Saudi Arabia.

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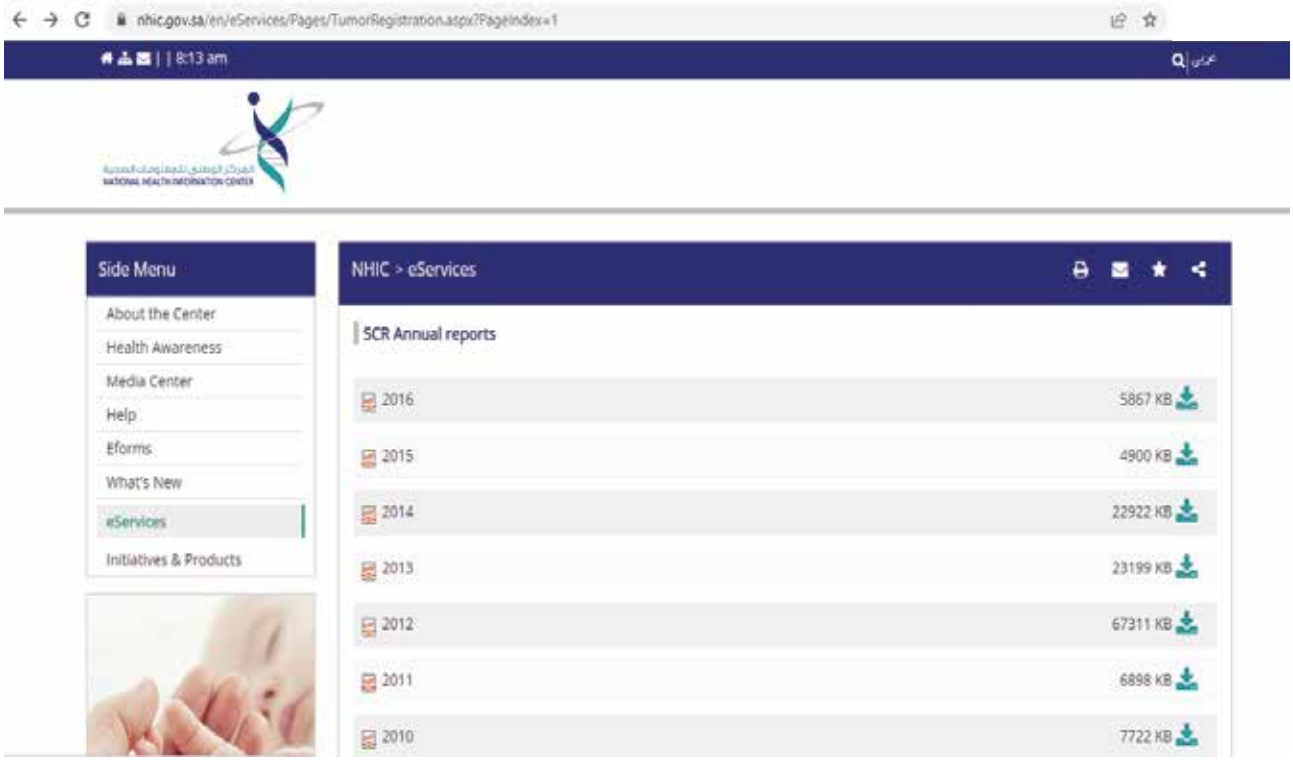

Figure legends

Figure 1: Incidence rate (Crude) of Cervix Uteri Cancer cases among Saudi women from 2004 to 2017.
Figure 2: Incidence rate (Crude) of Cervix Uteri Cancer cases among Saudi women by region from 2004 to 2017.
Figure 3: Incidence rate (age-standardised) of Cervix Uteri Cancer cases among Saudi women from 2004 to 2017.
Figure 4: Incidence rate (age-standardised) of Cervix Uteri Cancer cases among Saudi women by region from 2004 to 2017.

Research Article

The Prevalence of Post-traumatic Stress Disorder (PTSD) Symptoms in Women Hospitalized Due to COVID-19 Infection during Pregnancy in Indonesia and Its Association with Employment Status and Delivery Method: A Single Centre Study

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Abstract

Objective: To analyze the prevalence of post-traumatic stress disorder (PTSD) symptoms in pregnant women infected with COVID-19 in one of the Indonesian tertiary referral centers for COVID-19 cases and its association with maternal employment status and delivery method.

Method: Data from medical records and an online questionnaire were collected for a cross-sectional study. The study included pregnant women treated in the COVID-19 non-intensive isolation wards throughout 2021. The occurrence of PTSD symptoms was assessed using the PTSD Checklist for DSM-5 (PCL-5). Prevalence of PTSD symptoms was described and its correlation with employment status and delivery method were analyzed.

Results: The analysis involved data from 75 patients, with a mean PCL-5 total score of 17 ± 13.85. Among them, 16% met the PTSD symptoms criteria (PCL-5 total score ≥32). Of the total, 72% were women who had undergone caesarean section (CS), and the same percentage were unemployed. Comparisons revealed no significant difference in PTSD symptoms occurrence based on employment status (19% in employed women vs. 14.8% in unemployed women, p=0.729, 95% CI) and delivery method (14.8% in CS vs. 16.7% in spontaneous delivery, p=1, 95% CI).

Conclusion: This study revealed a significant prevalence of PTSD among pregnant women during the COVID-19 pandemic. Despite the fact that is no association found between the prevalence of PTSD symptoms and employment status or delivery method in this study, further research is needed to understand the psychological effects, clinical implications, and relevant factors impacting pregnant women in the acute-event settings.

Keywords: COVID-19, pandemics, pregnancy, post-traumatic stress disorders.

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INTRODUCTION

The World Health Organization (WHO) has declared the Coronavirus Disease 2019 (COVID-19) a new pandemic.[1] This global health crisis has had a profound effect on various aspects of healthcare, including maternity care.[2] In addition to the immediate impact on patients and healthcare systems, the pandemic has the potential to influence the mental health of a large population including pregnant women, whose emotional status is more susceptible to acute events.[3] Triggered by the COVID-19 outbreak as one of the major stressors, post-traumatic stress disorder (PTSD) can emerge in those populations. PTSD can manifest after an individual experiences a shocking, terrifying, or dangerous event, such as an emergency crisis or catastrophic disaster. However, despite the immediate focus on treating and controlling the spread of COVID-19, the mental health implications of this condition during the pandemic may be overlooked.[4]

A study reported an increased likelihood of PTSD in pregnant women.[5] A meta-analysis

involving 24,267 women reported a mean prevalence of 3.3% for PTSD during pregnancy, with a higher risk observed in 18.95% of women in the high-risk group.[6] A study conducted in Turkey during the COVID-19 pandemic revealed that obstetric, psychiatric, and social factors contribute to the risk of PTSD in pregnant women. Concerns regarding childbirth complications due to the pandemic, COVID-19 infections among close contacts, and the ongoing monitoring of the COVID-19 situation significantly affected PTSD symptoms in pregnant women[7].

Prenatal anxiety, depression, and stress have been linked to hyperemesis gravidarum, ectopic pregnancy, miscarriage, preterm birth, low birth weight, intrauterine growth retardation (IUGR) and fetal death.[8,9] A study from Indonesia reported that during COVID-19 pandemic, pregnant women who were anxious had a 3.761-fold risk of premature rupture of mambrane (PROM) compared to those who were not anxious.[10] Moreover, extensive evidence suggests that maternal depression, anxiety, and stress during pregnancy can have detrimental effects on the neurodevelopment of the child, leading to an increased risk of emotional, behavioral, and cognitive problems compared to children whose mothers did not experience these mental health challenges.[11]

Medical associations worldwide, including Indonesia, have issued clinical guidelines for the care of pregnant women and newborns during the pandemic. However, there has been limited empirical evidence and guidance regarding maternal mental health, particularly regarding PTSD.[12,13]This study aimed to fill this gap by providing data on the impact of the pandemic on maternal mental health, specifically PTSD, in the Indonesian population. The findings of this study will contribute to a better understanding of the mental health challenges faced by mothers during the pandemic and help inform future interventions and support strategies.

METHODS

A retrospective study was conducted at Fatmawati Hospital, involving pregnant patients who received treatment in the COVID-19 isolation room throughout 2021 and who were discarded in 3 months or more, prior to the study. Purposive total sampling to all the population with desired characteristics was conducted and data was collected from medical records and an online questionnaire. Prior informed consent was obtained from all participants before their inclusion in the study. The inclusion criteria for research subjects were as follows; obstetric patients with confirmed COVID-19, no previous history of mental health problems, and ability to participate in the study and complete the questionnaire. Exclusion criteria were; incomplete online questionnaire submission, and participants who have received treatment in high or intensive care units will be excluded. Patients receiving intensive treatment were excluded due to the complexity of severe COVID-19 cases involving more complex confounding factors outside of our scope of this study. We gathered data encompassing patient age, employment status, gestational status, obstetrical history, delivery method, newborn birthweight, Apgar score, and questionnaire responses.

The outcomes measured in this study were; the prevalence of PTSD symptoms, the association of PTSD symptoms and employment status, and the association of PTSD symptoms and delivery method. We consider analysis of employment status as a related factor to PTSD symptoms based on the findings that employment status has significant influence on a person's response to current critical events.[14] Method of delivery has also been reported to influence maternal psychological state.[15–18]

This study utilized The PTSD Checklist for DSM-5 (PCL-5), a 20-item questionnaire aligning with the DSM-5 symptom criteria for PTSD. The questionnaire employed a rating scale ranging from 0 to 4, with descriptors of "Not at all," "A little bit," "Moderately," "Quite a bit," and "Extremely" corresponding to scores of 0, 1, 2, 3, and 4, respectively. The PCL-5 provides a provisional diagnosis through two approaches: 1) summing all 20 items and using a cutoff point score of 31-33, or 2) considering any item rated as 2 or higher (equivalent to "Moderately" or above) as a symptom endorsed based on the DSM-5 diagnostic rule as follows: at least one item from Cluster B (re-experiencing symptoms, questions 1-5), one item from Cluster C (avoidance symptoms, questions 6-7), two items from Cluster D (negative alterations symptoms, questions 8-14), and two items from Cluster E (hyperarousal symptoms, questions 15-20). Generally, the use of a cutoff score tends to yield more reliable results compared to the DSM-5 diagnostic rule.[19] In this study, women who scored 32 or above on the PCL-5 were identified as having PTSD.[19,20]

Statistical analysis was conducted using IBM SPSS version 24.0 (IBM Corp., Armonk, NY). Descriptive statistics, including numbers (n), percentages (%), means, and standard deviations (Mean ± SD), were presented. The occurrence of PTSD was compared between employed and unemployed women, as well as between those who underwent cesarean section (CS) and those who had spontaneous delivery, using the chi-squared test. Statistical significance was defined as a p-value of < 0.05. The Cronbach's Alpha was computed to assess internal validity of PCL-5 questionnaire. In this study, the high Cronbach's Alpha value (0.93) indicates that the questionnaire items measuring the same underlying construct consistently, reinforcing the validity of our findings.

RESULTS

Out of 214 eligible subjects, 72 did not want to take part in the study, 67 could not be reached, and only 75 agreed to participate and complete the questionnaire. There is no incomplete questionnaire submission. The mean maternal age was 31 years old, and all participants were in their third trimester of pregnancy. Among them, 54 women (72%) had CS delivery, while three women were still pregnant at the end of data collection period. Fifty-five women (72%) were unemployed. Further details on the characteristics of the subjects are described in Table 1.

Table 1. Characteristics Description of Study Subjects

Variables	N	%	Mean ± SD
Age (years old) (Min-Max= 21-44)	75		31.47 ± 5.78
Gestational age (weeks)	75		37.4 ± 2.13
Gravidity			
1	18	25.0	2.49 ± 1.19
2	21	29.2	
3	17	23.6	
4	12	16.7	
5	4	5.6	
Parity			
0	22	30.6	1.24 ± 1.03
1	20	27.8	
2	21	29.2	
3	9	12.5	
History of abortion			
0	59	81.9	-
1	8	11.1	
2	4	5.6	
3	1	1.4	

The mean PCL-5 total score of the subjects was 17 (17.64 ± 13.85), ranging from 0 to 56. Twelve women (16%) met the criteria for PTSD with a PCL-5 score of ≥ 32. When classified based on the symptom clusters, the average score for cluster B (re-experiencing) was 4.5 (4.52 ± 3.5, 0-15), cluster C (avoidance) was 1.8 (1.85 ± 2, 0-6), cluster D (negative mood cognition) was 5 (5.09 ± 5.38, 0-18), and cluster E (hyperarousal) was 6 (6.17 ± 4.77, ranging from 0-19) (Table 2).

Table 2. Result from PCL-5 self-assessment questionnaire

PCL-5 scores	N (%)	Min-Max	Mean ± SD
Cluster B (reexperiencing)	65 (86.7)	0-15	4.52 ± 3.512
Cluster C (avoidance)	45 (60)	0-6	1.85 ± 2.005
Cluster D (negative mood cognition)	53 (70.7)	0-18	5.09 ± 5.376
Cluster E (hyperarousal)	67 (89.3)	0-19	6.17 ± 4.769
		0-56	17.64 ± 13.849
Total score of ≥32	12 (16)		42.75 ± 5.75

Among the three women who continued their pregnancy, one of them (33.3%) had PTSD. The prevalence of PTSD did not differ significantly between employed and unemployed women (19% vs. 14.8%, p=0.729) or between those who had a cesarean section (CS) delivery and those who had a spontaneous delivery (14.8% vs. 16.7%, p=1) (Table 3).

Table 3. PTSD Symptoms based on Delivery Method and Employment Status

Maternal Condition	N	PTSD		*P-value* (95% CI)
		No	Yes	
Pregnancy outcome		%	%	
Still Pregnant	3 (4)	2 (66.7)	1 (33.3)	
CS delivery	54 (72)	46 (85.2)	8 (14.8)	1.0
Spontaneous delivery	18 (24)	15 (83.3)	3 (16.7)	
Status				
Employed	21 (28)	17 (81)	4 (19)	0.73
Unemployed	54 (72)	46 (85.2)	8 (14.8)	
Total			12 (16)	

DISCUSSION

Pregnant women are particularly vulnerable to mental health issues, including PTSD, which can have significant impacts on both the mothers and their infants. Various factors contribute to the development of PTSD in pregnant women, such as obstetric complications, a history of perinatal and antenatal psychiatric conditions, exposure to traumatic life events, fear of childbirth, and lack of social support during the antenatal period.[7,21] While our study showed 12 (16%) COVID-19 positive pregnant women had PTSD, the prevalence of PTSD among pregnant women during the COVID-19 pandemic has varied in other studies, ranging from 0.9% to 43.2% (16-19). A meta-analysis involving 24,267 women indicated a mean prevalence of 3.3% (95% CI=2.44-4.54) for PTSD during pregnancy, with the high-risk group showing a higher risk of PTSD, estimated at 18.95% (95% CI=10.62-31.43).[6]

The COVID-19 pandemic has introduced significant stress and challenges for pregnant women, making pregnancy and childbirth more daunting. Nearly half of pregnant women infected with COVID-19 experienced clinically significant acute stress symptoms during delivery.[22] Studies by Berthelot et al. (2020) have reported a higher prevalence and more symptoms of PTSD in pregnant women during the COVID-19 pandemic compared to the pre-pandemic period.[12,23] However, a cross-sectional study involving 859 participants found that the frequency of PTSD was lower in pregnant women compared to non-pregnant women during the COVID-19 pandemic (0.9% vs. 5.7%, $p<0.05$). Possible explanations for this difference include the better mental and financial conditions of pregnant women due to their pre-pregnancy preparations and support from their families and healthcare providers.[24] These variations in findings may stem from differences in sample populations, diagnostic tools, and the timing of the studies conducted during the pandemic.

Our study revealed a similar unemployment rate among women (72%) to a descriptive study from Indonesia which found that 29 out of 41 (70.7%) pregnant women with COVID-19 were unemployed. The study also demonstrated a higher ratio for asphyxia babies born of unemployed women compared to those employed, although there is no available data concerning the psychological aspect of the mothers.[25] Our results indicate that COVID-19 positive pregnant women can experience PTSD symptoms regardless of their employment status and delivery method. Although we observed a higher number of PTSD cases among unemployed pregnant women compared to employed women, the difference was not statistically significant ($p>0.05$). This finding is consistent with previous studies.[7,26,27] One study reported that marital status, socioeconomic status, previous COVID-19 diagnosis, and pregnancy trimester were not associated with a PTSD diagnosis, while maternal age, education level, and obstetric condition showed significant differences between pregnant women with PCL-5 total scores <33 and ≥33.7 Other studies found that obstetric conditions, maternal age ≥35, presence of COVID-19-related symptoms were associated with a higher risk of PTSD.[28]

Although the number of PTSD cases was higher in women who had a cesarean section (CS) delivery, the difference was not statistically significant in our study. This finding is consistent with another study that reported no association between delivery method and PTSD.[27] A prospective study in Iran involving 240 women also found no significant correlation between the type of delivery and the incidence of PTSD, despite higher rates of PTSD in the CS group compared to normal vaginal delivery (7.3% vs.

5.1%, p=0.48).[17]

In a literature review, it has been suggested that vaginal delivery is associated with more positive experiences, while emergency cesarean section and instrumental vaginal delivery are associated with more negative experiences.15 A meta-analysis study further supported these findings, showing that cesarean section delivery was more closely associated with PTSD compared to vaginal delivery (P=0.005).16 Additionally, some studies have found that PTSD is more prevalent among women who had emergency CS compared to those who had elective CS.[17,18]

The prevalence of acute stress symptoms related to COVID-19 positivity suggests an increased risk of subsequent maternal psychopathology and potential impairments in mother-infant bonding, emphasizing the need for heightened attention to mental health concerns in this vulnerable population.22 Several factors contribute to the traumatic stress reaction experienced by COVID-19 positive mothers during delivery, including concerns about health risks and viral transmission to the baby. Additionally, the COVID-19 pandemic has resulted in social isolation for women during labor. Research indicates that around 40% of COVID-positive pregnant women had no visitors during their hospital stay, and the majority had no visitors during delivery due to hospital restrictions on visitation. Prior to the pandemic, the presence of a support person was recognized as a factor that enhanced obstetric and neonatal outcomes while reducing negative perceptions of birth experiences.[29,30]

COVID-19 positive pregnant women were more likely to experience physical separation from their newborns, as indicated by the lack of initial skin-to-skin contact and limited rooming-in. Partial breastfeeding was also less common in this group. Physical touch and closeness in the early hours of life have been shown to provide health benefits for both the mother and the child. It promotes mother-infant bonding, facilitates nursing, and reduces the risk of maternal psychological distress, all of which may contribute to increased acute stress experienced by COVID-positive women during childbirth.[22]

Our study on the impact of PTSD on peripartum women during the COVID-19 pandemic in Indonesia provides preliminary findings. However, it is important to acknowledge the limitations of our study. First, the sample size was limited, and data were collected from a single center, which may limit the generalizability of the findings. Additionally, as a cross-sectional study, we were unable to follow up with participants over time to assess the long-term effects of PTSD. Furthermore, we did not conduct a comprehensive analysis of the potential risk factors associated with PTSD or explore the broader impacts on both the mothers and babies.

CONCLUSIONS

The acute events experienced by pregnant women as a vulnerable population, including COVID-19 infection and receiving treatments at the hospital during pandemic, can have a profound impact on the well-being of both the mothers and the infants. Our findings showed that 16% of pregnant women with COVID-19 infection had PTSD symptoms. This study serves as a foundation for further exploration of related topics in future research. It is recommended that future studies analyze potential factors associated with the development of PTSD in this population and explore treatment options. Future research should aim conduct more extensive analyses to better understand the complexities and implications of PTSD in pregnant and peripartum women during the COVID-19 pandemic.

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Research Article

Vitamin D Levels and Risk Factors in Early Onset Preeclampsia, Late Onset Preeclampsia and Normal Pregnancy

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Abstract

Objective: To determine the frequency distribution and the relation of risk factors to vitamin D levels in women with early onset (EOSPE), late-onset severe preeclampsia (LOSPE), and normal pregnancy.

Methods: This study was cross-sectional with pregnant women diagnosed with EOSPE LOSPE at RSUP DR M Djamil Padang and normal pregnancy at the Pengambiran Health Center. The serum samples of the research subjects were examined for blood levels of Vitamin D using the ELISA kit.

Results: Frequency distribution of risk factors for EOSPE respondents aged 20-35 years 50%, mothers not working 85%, single pregnancy 95%, normal blood sugar 80%, and an increase in the number of leukocytes 90%. Frequency distribution of LOSPE respondents, maternal age 20-35 years 60%, mothers not working 95%, single pregnancy 95%, normal blood sugar 65%, and increasing leukocytes 75%. Based on the data analysis test EOSPE respondents with risk factors for preeclampsia history and body mass index had a significant relation with vitamin D levels with *P Value* 0.00 ($P < 0.05$). LOSPE mothers with gravid risk factors, history of preeclampsia, history of hypertension, and history of Diabetes Mellitus had a significant relation with Vitamin D levels *P Value* 0.00 ($P < 0.05$).

Conclusion: The results of statistical tests for LOSPE mothers had a significant relation with Vitamin D levels compared to the risk factors for EOSPE mothers. The incidence of preeclampsia is influenced by complex etiopathogenesis, one of which is influenced by vitamin D levels.

Keywords: EOSPE, LOSPE, Elisa-kit, Vitamin D, Etiopathogenesis**.**

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INTRODUCTION

According to the World Health Organization (WHO), the Maternal Mortality Rate (MMR) is still very high, around 810 women die from complications related to pregnancy or childbirth every day, and 295,000 women die during and after pregnancy and childbirth.[1] MMR in Indonesia in 2017 and 2019 did not change, 305 per 100,000 live births. The leading cause of maternal death is hypertension in pregnancy 27.2%.[2] According to WHO, incidence of preeclampsia ranges from 0.51% - 38.4%, while the incidence in Indonesia is around 3.4% - 8.5%.[3]

There are many theories about the causes of preeclampsia, including theories of placental vascular abnormalities, placental ischemia, nutritional deficiencies, and inflammation. increased VitD levels may improve the human extravillous trophoblast invasion, which is required for normal placentation.[4]

Vitamin D is produced through food or endogenous synthesis as ergocalciferol (vitamin D2) is obtained from plants while cholecalciferol (vitamin D3) is obtained from animals. The majority is produced endogenously in the skin from ultraviolet radiation.[5] About 50% of the pregnancies are classified as vitamin D deficient.

This deficiency is likely the result of increased melanin content that prevents adequate exposure to ultraviolet B radiation for conversion of 7-dehydrocholesterol to vitamin D. With an increased incidence of vitamin D deficiency, there is increased awareness of the potential impact on pregnancy outcome.[6]

In the antenatal population in London, vitamin D levels were less than 25 nmol/L, found 47% Asian Indian, 64% Middle Eastern, 58% Black and 13% Caucasian. United States, 33% deficient levels of vitamin D, 24% in Canada, 45% in Belgium, 44% in the Netherlands, 20% in Spain, and 77% in Germany. In Asia, the incidence of vitamin D deficiency is 90%, Turkey 67%, Iran 96%, and India 69%.[7]

Recent epidemiologic data has linked Vitamin D deficiency to adverse perinatal outcomes. More recently, data support associations between Vitamin D deficiency, preterm birth, decreased birth weight, and hypertensive disease in pregnancy.[8]

Preeclampsia is classified based on gestational age. Early onset preeclampsia (EOSPE) occurs before 34 weeks of gestation and is closely associated with impaired trophoblast invasion and failure of spiral artery remodelling. Late-onset preeclampsia (LOSPE) occurs at or after 34 weeks of gestation. It is caused by an increased susceptibility of the maternal vasculature to an inflammatory state to normal pregnancy or placental atherosis that initially develops normally.[9-10]

Preeclampsia is the second leading cause of maternal mortality in Indonesia, accounting for 27.1% of maternal deaths, and was the second largest cause of maternal mortality (12%) in West Sumatra in 2015. In 2016, it ranked as the leading cause of maternal death (25%) in Padang. Data from the medical records at Dr. RSUP M. Djamil Padang reported preeclampsia cases at 32.5% in 2016, 38.8% in 2017, and 15.7% in 2018. Given the issues and data presented, the researchers aimed to investigate the relationship between risk factors and vitamin D levels in pregnant women diagnosed with early-onset preeclampsia (EOSPE), late-onset preeclampsia (LOSPE), and those with normal pregnancies in Padang City in 2021.

METHODS

This study used a cross-sectional method, which linked risk factors to vitamin D levels in pregnancies diagnosed with EOSPE, LOSPE, and normal pregnancy at Padang in 2021. Data collection involved the use of a questionnaire, with the respondent's venous serum and a human vitamin D kit serving as the primary materials. The research instrument was an observation sheet that recorded the results of the vitamin D level assessments. Blood samples were collected from the maternity ward and the obstetrics polyclinic at RSUP Dr. M. Djamil, Padang City. The data gathered will be analyzed using both univariate and bivariate analyses, employing the Statistical Program for Social Science (SPSS). The ethical approval from the Faculty of Medicine Ethic Committee, Andalas University No 456/UN.16.2/ KEP-FK/2021.

RESULT

Subject Characteristics

Table 1. Frequency distribution of early-onset preeclampsia (EOSPE) and late-onset preeclampsia (LOSPE)

Characteristics		EOSPE		LOSPE	
		Frequency (F)	%	Frequency (F)	%
Age	< 20 years	1	5	-	-
	20 -35 years old	10	50	12	60
	35 years old	9	45	8	40
	Amount	20	100	20	100
Mom's job	Housewife	17	85	19	95
	working mom	3	15	1	5
	Amount	20	100	20	100
Pregnancy	SinglePregnancy	19	95	19	95
	Gameli	1	5	1	5
	Amount	20	100	20	100
Blood pressure	140 - < 160 mmHg	1	5	8	40
	≥ 160 mmHg	19	95	12	60
	Amount	20	100	20	100
blood sugar	Hypoglycemia	1	5	3	15
	Normalblood sugar	16	80	13	65

	Hyperglycemia	3	15	4	20
	Amount	20	100	20	100
Leukocytes	Normal	2	10	5	25
	Leukocytosis	18	90	15	75
	Amount	20	100	20	100

From Table 1 with EOSPE respondents, 50% of mothers 20-35 years, 85% a housewife, 95% of singleton pregnancies, mothers with blood pressure of 160 mmHg 95%, EOSPE mothers with normal blood sugar 80%, and increasing of leukocytes 90%. Mothers diagnosed with LOSPE found 60% aged 20-35 years, a housewife 95%, singleton pregnancies 95%, mothers with blood pressure 160 mmHg was 60%, normal blood sugar 65%, and had an increasing of leukocytes 75%.

Table 2. Distribution of the frequency of normal pregnancies

Normal Pregnancy		Frequency (F)	%
Age	< 20 years	1	3.1
	20 -35 years old	30	93.8
	35 years old	1	3.1
	Amount	32	100
Mom's job	A House Wife	26	81.2
	working mom	6	18.8
	Amount	32	100
Pregnancy	Single Pregnancy	32	100
	Gameli	-	-
	Amount	32	100
Blood pressure	Hypotension	3	9.4
	Normal	28	87.5
	Hypertension	1	3.1
	Amount	32	100

Table 2 shows that 93.8% of normal pregnant women were aged 20–35 years, 81.2% were housewives, 100% had singleton pregnancies, and 87.5% had normal blood pressure.

Table 3. Relation between risk factors for EOSPE and vitamin D levels

Risk Factors	Vitamin D Level				Amount		*P-Value*
	Deficiency		Severe Deficiency				
	F	%	F	%	F	%	
Age							
< 20 years	0	0	1	5	1	5	0.922 **
20 -35 years old	3	15	7	35	10	50	
35 years old	2	10	7	35	9	45	
Amount	5	25	15	75	20	100	
Gravida							
Primigravida	3	15	4	20	7	35	
Multigravida	2	10	11	55	13	65	0.290***
Amount	5	25	15	75	20	100	
PE history							
Never	4	20	14	70	18	90	0.000*
Ever	1	5	1	5	2	10	
Amount	5	25	15	75	20	100	
History of Hypertension							
Never	4	20	15	75	19	95	
Ever	1	5	0	0	1	5	0.250***
Amount	5	25	15	75	20	100	
History of Kidney Disease							
Never	5	25	15	75	20	100	Constant variables
Ever	0	0	0	0	0	0	
Amount	5	25	15	75	20	100	

DM history							
Never	5	25	15	75	20	100	Constant variables
Ever	0	0	0	0	0	0	
Amount	5	25	15	75	20	100	
IMT							
Underweight	0	0	0	0	0	0	
Normal	3	15	9	45	12	60	0.000*
Overweight	2	10	6	30	8	40	
Obesity	0	0	0	0	0	0	
Jumlah	5	25	15	75	20	100	
Hb level							
Normal	5	25	10	50	15	75	
Mild Anemia	0	0	3	15	3	15	0.150**
Moderate Anemia	0	0	2	10	2	10	
Severe Anemia	0	0	0	0	0	0	
Amount	5	25	15	75	20	100	

*Note : *chi-square Test*
***Mann Whitney Test*
****Fisher's Test*

Based on Table 3, 35% of women with early-onset preeclampsia (EOSPE) were aged between 20–35 years and over 35 years, while 55% were multigravida. Additionally, 70% of women with EOSPE had no history of preeclampsia, 75% had no history of hypertension, 75% had no history of kidney disease, and 75% had no history of diabetes mellitus. Among women with EOSPE, 45% had a normal BMI, and 50% had normal hemoglobin levels, with severe vitamin D deficiency observed.

Bivariate analysis revealed a significant relationship between risk factors and vitamin D levels in pregnant women with EOSPE, specifically for a history of preeclampsia (P-value = 0.000) and maternal Body Mass Index (BMI) (P-value = 0.000), both with P-values < 0.005. However, other risk factors such as maternal age (P = 0.922), gravidity (P = 0.290), history of hypertension (P = 0.250), and hemoglobin levels (P = 0.150) did not show a significant association with vitamin D levels, as their P-values exceeded 0.05. Maternal factors such as a history of kidney disease and diabetes mellitus could not be statistically tested against vitamin D levels due to constant variable data.

Table 4. Relation between late onset preeclampsia risk factors (LOSPE) and vitamin D levels

Risk Factors	Vitamin D Level				Amount		*P-Value*
	Deficiency		Severe Deficiency				
	F	%	F	%	F	%	
Age							
< 20 years	0	0	0	0	0	0	0.075**
20 -35 years old	0	0	12	60	12	60	
35 years old	2	10	6	30	8	40	
Amount	2	10	18	90	20	100	
Gravida							
Primigravida	0	0	4	20	4	20	0.000*
Multigravida	2	10	14	70	16	80	
Amount	2	10	18	90	20	100	
History of PE							
Never	2	10	17	85	19	95	0.000*
Ever	0	0	1	5	1	5	
Amount	2	10	18	90	20	100	
History of Hypertension							
Never	2	10	17	85	19	95	0.000*
Ever	0	0	1	5	1	5	
Amount	2	10	18	90	20	100	
History of Kidney Disease							
Never	2	10	18	90	20	100	Constant variables
Ever	0	0	0	0	0	0	
Amount	2	10	18	90	20	100	

History of DM							
Never	2	10	16	80	18	90	0.000*
Ever	0	0	2	10	2	10	
Amount	2	10	18	90	20	100	
BMI							
Underweight	0	0	0	0	0	0	
Normal	0	0	8	40	8	40	0.131**
Overweight	1	5	8	40	9	45	
Obesity	1	5	2	10	3	15	
Amount	2	10	18	90	20	100	
Hb level							
Normal	1	5	16	80	17	85	
Mild Anemia	1	5	2	10	3	15	0.154**
Moderate Anemia	0	0	0	0	0	0	
Severe Anemia	0	0	0	0	0	0	
Amount	2	10	18	90	20	100	

*Note: *chi-square Test*
***Mann Whitney Test*
****Fisher's Test*

Based on Table 4, 60% of women with LOSPE are aged 20-35 years, 70% multigravida, 85% have no history of preeclampsia, 85% have no history of hypertension, 90% have no history of kidney disease, 80% have no history of Diabetes Mellitus, 40% have normal and overweight BMI, and 80% have normal hemoglobin levels and have severe deficiency of Vitamin D levels.

The results of bivariate analysis between gravid (P = 0.000), history of preeclampsia (P = 0.000), history of hypertension (P = 0.000), and history of Diabetes Mellitus (P = 0.000) LOSPE had a significant relationship with vitamin D levels, P Value < 0.005.

Relation between LOSPE such as maternal age (P = 0.075), maternal body mass index (P = 0.131), maternal hemoglobin level (P = 0.154) with vitamin D levels didn't have a significant relationship because P Value > 0.05. Risk factors for a history of kidney disease cannot be statistically tested with vitamin D levels because the variable data are constant.

Table 5. Relation Between Normal Pregnancies and vitamin D levels in Pengambiran Health Center in 2021

Risk Factors	Vitamin D Level				Amount		*P-Value*
	Deficiency		Severe Deficiency				
	f	%	F	%	f	%	
Age							
< 20 years			1	3.1			
20 -35 years old			30	93.8			
35 years old			1	3.1			
Amount			32	100			
Gravida							
Primigravida			20	62.5			
Multigravida			12	37.5			
Amount			32	100			
History of PE							
Never			32	100			
Ever			0	0			
Amount			32	100			
History of Hypertension							
Never			31	96.9			
Ever			1	3.1			
Amount			32	100			
History of Kidney Disease							
Never			32	100			
Ever			0	0			
Amount			32	100			
History of DM							
Never			32	100			
Ever			0	0			
Amount			32	100			

BMI		
Underweight	2	6.3
Normal	17	53.1
Overweight	11	34.3
Obesity	2	6.3
Amount	32	100

For data on normal pregnancies, it was not possible to statistically test the relationships between maternal age, gravidity, history of preeclampsia, hypertension, kidney disease, diabetes mellitus, or body mass index with vitamin D levels, as the vitamin D levels in all normal pregnant women were constant, showing 100% severe deficiency.

DISCUSSION

Analysis Between Risk Factors for Early Onset Preeclampsia (EOSPE) and Vitamin D Levels

The results showed a significant relationship between maternal age and vitamin D levels as risk factors for early-onset preeclampsia (EOSPE). This finding aligns with research conducted at the Majalengka Health Center, which reported that 70% of preeclampsia cases occurred in women aged 20–35 years. This is consistent with the theory that, as the gestational period progresses, the body's organs must work harder to accommodate the demands of pregnancy, thereby increasing the risk of complications such as preeclampsia.[12]

Additionally, the low levels of vitamin D observed in women with EOSPE in this study are consistent with findings from case-control studies. Specifically, serum 25(OH)D levels below 37.5 nmol/L during early pregnancy (around 22 weeks) are associated with a fivefold increase in the incidence of preeclampsia, with the risk doubling for every 50 nmol/L decrease in serum 25(OH)D levels during pregnancy.[13]

Several studies explaining the age characteristics obtained p> 0.05 which stated that there was no difference between the two age groups of normal pregnancy and preeclampsia with vitamin D levels.[14]

There is no significant relationship between Gravid EOSPE and levels of Vitamin D. Research conducted by Leony in 2020 also obtained the same which stated there was no difference between gravid normal pregnancy and preeclampsia with vitamin D levels.[14]

Risk factors for the history of preeclampsia with EOSPE concluded a significant relationship between the history of preeclampsia in EOSPE and Vitamin D levels. This study was conducted by Robinson et al., regarding EOSPE, 25(OH)D levels were significantly lower than the control. In this study, logistic regression analysis was performed to assess the effect of maternal serum 25(OH)D levels and it was found that every 10 ng/mL increase in serum decreased EOSPE by 63%.[15]

Vitamin D levels assessed in early pregnancy were found to be lower among women who subsequently experienced preeclampsia. The study noted a two-fold increased risk for preeclampsia associated with a decrease of 20 ng/mL in serum vitamin D levels after adjusting for confounding factors.[16]

There is a significant relation between BMI of EOSPE and Vitamin D levels. The results are in accordance with the theory that low levels of vitamin D are one of the causes of being overweight, this is due to a decrease in the bioavailability of vitamin D from the skin and the presence of deposition in fat which causes vitamin D to be trapped in fat and cannot be easily excreted.[17]

Research conducted by the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, and Department of Pediatrics at the Medical University of South Carolina found that early-onset preeclampsia (EOSPE) was associated with an 8% increase in the odds of EOSPE when comparing the risk factors related to Body Mass Index (BMI) between the EOSPE group and a control group.[18]

50% of EOSPE with normal hemoglobin levels and vitamin D deficiencies. *P value* was 0.150 (p > 0.05), there is no significant relation between EOSPE maternal hemoglobin levels and vitamin D levels.[19]

Conclusion: Risk factors for history of preeclampsia and body mass index are significantly related to vitamin D levels for EOSPE with *P Value* <0.05.

Analysis Between Risk Factors for Late-Onset Preeclampsia (LOSPE) and Vitamin D Levels

The study results indicated that 60% of mothers with late-onset preeclampsia (LOSPE) aged

20–35 years experienced vitamin D deficiency, with a *p-value* of 0.075 (p > 0.05). This suggests that there is no significant relationship between LOSPE, age, and vitamin D levels. Vitamin D deficiency in LOSPE may be attributed to the normal physiological increase in vitamin D levels during pregnancy, which typically begins early in gestation and continues to rise, potentially doubling in the third trimester. Elevated vitamin D levels are essential for various functions, including bone metabolism, immunomodulation, blood pressure regulation, and the maintenance of insulin secretion by pancreatic beta cells. The increase in vitamin D levels during the third trimester may also have implications for LOSPE.[20]

Seventy percent of multigravida women with late-onset preeclampsia (LOSPE) experienced vitamin D deficiencies, with a *p-value* of 0.000 (p < 0.05), indicating a significant relationship between the gravid factor of LOSPE and vitamin D levels. Analysis by Septiasih demonstrated that gravida status was significantly related to the incidence of preeclampsia, as indicated by a *p-value* < 0.05. This suggests that vitamin D deficiencies are associated with preeclampsia, thereby establishing a significant relationship between gravida status and vitamin D levels.[21]

Eighty-five percent of women with late-onset preeclampsia (LOSPE) who had no prior history of preeclampsia experienced vitamin D deficiency. The *P-value* of 0.000 (p < 0.05) indicates a significant relationship between the history of preeclampsia and vitamin D levels in LOSPE. This study aligns with previous research, which found a significant difference in vitamin D status between women with and without preeclampsia (< 50 nmol/L versus ≥ 50 nmol/L, p = 0.002). Among pregnant women with vitamin D deficiency (< 50 nmol/L), 1.4% developed severe preeclampsia, whereas only 0.6% of those with sufficient vitamin D levels (≥ 50 nmol/L) experienced severe preeclampsia.[22]

Eighty-five percent of LOSPE did not have a history of hypertension having severe deficiency of vitamin D levels, *p-value* 0.000 (p < 0.05) there is a significant relationship between a history of hypertension in LOSPE and levels of Vitamin D. This study is in line with research about the relation between vitamin D levels and blood pressure in pregnancies which explains that vitamin D intake is significantly related to blood pressure (p = 0.028).20 The lower vitamin D intake, the systolic blood pressure will increase. The risk of hypertension in pregnancy increases when serum vitamin D levels are low. Vitamin D levels (1,25 dihydroxyvitamin D) can prevent hypertension in pregnancy by its effects on immune modulation and vascular function.[23]

In PE, circulating serum angiotensin I, angiotensin II, and aldosterone are lower compared to normotensive women, while plasma active rennin levels and autoantibodies to the Angiotensin II type I receptor, which stimulate receptor signaling to increase systemic blood pressure, are higher.[24]

Eighty percent of LOSPE have no history of Diabetes Mellitus having severe deficiency. The *p-value* was 0.000 (p < 0.05), so there is a significant relationship between the history of Diabetes Mellitus in LOSPE and the levels of Vitamin D. This study is in line with the theory that pregnant women with a history of gestational diabetes had significantly lower levels of 25-OHD than the comparison group. Gestational diabetes results from pregnancy-induced insulin resistance and impaired compensatory insulin secretion. Evidence suggests that vitamin D improves insulin sensitivity by enhancing the insulin response to glucose transport. In addition, vitamin D plays a role in early placental development through gene regulation and expression, which may influence the development of preeclampsia.[25]

Forty percent of LOSPE with normal BMI and 40% overweight have severe deficiency of Vitamin D levels. *P value* 0.131 (p > 0.05) shows no significant relationship between LOSPE maternal body mass index and vitamin D levels. Tested the relation between 2 groups of normal pregnancies and preeclampsia with BMI, it was found that 61.9% of obese women experienced preeclampsia during pregnancy, this was associated with vitamin D levels where the BMI factor was not significantly associated with vitamin D levels with *p value* 0.200.16 In normal BMI, increased levels of adiponectin can suppress the expression of adhesion molecules on vascular endothelial cells and cytokines. Production of macrophages to minimize the inflammatory process associated with preeclampsia.[26]

Eighty percent of LOSPEs with normal hemoglobin have severe deficiency of vitamin D levels. The *p-value* of 0.154 (p > 0.05) shows no significant relation between LOSPE maternal hemoglobin levels and Vitamin D levels. This study is not in line with the results of studies showing that mothers with vitamin D deficiency have the highest proportion of anemia, which may indicate that anemia has a stronger relation

with vitamin D levels than other factors.[27]

Thus, that gravid, history of preeclampsia, history of hypertension, and history of Diabetes Mellitus, had a significant relation with Vitamin D levels with late-onset preeclampsia (LOSPE).

Analysis Between Normal Pregnancies and vitamin D levels in Pengambiran Health Center

For data of normal pregnancies, it is not possible to do statistical tests between age, gravid, history of preeclampsia, history of hypertension, history of kidney disease, history of diabetes mellitus, maternal body mass index with vitamin D levels because the data is constant (100% Weight Deficiency).

In 2012, Wei et al. retrospectively analyzed vitamin D status for PE risk in 697 nulliparous women with singleton pregnancies in a randomized, placebo-controlled trial of Vitamin C and E supplementation to prevent PE. After controlling smoking habits, the results showed that in first trimester pregnant women (average 11 weeks), vitamin D deficiency was not associated with an increased risk of PE (P = 0.58).[28]

Observational studies evaluating the association between vitamin D and PE have shown inconsistent results and must be interpreted cautiously. This may result from study design and methodology issues, including a lack of adjustment of key confounding variables and methods of measuring vitamin D levels. [29]

Need for further research on populations with very low vitamin D levels to understand this observation and identify factors that predispose high-risk groups to PE and its more threatening clinical subtypes.[30]

CONCLUSIONS

Mothers with early-onset preeclampsia (EOSPE), a history of preeclampsia, and maternal body mass index showed a significant relationship with vitamin D levels. In mothers with late-onset preeclampsia (LOSPE), risk factors such as gravidity, a history of preeclampsia, a history of hypertension, and a history of diabetes mellitus were significantly related to vitamin D levels. Statistical analysis indicated that the risk factors associated with LOSPE (gravidity, history of preeclampsia, history of hypertension, and history of diabetes mellitus) had a more significant relationship with vitamin D levels compared to the risk factors for EOSPE respondents (history of preeclampsia and body mass index). The incidence of preeclampsia remains influenced by a complex etiopathogenesis.

CONFLICT of INTEREST

There are no conflicts of interest in this report.

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Research Article

An internal iliac artery ligation technique for bleeding control in the placenta accreta spectrum disorder

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Abstract

Objective: To assess the contribution of internal iliac artery ligation to bleeding control during surgery.

Methods: This retrospective study used secondary data from medical records. All patients diagnosed with PASD from January 2019 – to December 2022 were included in this study. Participants were grouped based on operation technique, and the blood loss and operative duration were evaluated. The tests used were the Kruskal-Wallis and the Mann-Whitney U tests.

Results: 108 PAS patients were discovered. The most age group was between 20-35 years with parity of more than or equal to 4, history of Cesarean section once, gestational age at termination 34-36 weeks, and maternal death in 7 out of 101 cases. There were 49 resections, 13 resections with internal iliac artery ligation, 34 hysterectomies, and 12 hysterectomies with internal iliac artery ligation. There was no difference in bleeding and operative duration between resection vs. resection with internal iliac artery ligation (p: 0.113; p: 0.639), hysterectomy vs. a hysterectomy with internal iliac artery ligation ((p:0.052; P:0.723), and resection with ligation vs hystetectomy with the internal iliac artery ligation (p:0.052; p:0.723). Bleeding and operative duration differed significantly between resection vs. hysterectomy (p:0.002; p:0.013). All patients underwent tourniquet placement.

Conclusion: An Internal iliac artery ligation was not shown to reduce bleeding in treating PASD.

Keywords: accreta, internal iliac artery, placenta.

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INTRODUCTION

Placental accreta spectrum disorder (PASD) is a condition in which implantation of the placenta is abnormal. The placenta cannot be delivered spontaneously, and if manual action is performed to release the placenta, it can cause profuse bleeding and is potentially life-threatening. [1] These conditions include placenta adherent or vera, which pathologists also refer to as placenta accrete, in which the villi adhere to the myometrium surface without invading it; placenta increta, in which the villi penetrate deeply into the myometrium to reach the uterus serous layer; and placenta percreta, in which the invasive villous tissue penetrates the serous layer and reaches surrounding tissues, vessels, and pelvic organs. Placental accreta spectrum disorder can be focal or diffuse.[2]

The incidence of PASD is increasing worldwide, from 1 in 2,500 pregnancies to 1 in 500 pregnancies, primarily attributed to an increase in C-section rates. The incidence of PASD in Indonesia in 2016 was around 2% and has increased so far. Increased morbidity and mortality in mothers and fetuses are a result of PASD. The primary cause of the maternal implications is the possibility of obstetric hemorrhage, while the primary cause of the fetal implications is iatrogenic preterm. Up to 90% of

patients require blood transfusions; the usual blood loss is 3000-5000 ml. Other complications include Sheehan syndrome, respiratory distress, kidney failure, and even death. Urine, bladder, and bowel damage, as well as hysterectomy, are examples of surgical complications. That has led to a high incidence of intensive care unit admissions and extended hospitalizations.[3-5] As a result of these complications, diagnosis needs to be made before delivery and allows for multidisciplinary planning to minimize the potential for maternal or neonatal morbidity and mortality.[3]

PASD is expected to be diagnosed during antenatal care; therefore, it can be referred to a referral canter hospital to get maximum treatment for a better outcome.[6] Managing the placenta accreta spectrum is challenging for obstetricians and gynecologists because they face the risk of massive bleeding during surgery; hence, the risk of morbidity and mortality of pregnant women increases. Keeping antepartum and intrapartum hemorrhage under control is the most considerable management challenge for PASD. The primary cause of PASD associated with hysterectomy, disseminated intravascular coagulation, and maternal mortality is massive hemorrhage. There are currently no international guidelines for the best way to operate PASDs, and the ones that exist mainly address how to handle PASDs optimally.[7]

Conservative treatment is a measure to prevent hysterectomy. It is primarily intended for women who still desire to have children or due to other psychosocial considerations related to status and self-esteem. There are four methods: an extirpative technique, leaving the placenta, one-step conservative surgery, and triple P procedure.[8,9]

Some literature reports that ligating the internal iliac artery may reduce the risk of bleeding in PASD, although other studies have shown different results. Based on this, we were interested in evaluating the effectiveness of internal iliac artery ligation in reducing the amount of bleeding in cases of placenta accreta.

METHODS

This study was retrospective; the data was taken from the medical records of patients treated with PASD at Dr. Wahidin Sudirohusodo Hospital, Makassar, Indonesia, from January 2019 – to December 2022. The placenta accreta spectrum disorder was diagnosed using grayscale 2-dimensional ultrasound with Doppler imaging to assess the placenta accreta index score. A definite diagnosis is made during intraoperative and anatomical pathology examination results.

The criteria used to establish the diagnosis of placenta accreta using the placenta accreta index (PAI): history of cesarean section 2 or more times = 3, lacunae grade 3 = 3.5, lacunae grade 2 = 1.0, myometrium thickness equal to 1 mm or less = 1, more 1 mm but less or equal 3 mm = 0.5, myometrium thickness more 3 mm but less or equal 5 mm = 0.25, anterior placenta previa = 1.0, found bridging vessels = 0.5.6

There were four types of management for PASD: resection of the uterine wall, resection with iliac internal artery ligation, hysterectomy, and hysterectomy with iliac internal artery ligation. The tourniquet was tied around the uterus onto the lower edge of the invasion of the placenta at the lower uterine segment for all the patients (Figure 1). A hysterectomy was performed based on the results of an ultrasound examination when bizarre lacunae, hypervascularization in the cervix, or decided intraoperatively if the placenta invaded the posterior wall or the parametrium. The determination of Internal iliac artery ligation was not random. The Wahidin Sudirohusodo Hospital PASD team performed all operations.

Statistical tests use non-parametric tests because the distribution was abnormal, using medians for ordinal data. The tests used were the Kruskal-Wallis test for data that are more than two and not paired with each other and the Mann-Whitney U test used to see differences using median values. The study committee of Universitas Hasanuddin Medical Faculty granted ethical approval for this research (Ethical Approval No: 590/UN4.6.4.5.31/ PP36/ Z0Z3).

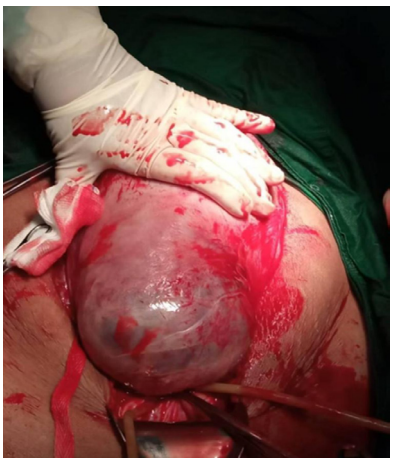

Figure 1. The tourniquet placement on the lower edge of the invasive placenta

RESULTS

During January 2019 – December 2022, 124 cases were diagnosed through ultrasound. Sixteen cases were excluded from the analysis, 4 cases because it was not a PASD by intraoperative, 5 cases of pathology examination results were not PASD, and 7 cases because the data was incomplete. Therefore, a total of 108 cases. The placenta accreta spectrum disorder in 2019 was 22 cases; in 2020, there were 16 cases; in 2021, there were 35 cases; and in 2022, there were 51 cases (Figure 1).

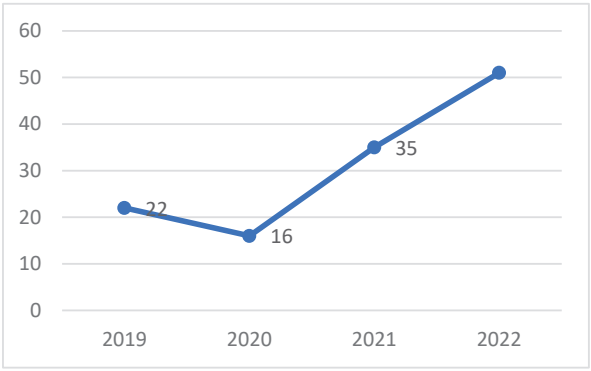

Figure 1. Number of PAS cases 2019 - 2022

In this study, PASD cases were found more in the reproductive age group, 20-35 years, multiparous, and the most was a history of cesarean section 1 time. The operative technique performed was uterine wall resection, resection accompanied by internal iliac artery ligation, hysterectomy, and hysterectomy accompanied by bilateral internal iliac artery ligation. Maternal deaths found 7 cases. (Table 1).

Table1. Sample Characteristics

Variable	Frequency	%
Age (y o)		
20 – 35	62	59.6
< 20 - > 35	42	40.4
Parity		
1	2	1.9
2	20	18.5
3	36	33.3
≥ 4	50	46.3
Number of S.C.s		
Never	4	3.7
1 x	47	43.5
2 x	45	41.7
Equal or more 3	12	11.1
Curettage history		
Never	90	83.3
Ever	18	16.7
Gestational age (weeks)		
Equal or more than 37	29	26.9
34-36	48	44.4
31-33	24	22.2
Less or equal to 30	7	6.5
Number of maternal deaths		
Live	101	93.5
Death	7	6.5

In Table 2, there was a significant difference between operation techniques on the amount of bleeding (0.003), but the duration of surgery was not different (0.082).

Table 2. Operation Technique on the Amount of Bleeding and Duration of Surgery

Operation Technique	N	Amount of Bleeding	*P-value*	Duration of Surgery	*P-value*
		Median		Median	
Resection	49	2000		135	
Resection+ ligation	13	2500		140	
Hysterectomy	34	3000	0.003	154	0.082
Hysterectomy + Ligation	12	4000		123	

Kurskal -Wallis test, $p<0.05$

The bleeding differs significantly between resection and hysterectomy cases. However, uterine artery ligation in resection did not show significant differences compared to no ligation (0.113), although the length of surgery in the two types of procedures was not significantly different (0.639). Internal iliac artery ligation in hysterectomy cases showed no significant difference in bleeding (0.210) or the length of surgery (0.154). The same was found between resection and hysterectomy with ligation; there was no difference in the bleeding or the surgery duration (Table 3).

Table 3. Comparison between Operation Technique with the Amount of Bleeding and Duration of Surgery

Operation Technique	N	Amount of Bleeding	*P-value*	Duration of Surgery	*P-value*
		Median		Median	
Resection	49	2000	0.113	135	0.639
Resection + ligation	13	2500		140	
Hysterectomy	34	3000		154	0.154
Hysterectomy + Ligation	12	4000	0.210	123	
Resection	49	2000		135	0.013
Hysterectomy	34	3000	0.002	154	
Resection + ligation	13	2500		140	0.723
Hysterectomy + Ligation	12	4000	0.052	123	

Mann-Whitney U test, P<0.05

Table 4 shows that there was a significant correlation between operative technique and the amount of bleeding (p:0.000, r 0.388), but the length of operation was not correlated (P:0.132)

Table 4. Correlation between Operation Technique to the Duration Of surgery and Amount of Bleeding

Operative action	N	r	*P-value*
Duration of operation	105	0.148	0.132
Amount of bleeding	108	0.388	0.000

DISCUSSION

The British study found that the risks for PASD were a history of cesarean section, maternal age, shorter pregnancy gap between previous cesarean section and current pregnancy, multiparity, placenta previa, assisted reproductive techniques, submucosal leiomyomas, smoking, and hypertension. Placenta previa and cesarean section were previously two of the most known risk factors.[10] PASD risk factors in Australia and New Zealand are older maternal age, previous cesarean section, placenta previa diagnosed before birth, and multiple births.[11]

In our study, PASD was more common in women of reproductive age, and in women with a history of cesarean section once, termination was primarily performed after more than 34 weeks of pregnancy with 6.5% maternal mortality.

Proper development of the placenta is the primary condition for a healthy pregnancy. The trophoblast invades the uterine decidua and then the uterine spiral artery; therefore, the artery dilates to ensure an adequate blood supply continues to the growing fetus. The invasion process is more enhanced in PASD. Extra villous trophoblast cells invade deeper and show more mesenchymal phenotypes than normal placentation, even continuing into the third trimester. In addition to excessive extra villous trophoblastic invasion, abnormal maternal vascular remodeling or neo-angiogenesis is one factor that triggers an increased invasion of the deeper placenta.[12]

PASD is linked to substantial aberrant neovascularization; therefore, blockage of several pelvic arteries can still result in more significant blood loss via collateral vessels. Several surgical procedures, i.e.,Internal iliac artery ligation, uterine devascularization, uterine compression sutures, uterine balloon tamponade, and pelvic tamponade, are performed to control severe intraoperative bleeding in women with PASD. There are no randomized controlled trials that examine how well various methods work to limit maternal blood loss during childbirth. Consequently, the operator's experience and resources should be considered while choosing the preferred procedure. According to a reasonable strategy, the easiest method with the lowest risk of complications should be used first.[1]

It is necessary to prepare preoperative maximum to avoid complications, such as preparing for the surgery team, blood transfusions, and stable preoperative maternal hemodynamics. Managing the PASD at Dr. Wahidin Sudirohusodo Hospital involves a multidisciplinary consisting of a maternal-fetal medicine consultant, oncologist, urologist, vascular surgery, anesthesiologist, and neonatologist. Blood preparation follows a massive transfusion procedure, providing as many as 24 bags of blood with eight packed red cells, eight whole blood, eight fresh frozen plasma, and eight thrombocyte concentrates.

Of the 108 cases of PASD operated on at Dr. Wahidin Sudirohusodo Hospital, most were carried out through planned surgery, and about 40.5% were treated conservatively. Conservative treatment was done by doing one-step conservative surgery. The operative action was preceded by the installation of a

ureteral stent by urologists to avoid ureteral trauma. Hysterotomy was performed in the corpus uteri to deliver the baby to avoid the placental insertion area in the uterus's lower segment. After the baby was born, intramural and intravenous fluid uterotonic injections were performed. Exploring the expansion of placental attachment and conducting trials by stretching the umbilical cord, if it appears that uterine tissue was attracted inward, it could be established that the placenta is attached to the endometrium unless there was bulging or percreta or purplish shadow. The following action was the installation of a tourniquet using ureteral catheter no. 16 in the lower segment of the uterus at the boundary of placental insertion for hemostatic purposes, then releasing the vesicouterine plica down while doing hemostatic with cautery or sutures. After the lower limit of the placenta was reached, resection of the involved uterine area was carried out, and suturing of the uterus was performed. In some cases, hemostasis was performed by iliac internal artery ligation first (12% resection and ligation and 11.1% hysterectomy and ligation).

In the case of total placenta previa, the placenta gets a significant amount of blood supply from the descending branches of the cervical and vaginal arteries. The artery continuously perfuses blood into the lower segment of the uterus even after the uterine artery ligation, thus still failing to control bleeding. In such cases, uterine artery ligation measures effectively reduce blood flow to uterine, cervical, and vaginal veins. Ligation of the internal iliac artery reduces the risk of a hysterectomy. It becomes easier if a hysterectomy is still performed, as ligation will help reduce bleeding.the Hence, the surgical field becomes more apparent and avoids trauma to other organs, especially the ureters.[13]

Our results showed that the operative type of action significantly differed in the amount of bleeding. The difference was significant, especially for resection compared to hysterectomy. Hysterectomy was decided if vascularization was found in the cervical region by ultrasound, large and numerous lacunae, and intraoperative if extensive attachment was found to the parametrium or the posterior corpus. The results showed that performing the internal iliac artery ligation for resection and hysterectomy did not significantly differ in bleeding. Operative action did not correlate with the length of surgery but did correlate with the amount of bleeding.

Our results were no different from those reported by Iwata et al. that among the 23 cases performed with or without the internal iliac artery ligation showed no significant difference in the amount of bleeding or length of hospitalization. Uterine artery ligation and cervical tamponade were simple and effective measures to control bleeding when releasing placenta accreta.[14,15]

According to Wagaarachchi and Fernando 18 and Nizard et al., bilateral internal iliac artery ligation was a safe and successful treatment for obstetric hemorrhage-related conditions that threatened life in order to save the uterus. Fifty percent of the twelve patients who had bilateral iliac artery ligation were able to conceive again.[16,17]

A meta-analysis of 795 patients and found that internal iliac artery ligation did not decrease the amount of bleeding and maintain the uterus. In contrast, uterine artery ligation accompanied by uterine tamponade significantly reduced bleeding volume.[18]

Surgical7 methods using tourniquets and forceps, when necessary, could improve the outcome of surgery in severe cases of placenta accreta spectrum, i.e., the amount of bleeding becomes less during surgery, especially for lower placental insertions.

STUDY LIMITATIONS

This study was retrospective. Consequently, it relies heavily upon medical records, which are often incomplete. Determining cases performed by resection or hysterectomy or accompanied or not by internal iliac artery ligation was not random but depended on the ultrasound examination results, whether focal or diffuse and during operation. The duration of the surgery and the amount of bleeding depend on the records in the operating room.

CONCLUSION

Internal iliac artery ligation during both uterine resection and hysterectomy did not show a significant difference in the amount of bleeding. However, the ligation did not affect the length of surgery compared to procedures without ligation. Further research needs to be done with a more significant number of samples and with randomization methods to obtain better results. In all cases, a tourniquet was applied to prevent bleeding; this can be considered a modality to reduce the bleeding. However, further research is still needed.

CONFLICT of INTERESTS

The authors declare that there is no conflict of interest.

Informed Consent

Informed consent was obtained from each subject and their husband or parents.

ETHICAL APPROVAL

This study was conducted with ethical approval from the Hasanuddin University Research Ethics Commission, under Number 590/UN4.6.4.5.31/ PP36/ Z0Z3.

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Research Article

The Value of Intrapartum Ultrasound in Predicting Mode of Delivery: a Prospective Cohort Study

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Abstract

Objective: The main aim of this study was to explore the value of several intrapartum ultrasonographic parameters in predicting mode of birth following spontaneous labor.

Methods: This prospective observational cohort study included two groups of primparous term singleton vertex presentation pregnant patients >18 years old admitted in the first stage of labor between January 2021 and May 2023: a cesarean section (CS) group and a normal vaginal delivery (NVD) group. All patients provided informed written consent. The investigation utilized both transabdominal and transperineal ultrasonography for comprehensive fetal and pelvic floor assessment. Transabdominal ultrasound evaluated standard parameters including fetal occiput position, biometry number, viability, presentation, and estimated fetal weight. Intrapartum transperineal ultrasonography, specifically performed during the first stage of labor, focused on the Levator Hiatus, measuring its anteroposterior diameter (APD) at rest and during Valsalva maneuver, as well as the angle of progression (AOP).

Results: The study population comprised 609 participants with a mean age of 22.8 ± 4.3 years and a high prevalence of being overweight and obesity (38.8% and 57.8%, respectively). When comparing patients who had an intrapartum CS to those who had a normal vaginal delivery (NVD). However, on logistic regression, age, BMI, gestational age, posterior occiput presentation, head circumference (HC), AOP (V) and APD (V) as significant predictors for both ICS and 2nd stage CS ($p<0.05$).

Conclusion: This study demonstrated that maternal age, BMI, gestational age, occiput posterior position, Angle of progression at Valsalva and levator hiatus anteroposterior diameter at Valsalva were independent significant predictors for Intrapartum cesarean section in primiparous women at term When the ratio between HC/APD at rest, BPD/APD at Valsalva and HC/APD at Valsalva is high, while the APD at Valsalva, AoP Valsalva values are low, cesarean section was more likely to be the mode of delivery.

Keywords: Angle of progression, Anteroposterior diameter of levator hiatus, Intrapartum cesarean section, Intrapartum ultrasound, Mode of delivery.

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INTRODUCTION

Normal vaginal delivery (NVD) is the preferred mode of childbirth for most mothers and infants due to its well-established benefits compared to cesarean sections (CS) [1]. NVDs are associated with faster recovery, shorter hospital stays, and a lower risk of complications [2]. Additionally, newborns born vaginally gain exposure to maternal microbiota, which seem to have immunological and respiratory benefits [3,4]. However, there has been a trend in recent decades towards an increasing CS rate, particularly those performed without a clear medical indication [5]. This not only strains healthcare resources but also carries potential risks for both mothers and neonates [6].

A crucial challenge to optimizing birth outcomes lies in the limitations of methods used for assessing labor progress, including traditional clinical examinations that can be subjective and lack consistency between practitioners [7, 8]. This highlights the need for more objective assessment tools to guide decision-making during labor [9]. Indeed, by providing a more objective and

quantifiable assessment of fetal station and position within the birth canal, ultrasound has the potential to omit the subjectivity of such assessments [10].

Furthermore, precise determination of fetal head position and presentation are critical for a safe operative vaginal delivery. Similarly, distinguishing between a face and brow presentation is particularly crucial, as the larger engaging diameters of a brow presentation in a term neonates preclude vaginal birth [11]. Sonographic evaluation with trans-abdominal imaging in both sagittal and axial planes offers the optimal approach for this assessment. Several studies have demonstrated that intrapartum ultrasound offers superior accuracy and reproducibility compared to clinical examination in the diagnosis of fetal head station and position [12, 13]. However, there is paucity of information on any association between intrapartum ultrasound scan parameters performed early in labor and mode of birth. Moreover, there is no general agreement regarding, which measurements should be obtained and how useful they are if integrated with demographic and clinical parameters.

This was a nested study within the Reliability, Effectiveness and Acceptability of Sequential Stage Ultrasonographic Routine Examination (REASSURE) program. The main aim of this study was to explore the value of several intrapartum ultrasonographic parameters in predicting mode of birth following spontaneous labor.

METHODS

This prospective, single center, cohort study was conducted at Kasr Al Ainy Maternity Hospital, Cairo, Egypt between January 2021 and May 2023. A total of 609 pregnant primiparous participants presenting in the first stage of labor were enrolled into the study. All participants provided a written informed consent for participation. In addition, an ethical approval was obtained from Research Ethics Committee, Faculty of Medicine, Cairo University under No. MD-22-2021.

Primiparous term singleton pregnant patients aged 18 or older presenting in spontaneous labor with a baby in vertex presentation were considered potentially eligible for inclusion. Exclusion criteria for this study included individuals who were multiparous, had a history of preterm labor, presented with a non-vertex fetal presentation, were carrying a multiple pregnancy, had a planned elective Cesarean Section, had underlying medical conditions, or were unwilling to participate in the research. Patients were only recruited when at least one of the three obstetricians trained to measure the intrapartum ultrasonographic parameters of interest were available on labor ward. In addition, patients who developed prolonged labor were included in the study.

The primary endpoint of the study was the predictive accuracy of intrapartum ultrasound in predicting the need for cesarean section due to failure to progress in labor. Secondary endpoints included the rate of operative vaginal deliveries, (composite neonatal outcomes).

Both transabdominal and transperineal ultrasound scans were performed on all participants by trained operators on the use of both modalities. Transabdominal ultrasound was used to assess fetal presentation, biometry, and estimated fetal weight (EFW) (measured according to established protocols by Salomon et al., 2011) [14]. Intrapartum transperineal ultrasound was performed during the first stage of labor by experienced obstetricians who underwent standardized training on the measurement techniques. The anteroposterior diameter (APD) of the levator hiatus and angle of progression (AoP) were measured using a consistent protocol, with the transducer positioned at a specific angle and depth. To minimize intraobserver variability, measurements were performed by the same obstetrician for each patient. Additionally, a quality control process was implemented to regularly review and calibrate the ultrasound equipment.

Scans were performed by one of 3 trained operators. A single operator performed both the transabdominal and transperineal ultrasound scan measurements for each of the participants. A Samsung SONOACE R3 portable ultrasound machine was used to perform both transabdominal and transperineal scans on all participants. Transabdominal ultrasound was performed in the sagittal and axial planes and used as the primary method for evaluating fetal head position, following established protocols [14]. The probe placement on the maternal abdomen allowed visualization of the fetus spine and head. Ultra-sonographic identification of specific fetal landmarks, including the fetal orbits (occiput posterior presentation), midline cerebral echo (occiput transverse presentation), and the occipitocervical approximation (occiput anterior

presentation), facilitated the determination of fetal head position. The choroid plexus further assisted in some cases. In situations with a low fetal head, where visualizing midline structures was difficult, a combined transperineal and transabdominal ultrasound approach ensured accurate positioning [15, 16]. Additionally, a transperineal ultrasound in the midsagittal plane was used to assess fetal head station. The symphysis pubis served as a landmark for quantitative assessments of the AoP [17]. The AoP, or the angle of descent, is the angle between the long axis of the pubic bone and a line from the lowest edge of the pubis drawn tangential to the deepest bony part of the fetal skull. The APD was measured as distance between the distal symphysis pubis and the proximal puborectalis muscle in line with previous reports [18]. Intrapartum transperineal ultrasound measurements were performed sequentially during the first stage of labor, with intervals determined by the clinical course and progress of labor.

The clinical labor ward team managed the patient's labor according to the unit's protocol and were blinded to any ultrasound scan findings related to this study. In addition to baseline demograhic details and ultrasonographic measurements, and mode of birth, we collected data on the clinical indication for CS if one was performed, the decision-making process for CS (e.g., based on maternal or fetal factors, failure to progress in labor, or other clinical indications), duration of second stage of labor, fetal sex, actual birthweight, any immediate adverse neonatal outcomes and/or need for NICU admission.

Descriptive statistics (means and standard deviations for continuous variables; frequencies and percentages for categorical variables) were calculated using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY). Chi-square or Fisher's exact tests were used to assess group differences for categorical variables. Due to skewed data, the Mann-Whitney U test was used to compare quantitative variables between groups. Multivariate logistic regression models with standard enter technique were used to identify independent predictors of both overall intrapartum caesarean section (ICS) and second-stage CS. Ultrasound parameters derived from more than one measurement (i.e ratios and delta measurements) were not included in the multivariate analysis. A p-value less than or equal to 0.05 was considered statistically significant.

In this study our main focus was to examine 8 ulatrsound scan intrapartum parameters for being potential candidate predictors for mode of borth in spontaneous labour in primaprous patients. These parameters were APD (R), APD (V), AoP (R), AoP(V), HC, BPD, occiput posterior position and estimated fetal weight. Simulation studies examining predictor variables for inclusion in logistic regression models suggest that approximately 10 events are necessary for each candidate predictor to avoid overfitting [19-21]. Therefore, we needed at least 80 patients with the primary outcome of interest (which is intrapartum caesarean section) in our cohort. Based on a recent study [22] the ICS rate from their large size multicentre study was 7-9% we assumed that 8% of our population will have an ICS and hence we estimated that 1000 would be required for 80 to have an ICS. Allowing a 15% attrition rate, we intend to recruit into the study till we have 92 ICSs or a total of 1150 patients recruited, whichever is reached first.

RESULTS

A total of 609 patients were enrolled into the study. The mean age of the study cohort was 22.8 ± 4.3 years and a mean BMI of 31 ± 3.9 kg/m^2 with 38.8% and 57.8% categorized as overweight and obese respectively. The full cohort charcateristics and, birth outcomes and sonographic paramters are presented in (Table 1).

Table 1. Population characteristics, ultrasound parameters and birth outcomes

Variable		Description (n=609)
Age (years)	Range	18 - 40
	Mean ± SD	22.8 ± 4.3
BMI (kg/m^2)	Range	19.5 - 44.3
	Mean ± SD	31 ± 3.9
	Normal	21 (3.4)
BMI (kg/m^2)	Overweight	236 (38.8)
	Obese	352 (57.8)
EFW by U/S (grams)	Range	2100 - 4600

	Mean ± SD	3242.3 ± 397
Gestational age (Weeks)	Range	37 - 42
	Mean ± SD	38.6 ± 1.3
HC (mm)	Range	279 - 359
	Mean ± SD	319.7 ± 12.2
BPD (mm)	Range	84 - 99
	Mean ± SD	92 ± 2.9
Occiput	Anterior	386 (63.4)
	Posterior	177 (29.1)
	Transverse	46 (7.6)
APD (R) (mm)	Range	45 - 75
	Mean ± SD	56.1 ± 5.7
BPD/APD (R)	Range	1.24 - 2.11
	Mean ± SD	1.66 ± 0.16
HC/APD (R)	Range	4.43 - 7.28
	Mean ± SD	5.75 ± 0.58
APD (V) (mm)	Range	50 – 80
	Mean ± SD	63.7 ± 6.2
BPD/APD (V)	Range	1.11 - 1.83
	Mean ± SD	1.46 ± 0.14
HC/APD (V)	Range	3.95 - 6.48
	Mean ± SD	5.06 ± 0.5
AoP (R) (Degrees)	Range	68 – 132
	Mean ± SD	98.5 ± 11.7
AoP (V) (Degrees)	Range	68 – 142
	Mean ± SD	109.3 ± 12
ΔAoP (Degrees)	Range	1 – 28
	Mean ± SD	11 ± 4.8
ΔAPD (mm)	Range	1 – 18
	Mean ± SD	7.7 ± 2.8
ΔBPD/APD	Range	0.02 - 0.49
	Mean ± SD	0.2 ± 0.08
ΔHC/APD	Range	0.07 - 1.66
	Mean ± SD	0.7 ± 0.27
Mode of delivery	CS	93 (15.3)
	NVD	516 (84.7)
CS stage (n=93)	1st stage	13 (14)
	2nd stage	80 (86)
	Obstructed labor	72 (77.4)
	Fetal distress	11 (11.8)
Cause of CS (n=93)	Antepartum hemorrhage	6 (6.5)
	Macrosomia	3 (3.2)
	Elderly primigravida	1 (1.1)
Duration of 2nd stage (min)	Range	20 – 150
	Mean ± SD	40.8 ± 21.7
Fetal sex	Male	293 (48.1)
	Female	316 (51.9)
Actual Birthweight weight (gram)	Range	2300 – 4500
	Mean ± SD	3232.5 ± 412.7
Adverse neonatal Outcomes	Yes	35 (5.9)
	No	573 (94.1)
Outcomes (n=35)	Respiratory distress	30 (86)
	Shoulder dystocia	1 (2.8)
	Hypoxic insult / convulsions	1 (2.8)
	Hypoglycemia	1 (2.8)
	Fracture clavicle	1 (2.8)
	Erbs palsy	1 (2.8)

SD: standard deviation, BMI: body mass index, EFW: estimated fetal weight, U/S: ultrasound. HC: Head circumference, BPD: Biparietal diameter. APD: Antero-posterior diameter of the levator hiatus, R: at rest, BPD: Biparietal diameter, HC: head circumference, V: at Valsalva, AoP: Angle of progression, Δ: Delta (The difference between the measurements at rest and Valsalva), CS: cesarean section, NVD: normal vaginal delivery.

There was no significant difference between patients who had an ICS compared to those who had an NVD with regards to APD (R) (p=0.964), BPD/APD (R) (p=0.082), AoP (R) (p=0.441). However, there were statistically significant difference between both groups with regards to HC, BPD, occiput posterior position, HC/APD (R), APD (V), BPD/APD (V), HC/APD (V), AoP (V), ΔAOP, ΔAPD, ΔBPD/APD, ΔHC/APD (0.001, 0.001, 0.011, 0.023, 0.004, 0.001, 0.001, 0.001, 0.001, 0.001, 0.001 respectively). There were also differences between both groups in mean age, mean BMI, gestational age, estimated fetal weight and fetal sex (Table2).

Table 2. Comparison between ICS and NVD outcomes.

		ICS (n=93)	**NVD (n=516)**	***P-value***
Age (years)	Range	18 - 40	18 - 40	<0.001
	Mean ± SD	25.3 ± 6.3	22.4 ± 3.7	
BMI (kg/m²)	Range	23.9 - 43.9	19.5 - 44.3	<0.001
	Mean ± SD	33.1 ± 4	30.7 ± 3.8	
BMI	Normal	1 (1.1)	20 (3.9)	<0.001
	Overweight	19 (20.4)	217 (42.1)	
	Obese	73 (78.5)	279 (54.1)	
EFW by U/S (grams)	Range	2750 - 4600	2100 - 4270	<0.001
	Mean ± SD	3492.2 ± 412.9	3197.3 ± 377.2	
Gestational age (Weeks)	Range	37 - 42	37 - 42	0.001
	Mean ± SD	39.1 ± 1.5	38.5 ± 1.3	
HC (mm)	Range	307 - 358	279 - 359	<0.001
	Mean ± SD	327.4 ± 9.8	318.3 ± 12	
BPD (mm)	Range	87 - 98	84 - 99	<0.001
	Mean ± SD	93.7 ± 2.3	91.7 ± 2.8	
Occiput	Anterior	52 (55.9)	334 (64.7)	0.011
	Posterior	38 (40.9)	139 (26.9)	
	Transverse	3 (3.2)	43 (8.3)	
APD (R)	Range	45 - 72	46 - 75	0.964
	Mean ± SD	56.3 ± 6.5	56.1 ± 5.6	
BPD/APD (R)	Range	1.27 - 2.11	1.24 - 2.02	0.082
	Mean ± SD	1.68 ± 0.18	1.65 ± 0.16	
HC/APD (R)	Range	4.51 - 7.27	4.43 - 7.28	0.023
	Mean ± SD	5.88 ± 0.61	5.73 ± 0.57	
APD (V)	Range	50 - 79	52 - 80	0.004
	Mean ± SD	62.2 ± 7	64 ± 6	
BPD/APD (V)	Range	1.18 - 1.83	1.11 - 1.79	<0.001
	Mean ± SD	1.52 ± 0.16	1.44 ± 0.13	
HC/APD (V)	Range	4.08 - 6.48	3.95 - 6.44	<0.001
	Mean ± SD	5.33 ± 0.54	5.01 ± 0.48	
AoP (R)	Range	76 - 130	68 - 132	0.441
	Mean ± SD	97.9 ± 11.6	98.6 ± 11.8	
AoP (V)	Range	77 - 135	68 - 142	<0.001
	Mean ± SD	105.4 ± 12.2	110 ± 11.9	
ΔAOP	Range	1 - 22	4 - 28	<0.001
	Mean ± SD	7.7 ± 3.6	11.5 ± 4.8	
ΔAPD	Range	2 - 12	1 - 18	<0.001
	Mean ± SD	6.2 ± 2.2	8 ± 2.8	
ΔBPD/APD	Range	0.04 - 0.38	0.02 - 0.49	<0.001
	Mean ± SD	0.17 ± 0.06	0.21 ± 0.08	
ΔHC/APD	Range	0.15 - 1.31	0.07 - 1.66	<0.001
	Mean ± SD	0.59 ± 0.22	0.72 ± 0.27	
Fetal sex	Male	53 (57)	240 (46.5)	0.063
	Female	40 (43)	276 (53.5)	
Actual fetal weight (gram)	Range	2650 - 4500	2300 - 4300	<0.001
	Mean ± SD	3499.5± 488.9	3184.4± 378.4	
Adverse neonatal outcomes	Yes	6 (7.5)	29 (5.6)	0.473
	No	87 (92.5)	487 (94.4)	
Outcomes (n=35)	Respiratory	5 (83.3)	25 (86.2)	0.152
	Shoulder dystocia	0 (0)	1 (3.4)	
	Hypoxic insult/convulsions	0 (0)	1 (3.4)	
	Hypoglycemia	1 (16.7)	0 (0)	
	Fracture clavicle	0 (0)	1 (3.4)	
	Erbs palsy	0 (0)	1 (3.4)	

P values less than or equal to 0.05 were considered statistically significant, SD: standard deviation, BMI: body mass index, EFW: estimated fetal weight, U/S: ultrasound. HC: Head circumference, BPD: Biparietal diameter. APD: Antero-posterior diameter of the levator hiatus, R: at rest, BPD: Biparietal diameter, V: at Valsalva, AoP: Angle of progression, ICS: intrapartum cesarean section

On multivariate analysis, variables that continued to be significant were the mean maternal age (aOR: 1.111, 95% CI: 1.053-1.173; P<0.001), BMI (OR: 1.071, 95% CI: 1.001-1.146; P=0.047), gestational age (aOR: 1.265, 95% CI: 1.046-1.529; P=0. 015), posterior occiput (aOR: 3.187, 95% CI: 1.849-5.492; P<0.001), AOP (V) (aOR: 0.972, 95% CI: 0.950-0.995; P=0.016) and APD (V) (aOR: 0.925, 95% CI: 0.884-0.968; P=0.001) (Table 3). In addition, on multivariate analysis, mean maternal age (aOR: 1.116, 95% CI: 1.054-1.181; P<0.001), BMI (aOR: 1.083, 95% CI: 1.009-1.162; P=0.027), gestational age (aOR: 1.257, 95% CI: 1.029-1.535; P=0. 025), occiput posterior (aOR: 3.236, 95% CI: 1.842-5.686; P<0.001), and APD (V) (OR: 0.916, 95% CI: 0.872-0.962; P<0.001) continued to be significant (Table 3).

Table 3. Multi variate analysis to explore potential ICS predictors and 2nd stage CS.

Multi variate analysis to explore potential ICS predictors	***P-value***	**aOR**	**95% CI for OR**		
Age (years)	<0.001	1.111	1.053	-	1.173
BMI (kg/m²)	0.048	1.071	1.001	-	1.146
Gestational age (Weeks)	0.015	1.265	1.046	-	1.529
EFW by U/S (grams)	0.151	1.001	1.000	-	1.002
Occiput (posterior VS anterior or transverse)	<0.001	3.187	1.849	-	5.492
AoP (V)	0.016	0.972	0.950	-	0.995
APD (V)	0.001	0.925	0.884	-	0.968
Head circumference (mm)	0.091	1.033	0.995	-	1.072
Biparietal diameter (mm)	0.591	1.049	0.881	-	1.250
Multi variate ananlysis to explore the predictors of 2nd stage CS	***P-value***	**OR**	**95% CI for OR**		
Age (years)	<0.001	1.116	1.054	-	1.181
BMI (kg/m²)	0.027	1.083	1.009	-	1.162
Gestational age (Weeks)	0.025	1.257	1.029	-	1.535
EFW by U/S (grams)	0.254	1.001	1.000	-	1.002
Occiput posterior VS anterior or transverse	0<0.001	3.236	1.842	-	5.686
AoP (V)	0.198	0.984	0.961	-	1.008
APD (V)	<0.001	0.916	0.872	-	0.962
HC (mm)	0.392	1.017	0.978	-	1.058
BPD (mm)	0.328	1.097	0.911	-	1.322

P values less than or equal to 0.05 were considered statistically significant, OR= odds ratio, CI= Confidence Interval, BMI: body mass index, EFW: estimated fetal weight, U/S: ultrasound, AoP: Angle of progression, APD: Antero-posterior diameter of the levator hiatus, HC: Head circumference, BPD: Biparietal diameter. P values less than or equal to 0.05 were considered statistically significant, OR= odds ratio, CI= Confidence Interval, BMI: body mass index, EFW: estimated fetal weight, U/S: ultrasound, AoP: Angle of progression, APD: Antero-posterior diameter of the levator hiatus, HC: Head circumference, BPD: Biparietal diameter.

When comparing patients who had a 2nd stage CS and those who had an NVD, there were no significant differences in APD (R) and AoP (R) measurements. However, we found a statistically significant difference in mean maternal age, BMI, estimated fetal weight, gestational age, HC, BPD, occiput, HC/APD (R), APD (V), BPD/APD (V), HC/APD (V), AoP (V), ΔAOP, ΔAPD, ΔBPD/APD, ΔHC/APD, fetal sex, and birthweight (Table 4).

Table 4. Comparison between 2nd stage CS and NVD outcomes.

		2nd stage CS (n=80)	NVD (n=516)	*P-value*
Age (years)	Range	18 - 40	18 - 40	<0.001
	Mean ± SD	25.3 ± 6.1	22.4 ± 3.7	
BMI (kg/m²)	Range	23.9 - 43.9	19.5 - 44.3	<0.001
	Mean ± SD	33.2 ± 4.1	30.7 ± 3.8	
BMI	Normal	1 (1.3)	20 (3.9)	<0.001
	Overweight	16 (20)	217 (42.1)	
	Obese	63 (78.8)	279 (54.1)	
EFW by U/S (grams)	Range	2750 - 4200	2100 - 4270	<0.001
	Mean ± SD	3462.8 ± 391.3	3197.3 ± 377.2	
Gestational age (Weeks)	Range	37 - 42	37 - 42	0.003
	Mean ± SD	39.1 ± 1.5	38.5 ± 1.3	
HC (mm)	Range	307 - 348	279 - 359	<0.001
	Mean ± SD	326.3 ± 8.9	318.3 ± 12	
BPD (mm)	Range	87 - 98	84 - 99	<0.001
	Mean ± SD	93.6 ± 2.3	91.7 ± 2.8	
Occiput	Anterior	43 (53.8)	334 (64.7)	0.011
	Posterior	34 (42.5)	139 (26.9)	
	Transverse	3 (3.8)	43 (8.3)	
APD (R)	Range	45 - 72	46 - 75	0.991
	Mean ± SD	56.2 ± 6.2	56.1 ± 5.6	
BPD/APD (R)	Range	1.27 - 2.11	1.24 - 2.02	0.085
	Mean ± SD	1.69 ± 0.18	1.65 ± 0.16	
HC/APD (R)	Range	4.51 - 7.27	4.43 - 7.28	0.040
	Mean ± SD	5.87 ± 0.61	5.73 ± 0.57	
APD (V)	Range	50 - 79	52 - 80	0.006
	Mean ± SD	62 ± 6.8	64 ± 6	
BPD/APD (V)	Range	1.18 - 1.83	1.11 - 1.79	<0.001
	Mean ± SD	1.53 ± 0.16	1.44 ± 0.13	
HC/APD (V)	Range	4.08 - 6.48	3.95 - 6.44	<0.001
	Mean ± SD	5.32 ± 0.54	5.01 ± 0.48	
AoP (R)	Range	80 - 130	68 - 132	0.982
	Mean ± SD	99 ± 11.6	98.6 ± 11.8	
AoP (V)	Range	77 - 135	68 - 142	0.006
	Mean ± SD	106.5 ± 12.1	110 ± 11.9	
ΔAOP	Range	1 - 22	4 - 28	<0.001
	Mean ± SD	7.7 ± 3.6	11.5 ± 4.8	
ΔAPD	Range	2 - 12	1 - 18	<0.001
	Mean ± SD	6.3 ± 2.2	8 ± 2.8	
ΔBPD/APD	Range	0.05 - 0.38	0.02 - 0.49	<0.001
	Mean ± SD	0.17 ± 0.07	0.21 ± 0.08	
ΔHC/APD	Range	0.18 - 1.31	0.07 - 1.66	<0.001
	Mean ± SD	0.6 ± 0.23	0.72 ± 0.27	
Fetal sex	Male	47 (58.8)	240 (46.5)	0.042
	Female	33 (41.3)	276 (53.5)	
Actual fetal weight (gram)	Range	2700 - 4500	2300 - 4300	<0.001
	Mean ± SD	3486.9±485.5	3184.4±378.4	
Adverse neonatal outcomes	Yes	5 (6.3)	29 (5.6)	0.796
	No	75 (93.8)	487 (94.4)	
Outcomes (n=35)	Respiratory	4 (80)	25 (86.2)	0.260
	Shoulder dystocia	0 (0)	1 (3.4)	
	Hypoxic insult/convulsions	0 (0)	1 (3.4)	
	Hypoglycemia	1 (20)	0 (0)	
	Fracture clavicle	0 (0)	1 (3.4)	
	Erbs palsy	0 (0)	1 (3.4)	

P values less than or equal to 0.05 were considered statistically significant, SD: standard deviation, BMI: body mass index, EFW: estimated fetal weight, U/S: ultrasound. HC: Head circumference, BPD: Biparietal diameter. APD: Antero-posterior diameter of the levator hiatus, R: at rest, V: at Valsalva, AoP: Angle of progression.

DISCUSSION

Our study included 609 primarous term singleton patients who had transabdominal and transperineal ultrasound examinations to explore parameters that could potentially be useful in predicting mode of birth in spontaneous labor. Participants who had a CS were older, had higher BMI and gestational age compared to those who delivered vaginally. On trasnsbdominal scan measurements, patients in the CS cohort had bigger HC, BPD and were more likely to have their baby in an occiput posterior position. While on transperineal ultrasound scanning, the levator hiatal APD and AoP were lower in the group of participants who had a CS compared to NVD. Nevertheless, these differences were only significant in measurements taken during a Valsalva maneuver. Additionally, multivariate analysis identified that maternal age, BMI, gestational age, occiput posterior position, AOP (V) and APD (V) to be independent factors associated with ICS. Notably, AOP (V) and APD (V) were inversely associated with CS risk. When comparing patients who had a CS during the 2nd stage to those who had an NVD, the findings were comparable, nonetheless, the difference in AOP (V) between the two group was not significant on multivariate analysis.

Traditionally, labor management heavily relies on a series of subjective clinical assessments to determine cervical evaluation, head descent and fetal position. However, the accuracy and reproducibility of these examinations are limited, particularly in the presence of caput [13]. Since the 1990s, ultrasound has emerged as a potential tool to improve labor management [23, 24]. Studies suggested it surpasses clinical examination in accuracy and reproducibility for fetal head position, station, and predicting arrest of labor [13, 25]. It has even been proposed that ultrasound may even hold promise in stratifying patients likely to achieve a spontaneous vaginal delivery from those requiring an operative intervention [24, 26]. However, despite these advantages, ultrasound currently remains a secondary tool in delivery room settings, with clinical evaluation still taking precedence [27]. Uncertainties persist regarding the optimal timing of ultrasound examinations, the most relevant parameters to assess, and how to effectively integrate sonographic findings into clinical practice to optimize patient management [13].

Our study population exhibited similar characteristics to those reported in comparable studies in terms of maternal BMI, estimated fetal weight, and gestational age. However, the mean maternal age in our cohort (22.8 ± 4.3) years was lower than other studies exploring the intrapartum ultrasonographic parameters [12,28, 29]. These differences are probably secondary to the potential influence of social background variations across the studied populations. Moreover, there was disparity in the use of epidural where none of our study cohort had an epidural compared to the 57.7% [29] and 93.4% [30] reported by other studies. The limited resources and high birth rate healthcare setting are the most likely reasons for this disparity. Among the 609 laboring women enrolled into the study, 93 (15.3%) had an ICS. This rate was much higher than that reported. [29] of 7.7% and (7-9%) [22]. We believe that an important reason for this discrepancy is related to the absence of operative vaginal deliveries in our cohort. This is mainly linked to limited availability of midwavies with the required skill to perform a vacuum extraction and limited resources to maintain the vacuum extraction equipment. An issue that is currently being addressed.

We were able to accurately determine the fetal head position in all of the studied labors via transabdominal ultrasound, which concurs with with the findings reported by other groups [13,27, 28]. Notably, a recent meta-analysis by Yaw Amo Wiafe et al. [31] encompassing 31 studies and 3,370

subjects reported that that ultrasound is preferred to digital vaginal examinationfor fetal head position assessment. Furthermore, our study identified a significant association between fetal head position and mode of birth. This association aligns with findings [32], our study also demonstrated significant associations between maternal age, sonographic fetal occiput position, and AoP as potential predictors for labor outcome. Nevertheless, Kamel et al investigated the use of these paramteres in the context of induced rather than spontaneous labor.

Our investigation innovatively assessed the AOP during both resting and Valsalva maneuvers, investigating its potential for predicting normal and abnormal labor progress. To our knowledge, this is the first study to explore this specific

application. While prior research successfully measured AOP using transperineal ultrasound, their focus primarily remained on delivery mode rather than the entire labor process [28, 12]. Building upon the concept of a "sonopartogram" proposed by Hassan et al. [28],we aimed to establish AOP's predictive value. Unlike their study, however, we explored its correlation with normal or abnormal labor progression.

Previous studies have established a connection between AOP and vaginal delivery rates. reported a vaginal delivery rate of 87% when the AOP was ≥110° compared to 38% with an AOP <100° [33]. Found that 58% of vaginal deliveries had an AOP exceeding 110°.

Our study revealed a significant association between various cephalometric measurements obtained at rest and during Valsalva maneuvers with the mode of delivery. Specifically; HC to APD ratio at Valsalva, BPD to APD ratio at Valsalva, and HC to APD ratio at Valsalva were all significantly higher in women who underwent CS compared to those in NVD. Conversely, APD at Valsalva, AOP at Valsalva, AOP change (Delta), APD change (Delta), and the ratios and change values of BPD/APD were all significantly lower in the CS group. Notably, no statistically significant differences were observed between the groups regarding resting measurements of APD, the BPD/APD ratio, or AOP.

To address multicollinearity, or the interdependence between variables, our model selection process for multivariate analysis only included resting and Valsalva maneuver measurements, excluding other ratios and change values. While prior studies have employed multivariate analysis, none have investigated the combined influence of both APD and AOP. Existing research has focused on models incorporating AOP with other factors including, cervical dilation, fetal head position, and presence of caput [28, 12].

While numerous studies explored the potential of transperineal intrapartum ultrasound for predicting vaginal delivery, the optimal timing for its use during labor remains unclear [13]. Proposals range from utilizing TPUS in situations where vaginal exams are discouraged (e.g., PROM, preterm labor, placenta previa) [34] to incorporating it as part of pre-induction assessment in postterm pregnancies. Notably, Hassan et al. [28] proposed a "sonopartogram" involving repeated TPUS measurements during active labor, mimicking the clinical partogram. High-resource centers have even explored automated software for continuous TPUS monitoring throughout active labor [35].

Our study aimed to address this gap in knowledge by investigating the value of TPUS measurements of APD and AOP at rest and during Valsalva for implementing a clinical labor sonopartogram. This approach prioritizes situations where early intervention might be necessary due to potential deviations from normal labor progress.

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CONFLICT of INTEREST

None.

CONCLUSION

In conclusion, intrapartum ultrasound offers a more objective and reliable method for diagnosing fetal head position and station compared to clinical examination. Our findings indicate that specific ultrasound measurements, such as the anteroposterior diameter (APD) and angle of progression (AOP) during Valsalva maneuvers, in conjunction with head circumference (HC) to APD ratios, can effectively predict the need for cesarean section. Additionally, maternal age, body mass index (BMI), gestational age (GA), fetal occiput position, and AOP and APD measurements during Valsalva are significant independent predictors of instrumental cesarean section (ICS).

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Supplementary file 1

Code: MD-22 -2021

Cairo University
Faculty of Medicine
Research Ethics Committee

NOTICE OF APPROVAL

Date 28 -2-2021
Protocol title: **The value of intrapartum ultrasound in predicting mode of delivery**

Supervisor: **Prof. Rasha A.M. Kamel**

Candidate: **Dr. Gamal Omar Abdel Ghany**
Institution: **Cairo University**

Decision: APPROVAL

The Research Ethics Committee **(REC),** has reviewed and **approved** the above mentioned **protocol. You may begin your investigation**. Approval is granted for one year from the date of initial approval. At the end of this period, the principal investigator will submit the required documents for continuing review.

The principal investigator will need to:

- Notify the REC Chair immediately after any **serious adverse events** experienced by participants of the investigational study or as reported to you by the sponsor/manufacturer/co- investigators.
- Submit End of trial notification at the end of trial.
- Submit Clinical study report at the end of trial.
- You may not initiate **changes** in approved research protocol without REC review and approval except where necessary to eliminate apparent immediate hazards to the human subjects.
- يحظر سفر أى عينات بشريه من المبحوثين خارج جمهوريه مصر العربية الا بعد موافقة الجهات الامنية .

Sincerely,

REC Subcommittee Chairman

Prof. Maher Fawzy, MD

Professor of Anaesthesia,

Cairo University

REC Chairman

Prof. M. Mohsen Ibrahim

Professor of Cardiology,

Cairo University

Research Article

Evaluation of Estradiol and Pro-Inflammatory Marker Level with VAS Score After Progestin Therapy in Endometriosis Patients

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Abstract

Objectives: To compare estradiol levels and proinflammatory biomarkers (IL-6, IL-1β, and COX-2) in endometriosis patients with pain and without pain after progestin therapy.

Methods: This observational cross-sectional study involved 47 endometriosis patients undergoing three months of progestin therapy at RSUPN Cipto Mangunkusumo from March to June 2024. Serum levels of COX-2, IL-6, IL-1β, and estradiol will be measured using ELISA and Microplate Enzyme Immunoassay, with pain status assessed to determine associations between biomarkers and pain presence.

Results: A significant difference in COX-2 levels between patients with pain and those without, with higher levels in the pain group [1.845 (1.24-10.26) vs 1.55 (0.32-3.07), p = 0.004]. A significant positive correlation was found between IL-1β and IL-6 (r = 0.471, p = 0.001). COX-2 levels also exhibited a weak but statistically significant positive correlation with VAS scores (r = 0.360, p = 0.013).

Conclusion: There is a difference in inflammatory markers IL-6, IL-β and COX-2 in endometriosis patients with progestin therapy who experience pain and painlessness.

Keywords: endometriosis, estradiol, proinflammation, progestin therapy.

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INTRODUCTION

Endometriosis is a condition characterized by the growth of endometrial-like tissue outside the uterus. Endometriosis is estrogen-dependent, so clinical symptoms typically manifest when a woman reaches reproductive age.[1, 2] The condition affects approximately 10% of young women, representing around 200 million women of reproductive age. Its prevalence increases to up to 50% among patients with chronic pelvic pain, infertility, or both.[3]

One evolving theory regarding the pathophysiology of endometriosis is the Tissue Injury and Repair (TIAR) concept. This theory is based on the idea that the disease develops spontaneously due to repeated microtrauma from chronic uterine peristaltic activity affecting the endometrium-myometrium boundary.[4] This condition heightens the inflammatory response by increasing COX-2 levels, elevating prostaglandin E2 (PGE2) levels. Elevated PGE2 activates STAR (steroidogenic acute regulatory protein) and P450 aromatase.[4]

In endometriosis patients, there is an increased expression of COX-2 and PGE2, which induces inflammation. The elevated levels of these compounds further increase estradiol levels, exacerbating pro-inflammatory factors.[5] The increased pro-inflammatory factors are the secretion of IL-6 and IL-1β.[6] The increase in pro-inflammatory factors enhances the recruitment of peripheral nerve fibres associated with pain sensitization, specifically C-fibers. The elevated

density of these nerve fibers contributes to the development of pain.[6,7]

Based on this pathophysiology, estrogen plays a crucial role in the development of endometriosis and is closely associated with the production of pro-inflammatory cytokines, which contribute to pain.1 Progestin therapy is one of the hormonal treatments that can suppress estrogen production, both from the ovaries and within endometriotic lesions, thereby reducing pain stimulation caused by endometriosis lesions. In the hypothalamic-pituitary-ovarian axis, progestin therapy can inhibit the release of GnRH, and the levels of FSH and LH. The decrease in gonadotropin levels subsequently leads to reduced ovarian steroid hormone levels, which can cause amenorrhea and prevent endometrial reflux.[8]

Progestin has antimitotic effects on endometrial cells, inhibiting aromatase enzyme activity, COX-2 expression, and PGE2 production in endometrial cell cultures. This action helps to reduce or suppress inflammatory reactions in the pelvic cavity and alleviate pain.[9, 10] In addition to inducing apoptosis and atrophy in endometriotic implants, progestin also plays a role in reducing angiogenic factors such as VEGF, which can decrease the survival rate of endometriosis lesions.[10,11] Compared to GnRH agonist therapy, progestin provides similar effects with higher tolerability and without the side effects commonly associated with GnRH agonists, such as vasomotor symptoms, loss of bone mineral density, vaginal dryness, and decreased libido.[12-14]

Despite this, progestin therapy does not completely eliminate pain complaints in patients with endometriosis. There are phenotypic subgroups of patients who continue to experience pain despite receiving therapy. The persistence of pain in these patients may be attributed to elevated levels of pro-inflammatory compounds and serum estrogen. Therefore, this study aims to evaluate the levels of pro-inflammatory markers IL-6, IL-1β, and COX-2, as well as serum estradiol, in endometriosis patients with and without pain undergoing progestin therapy.

METHODS

This observational cross-sectional study aims to compare serum levels of COX-2, IL-6, IL-1β, and estradiol in patients with endometriosis who are undergoing long-term (3-month) progestin therapy. The research will differentiate between those experiencing pain and those who are not. Conducted at RSUPN Cipto Mangunkusumo in Jakarta from March to June 2024, or until the sample size is achieved, the study will adhere to the guidelines set by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia - RSUPN Cipto Mangunkusumo. Approval has been granted under the number KET-1181/UN2.F1/ETIK/PPM.00.02.2024, and written informed consent will be obtained from all participants regarding the procedures involved.

The target population for this study consists of endometriosis patients receiving long-term (three months) progestin therapy at RSUPN Cipto Mangunkusumo from March to June 2024, who meet the inclusion and exclusion criteria. Inclusion criteria are patients with endometriosis diagnosed via ultrasound or laparoscopy, receiving three months of progestin therapy, exclusively using progestin without other modalities (such as GnRH agonists or aromatase inhibitors), and aged 18–45 years. Exclusion criteria include patients with autoimmune diseases (lupus, Sjögren's syndrome, rheumatoid arthritis), PCOS, a history of hypertension, diabetes mellitus, or dyslipidemia, those undergoing treatment for other infections, and patients with pelvic inflammatory disease. Consecutive sampling will be used to obtain 47 subjects who meet the inclusion and exclusion criteria.

The study procedure involves identifying and selecting endometriosis patients who have received long-term progestin therapy from March to June 2024 at the RSCM outpatient clinic, based on inclusion and exclusion criteria. Patients who meet the criteria will be evaluated through medical records concerning endometriosis phenotype, surgical history, progestin use, other treatments, pain complaints, abnormal uterine bleeding, and the volume of blood loss. Blood samples will be collected at the clinic, analyzed at the IMERI laboratory, and the results will be processed statistically.

The levels of pro-inflammatory markers IL-1β, IL-6, and COX-2 will be measured using the sandwich ELISA method. For the analysis of IL-1β and IL-6, samples will be diluted and analyzed through a procedure involving incubation, washing, addition of antibodies and substrates, followed by reading the results using a microplate reader at 450 nm. Serum estrogen levels will be assessed using Microplate Enzyme Immunoassay. Serum samples, calibrators, and controls will be tested in duplicate.

The procedure includes the addition of reagents, incubation, washing, addition of substrate solution, and reading the results at 450 nm. Data analysis will be conducted using SPSS version 23.0. The independent variables in this study are serum levels of COX-2, IL-6, IL-1β, and estradiol. The dependent variable is the presence or absence of pain. Data will be presented as mean ± standard deviation (SD) for normally distributed data or median (interquartile range, IQR) for non-normally distributed data. Statistical tests used to evaluate the effectiveness of the treatment include the independent T-test for normally distributed data and the Mann-Whitney U test for non-normally distributed data.

RESULTS

Table 1. Basic Characteristics of Patients

Variables	Total (n = 47)
Age: Median (min-max), years	40(27-45)
Body Weight: Mean ± SD, kg	62.4±11.8
Height: Mean ± SD, cm	155.9±5.6
Body Mass Index (BMI): Mean ± SD, kg/m²	25.6±4.6
Endometriosis Phenotype	
Endometrioma	
Yes	26 (55.3)
No	21 (44.7)
Endometrioma Location	
Unilateral	15 (57.7)
Bilateral	11 (42.3)
Adenomyosis	
Yes	42 (89.4)
No	5 (10.6)
Deep Endometriosis	
Yes	1 (2.1)
No	46 (97.8)
Surgical Intervention	
Yes	22 (46.8)
No	25 (53.2)
Progestin Used	
Type of Medication	
Oral	44 (93.6)
Implant	3 (6.4)
Medication Type	
Lynestrenol	11 (23.4)
Dienogest	27 (57.5)
Norethisterone	3 (6.4)
Microlut	2 (4.3)
Drospirenone	1 (2.1)
Duration of Progestin Therapy (months): median (min-max)	12 (3-240)
Pain Symptoms before Progestin Therapy: median (min-max), VAS	9(5-10)
after Progestin Therapy: median (min-max), VAS	3(0-7)
Abnormal Uterine Bleeding	
Yes	18 (38.3)
No	29 (61.7)
Bleeding Pattern	
Cyclical	1 (5.6)
Non-Cyclical	17 (94.4)
Amount of Blood	
Same as Menstruation	1 (5.6)
Less than Menstruation	16 (88.9)
More than Menstruation	1 (5.6)

Table 1. showed that the median age of the subjects was 40 years, with an average weight of 62.4 ± 11.8 kg, height of 155.9 ± 5.6 cm, and BMI of 25.6 ± 4.6 kg/m². Of the subjects, 26 (55.3%) had endometrioma, with 15 (57.7%) being unilateral and 11 (42.3%) bilateral. The majority of subjects (42 subjects, 89.4%) had adenomyosis, and 1 subject (2.1%) had deep endometriosis lesions. A total of 22 subjects (46.8%) had a history of surgery. Most subjects (44 subjects, 93.6%) received oral progestin therapy with a median duration of 12 months (range 30-240 months), and 3 subjects (6.4%) used implants. The most commonly used oral progestins were dienogest (57.5%) and lynestrenol (23.4%). Among the 47 subjects, 18 (38.3%) experienced abnormal uterine bleeding, with 17 subjects (94.4%) having a non-cyclical bleeding pattern and 16 subjects (88.9%) experiencing bleeding less than menstruation.

Table 2. Basic Patient Characteristics Based on Pain Group

Variables	Pain (n = 24)	No Pain (n = 23)	*P-Value*
Age: Median (min-max), years	40 (27-45)	40 (25-48)	
Body Weight: Mean ± SD, kg	64 (36-96)	58 (48-85)	
Height: Mean ± SD, cm	156.2±5.9	155.6±5.4	
Body Mass Index (BMI): Mean ± SD, kg/m²	26.1 (17.6-37.5)	23.3 (20-36.3)	0.333
Endometriosis Phenotype			
Endometrioma			0.453
Yes	12 (50)	14 (60.1)	
No	12 (50)	9 (39.9)	
Endometrioma Location			0.462

Unilateral	6 (50)	9 (64.3)	
Bilateral	6 (50)	5 (35.7)	
Adenomyosis			0.600
Yes	22 (91.7)	20 (86.9)	
No	2 (8.3)	3 (13.1)	
Deep Endometriosis			0.322
Yes	1 (4.2)	0 (0.0)	
No	23 (95.8)	23 (100)	
Surgical Intervention			
Yes	12 (50)	10 (43.5)	
No	12 (50)	13 (56.5)	
Progestin Used			
Type of Medication			
Oral	24 (100)	20 (86.9)	
Implant	0 (0.0)	3 (13.1)	
Medication Type			0.067
Lynestrenol	6 (26.1)	5 (23.8)	
Dienogest	14 (60.9)	13 (61.9)	
Norethisterone	1 (4.3)	2 (9.5)	
Microlut	1 (4.3)	1 (4.8)	
Drospirenone	1 (4.3)	0 (0)	
Duration of Progestin Therapy (months): median (min-max)	8.5 (3-240)	18 (3-96)	0.849
Pain Symptoms			
before Progestin Therapy: median (min-max), VAS	9 (7-10)	9 (5-10)	0.031
After Progestin Therapy: median (min-max), VAS	4 (3-7)	1(0-2)	
Abnormal Uterine Bleeding			
Yes	9 (37.5)	9 (39.1)	
No	15 (62.5)	14 (60.9)	
Bleeding Pattern, n (%)			0.908
Cycklical	1 (11.1)	0 (0)	
Non-Cyclical	8 (88.9)	9 (100)	
Amount of Blood, n (%)			1.000
Same as Menstruation	1 (11.1)	0 (0.0)	
Less than Menstruation	7 (77.8)	9 (100)	
More than Menstruation	1 (11.1)	0 (0.0)	

The study categorized participants into two groups based on pain presence after at least 3 months of progestin therapy. The pain group had a median age of 40 years and a median weight of 64 kg, with a mean height of 156.2 cm and a median BMI of 26.1 kg/m^2. The non-pain group had a similar median age but a lower median weight of 58 kg, mean height of 155.6 cm, and median BMI of 23.3 kg/m^2, with no significant difference in BMI between groups (p = 0.333). Phenotypic analysis revealed that 50% of the pain group and 60.1% of the non-pain group had unilateral endometriomas. Adenomyosis was common in both groups (91.7% and 86.9%, respectively), while endometriosis lesions were rare, found in only 4.2% of the pain group. 50% of the pain group and 43.5% of the non-pain group had a history of surgery. Progestin therapy was predominantly oral in both groups, with all subjects in the pain group receiving oral progestin, primarily dienogest (60.9%), and 86.9% of the non-pain group also receiving oral progestin, including 61.9% on dienogest and 13.1% on implant therapy. The median duration of progestin use was shorter in the pain group (8.5 months) compared to the non-pain group (18 months). Pre-therapy, the median VAS scores were similar in both groups (9). Post-therapy, the median VAS score improved to 4 in the pain and 1 in the non-pain group. Both groups had similar rates of abnormal uterine bleeding (37.5% in the pain group and 39.1% in the non-pain group), with no significant difference (Chi-Square p = 0.908) (Table 2).

Table 3. Differences in IL-1β, IL-6, COX-2, and Estradiol Levels Based on Pain Group

Variables	Pain (n = 24)	No Pain (n = 23)	*P-Value*
IL-1β	1.54(1.15-43.12)	1.41(1.02-7.45)	0.303
IL-6	0.25(0.08-5.12)	0.29(0.09-2.01)	0.725
COX-2	1.845(1.24-10.26)	1.55(0.32-3.07)	0.004
Estradiol	47.35(17.3-314.1)	37.7(14.4-299.3)	0.587

The study compared serum levels of IL-1β, IL-6, COX-2, and estradiol between the pain and non-pain groups. Using the Mann-Whitney test, there were no significant differences in median levels of IL-1β (1.54 [1.15-43.12] vs. 1.41 [1.02-7.45], p = 0.303), IL-6 (0.25 [0.08-5.12] vs. 0.29 [0.09-2.01], p = 0.725), and estradiol (47.35 [17.3-314.1] vs. 37.7 [14.4-299.3], p = 0.587). However, a significant difference was found in COX-2 levels (1.845 [1.24-10.26] vs. 1.55 [0.32-3.07], p = 0.004) (Table 3).

Table 4. Correlation between IL-1β, IL-6, COX-2, and Estradiol

Variables		Correlation Coefficient	*P-Value*
IL-1B	Estradiol	-0.084	0.573
IL-6	Estradiol	-0.088	0.555
COX-2	Estradiol	0.035	0.817
IL-1B	IL-6	0.471	0.001
IL-1B	COX-2	0.141	0.346
IL-6	COX-2	0.045	0.766

Correlation analysis using Pearson's test evaluated the relationships between pro-inflammatory cytokines and estradiol levels. The results showed no significant correlations between IL-1β and estradiol (r = -0.084, p = 0.573), IL-6 and estradiol (r = -0.088, p = 0.555), COX-2 and estradiol (r = 0.035, p = 0.817), IL-1β and COX-2 (r = 0.141, p = 0.346), and IL-6 and COX-2 (r = 0.045, p = 0.766). However, a significant positive correlation was found between IL-1β and IL-6 (r = 0.471, p = 0.001) (Table 4 and Figure 1).

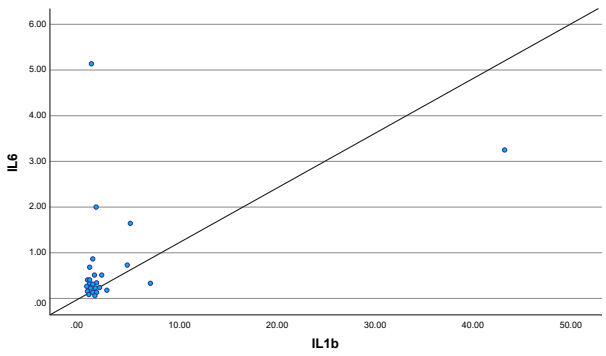

Figure 1. Correlation between IL-1β and IL-6

In this study, Pearson's correlation analysis assessed the relationships between COX-2, IL-1β, IL-6, and VAS scores. COX-2 showed a weak but statistically significant positive correlation with VAS scores (r = 0.360, p = 0.013). In contrast, IL-1β and VAS scores had a weak positive correlation that was not statistically significant (r = 0.144, p = 0.333). Similarly, IL-6 and VAS scores exhibited a weak correlation (r = 0.132, p = 0.376), and estradiol showed a weak negative correlation with VAS scores (r = -0.036, p = 0.808) (Table 5 and Figure 2).

Table 5. Correlation Between COX-2, IL-1β, IL-6, and Post-Therapy VAS ScoreS

Variables		Correlation Coefficient	*P-Value*
COX-2	VAS	0.360	0.013
IL-1B	VAS	0.144	0.333
IL-6	VAS	0.132	0.376
Estradiol	VAS	-0.036	0.808

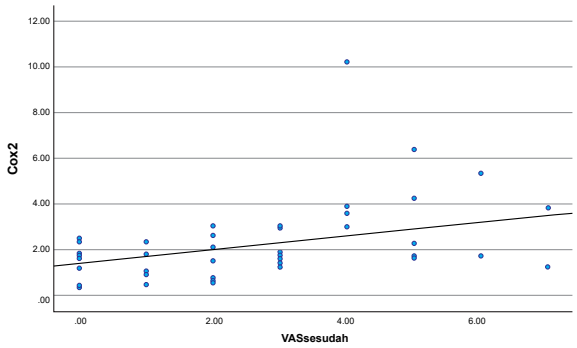

Figure 2. Correlation between COX-2 levels and post-therapy VAS scores

DISCUSSION

Dysmenorrhea, dyschezia, dyspareunia, and chronic pelvic pain are primary complaints in patients with endometriosis. Progestin is a long-term therapeutic option for managing these pains, with several studies indicating that progestin can suppress the activity of endometriosis cells and associated inflammation, thereby alleviating pain. However, the effectiveness of progestin therapy can vary, and in some cases, pain does not fully improve. This study included 47 endometriosis patients undergoing progestin therapy at RSUPN dr. Cipto Mangunkusumo. The patients were categorized into two groups: the pain and the non-pain group. The characteristics of both groups, including age, weight, height, body mass index (BMI), endometriosis phenotype, and abnormal uterine bleeding, showed no significant differences. The patients' characteristics

showed that both groups had similar baseline profiles, with a median age of approximately 40 years, consistent with the epidemiology of endometriosis in women of reproductive age, ranging from 18 to 45 years.[15] BMI also did not show a significant difference, and although biological risk factors such as BMI may be associated with endometriosis, these results align with other studies suggesting that BMI cannot be conclusively identified as a primary risk factor.[16, 17]

Before progestin therapy, the median VAS score was 9 in both groups. After treatment, the VAS score decreased to 4 in the pain group and 1 in the no pain group, consistent with several studies indicating that progestin is effective in reducing endometriosis-related pain. A meta-analysis by Mitchell et al. demonstrated that progestin improves pain symptoms (SMD = -0.61, 95% CI (-0.77, -0.45), P <0.00001). A prospective study also showed a significant reduction in VAS scores during the first three and six months of treatment. Progestin alleviates endometriosis-related pain by decreasing FSH and LH hormone secretion, leading to anovulation and reduced estrogen levels, thus inhibits the growth of endometriotic tissue through anti-inflammatory, antiproliferative, and anti-angiogenic effects, particularly by suppressing PGE2 and COX2.[18]

The study found a significant difference in COX-2 levels between the pain and non-pain groups, with median COX-2 levels being higher in the pain group compared to the non-pain group (1.845 vs. 1.55; p = 0.004). In contrast, IL-1β, IL-6, and estradiol levels did not differ significantly between the groups. This may be attributed to the localized nature of the inflammatory response in endometriosis lesions, which might not be reflected in serum levels. Endometriosis lesions can produce estrogen and elevate pro-inflammatory compounds such as IL-1β, IL-6, and COX-2.[2, 6] Although IL-6 levels increase with the extent of endometriotic tissue, IL-1β does not show a significant increase in peritoneal fluid. Serum blood tests reveal elevated IL-1β and IL-6 levels compared to controls, but there are no significant differences among various stages of endometriosis.[3]

No significant correlation was found between estradiol and the pro-inflammatory cytokines IL-1β, IL-6, and COX-2. Theoretically, estradiol can increase pro-inflammatory cytokine levels through a positive feedback mechanism,[2] however, in this study, increased estrogen may be localized to endometriosis lesions, thus not affecting serum cytokine levels. The correlations among IL-1β, IL-6, and COX-2 revealed that while IL-1β and IL-6 are correlated, there was no significant correlation between IL-1β and COX-2, or IL-6 and COX-2. This is consistent with previous studies that identified a correlation between IL-1β and IL-6 in endometriosis patients but not with COX-2. Pro-inflammatory cytokines may be more specific to endometriotic tissue rather than serum, which could explain the absence of significant correlations.[3] These findings suggest that pro-inflammatory cytokines are more specific to endometriotic tissue rather than serum blood.

The analysis indicates that COX-2 has a weak positive correlation with VAS pain scores (r=0.360; p=0.013), while no significant correlation was found between IL-1β and VAS, or IL-6 and VAS. This suggests that COX-2 may play a more significant role in the pain mechanism of endometriosis compared to IL-1β and IL-6. Chronic inflammation in endometriosis leads to alterations in pain signalling and increased sensitivity to stimuli. Inflammatory mediators such as prostaglandins, VEGF, TNF-α, NGF, and various interleukins play a role in this process. Cytokines like IL-1β and IL-6 are involved in pathological pain, with expression observed in the dorsal root ganglia and spinal cord.[6] Excessive COX-2 is associated with resistance to apoptosis and phenotypic changes in chronic diseases. COX-2 induces the production of prostaglandins, which trigger angiogenic factors such as VEGF. COX-2 expression is upregulated by IL-1β, as demonstrated in studies on colorectal cancer cells and diabetic wounds, where IL-6 levels decreased while COX-2 increased. This suggests that IL-1β in chronic pain may drive the upregulation of COX-2, explaining the significant increase in COX-2 compared to IL-1β and IL-6.[19, 20]

CONFLICT of INTEREST

The authors declare no potential conflicts of interest.

CONCLUSION

This study demonstrates that there are no significant differences in the levels of the pro-inflammatory markers IL-1β and IL-6, as well as estradiol, in the serum between endometriosis patients with and without pain who received progestin therapy. However, a significant difference was found in the COX-2 levels, which

were higher in the pain group. There was no correlation between estradiol levels and IL-1β, IL-6, or COX-2. A correlation was observed between COX-2 levels and pain intensity, and a reduction in pain intensity was noted in both groups receiving progestin therapy, as indicated by the decrease in VAS scores.

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Research Article

The SDGs Perspective of TeleDoVIA Reliability for Cervical Cancer Elimination in 2030: A Cross-Sectional Study in Indonesia

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Abstract

Objective: To describe the prevalence of HPV infection in women with negative Visual Inspection with Acetic Acid (VIA) and introduce Teleconsultation of Documented VIA (TeleDoVIA) as an objective test and provide a rationalization for recommending TeleDoVIA as a "high-performance" test for cervical cancer screening in lower resource settings, from SDGs perspective, to accelerate the achievement of second pillar elimination and the third SDGs target in 2030.

Methods: This is a 7-year cross-sectional study. Subjects were recruited consecutively from several public and private health providers in Jakarta. VIA test was documented and consulted with the experts panel (TeleDoVIA). Negative VIA women underwent HPV-DNA testing using SPF10-DEIA-LiPA25 for PCR and electrophoresis.

Results: A total of 1,397 negative VIA subjects were collected, including 52 HPV-DNA positive. The false-negative for VIA was 3.7% (95% CI 0.027–0.047).

Conclusion: VIA is a reliable screening method with a low false-negative rate. TeleDoVIA could be recommended as a reliable cervical cancer screening method in low-resource settings such as Indonesia, which is in line with the third SDG: good health and well-being.

Keywords: asia, public health, southeast asia.

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INTRODUCTION

Cervical cancer is the leading cause of gynecological death worldwide, especially in developing countries or LMICs. The 5-year prevalence of cervical cancer globally and in Indonesia, based on GLOBOCAN 2020, is 1,495,211 and 92,930, respectively.[1-3] The World Health Organization (WHO) has set a triple intervention target of 90% vaccination coverage before age 15.70% screening with high-performance tests, and 90% treatment to be achieved in 2030.[4] In addition, eliminating cervical cancer is consistent with the third goal of the SDGs stated by the United Nations (UN): good health and well-being.[5] HPV-DNA testing is a high-performance test generally used as a cervical cancer screening test.[6-8] It has been recommended by the WHO as the primary screening tool over the other screening methods.[9] However, in LMICs, where facilities and resources are limited, this method is unreachable due to higher costs.[10-12] VIA is a screening method for cervical cancer precancerous lesions recommended by WHO for low-resource settings because it is simple, inexpensive, and sensitive.[13-15] In the

WHO decision-making flowchart for program managers, VIA is no longer just a screening test but a triage for treatment implementation after a positive HPV-DNA test.9 In order to avoid subjectivity of VIA expertise, in Indonesia, we have developed Documented VIA (TeleDoVIA): a method of documenting VIA results using a cell phone camera and a specific technique that provides results comparable to colposcopy. The Documentated VIA (DoVIA) results are consulted and discussed by gynecologists oncologist experts panel on the easy-to-use Whatsapp Messenger telecommunication system, which is called Teleconsultation of Documented VIA (TeleDoVIA). These experts' responses have been reported to be immediate or, at maximum, within six hours, providing accurate VIA interpretations.[16,17] A study by Utami et al. reported a low false positive rate of 3.21% for DoVIA, suggesting that TeleDoVIA is likely also to maintain a low false negative rate. Given this, our primary concern with this teleconsultation platform remains the potential for false positives.[18]

This modality is expected to provide an alternative screening method with comparable performance to the HPV-DNA test. Hence, the goal of cervical cancer elimination can be achieved in LMICs. Poverty is a significant risk factor for non-communicable diseases, including cancer.[18] Poverty is the first goal of SDGs. This study aims to describe the prevalence of HPV-DNA positive in the negative VIA women and rationalize the recommendation of TeleDoVIA as an objective, even as a "high-performance" test for cervical cancer screening in lower resource settings, from SDGs perspective, to accelerate the achievement of second pillar elimination and the third SDGs target in 2030.

METHODS

The research method is a descriptive cross-sectional study conducted for approximately seven years (January 2012 - July 2018). The subjects were women enrolled in primary health care (PHC) and other health care facilities appointed in the "See and Treat" Female Cancer Program (FCP) Jakarta, as well as gynecologists, colposcopies, and gynecological outpatients from the private clinic of the Department of Obstetrics and Gynaecology of Dr. Cipto Mangunkusumo General Hospital (RSCM).

Ethical approval for this study was obtained from the University of Indonesia Review Boards. Subjects were recruited consecutively, and the inclusion criteria were: married or sexually active, residing in Jakarta, the squamocolumnar junction (SCJ) was fully seen, and willingness to participate in the study. The exclusion criteria were pregnancy, recent genital infection, SCJ was not fully seen, and cervical cancer or precancerous lesion. With an assumed proportion of positive HPV in the hostile VIA population of 5 % and a precision of 1.25 %, a minimum of 1,167 subjects were required.

After ethical approval was obtained, the data were collected. The information about the study was explained to the patients by trained medical staff. Each subject who participated in this study signed an informed consent form. Before a thorough physical examination, each woman was administered a structured socio-demographic and complications and medical history questionnaire. The questions addressed socio-demographic variables such as age, occupation, education level, smoking, contraceptive use, age at menarche, previous cervical screening history, and sexual behaviour questions.

A well-trained general practitioner or midwife performed the procedures under the direct supervision of an oncological gynecologist. The subject was in the lithotomy position after being informed about the indications, the procedure, possible side effects, and the treatment. The examiner inserted a speculum into the vagina to expose the cervix. Before the VIA test, an initial inspection was performed to assess whether the cervix was normal, severely inflamed, or suspicious for precancerous or invasive cervical cancer. In addition, the examiner applied a 5% acetic acid solution to the cervix, especially in the transformation zone, including the squamocolumnar junction site. The result was evaluated after 60 seconds. We make documentation of VIA (DoVIA) using a cell phone camera and a specific technique that provides results comparable to colposcopy. The DoVIA results are consulted and discussed by an expert panel on the Whatsapp Messenger telecommunication system (TeleDoVIA).

The result was considered positive VIA due to the presence of white epithelial lesions (WEL). Meanwhile, the absence of WEL was stated as a negative VIA. Patients with negative VIA results were then subjected to HPV-DNA testing.

HPV-DNA tests were performed by Leiden University Medical Center (LUMC) and KALGen Laboratory Jakarta. HPV-DNA status was defined

as a positive or negative HPV-DNA test result determined by PCR SPF10-DEIA-LiPA25. A standardized protocol was used for cervical sampling.

Samples from cervical mucus were collected by swabbing with a cytobrush rotated 2x360° and then placed in a tube containing a 20 ml volume buffer solution. The tubes were centrifuged at 4,500 rpm for 10 min the same day. The residue was then placed in a 2 ml save-lock tube and stored at -50°C for further analysis.

HPV-DNA detection. The assay was performed in the pre-PCR laboratory, specifically in the HPV cabinet, to avoid contamination. Each well (A1-H9) contained 20 ul SPF10 PCR, 2 l H2O, and 3 ul DNA samples; the H10 well contained 20 ul SPF10 PCR and 5 ul H2O; the H11 well contained 20 ul SPF 10 PCR and 5 ul Siha; the H12 well contained 20 ul SPF 10 PCR and 5 ul SPF 10 positive control. After all, well were ready, the plate was covered with the Bio-rad RT-PCR shield, and short centrifugation was performed (± 1 min). Then the thermal cycle PCR process was performed with an additional LIPA program in the PCR lab. Subsequently, electrophoresis is continued to determine HPV-positive samples.

DNA quality PCR (Q-PCR). All A1-H9, H10, and H11 were prepared. Each well (A1-H9) contained 11 ul qPCR mix and 1 ul DNA samples; the H10 well contained 11 ul qPCR and 1 ul H2O; the H11 well contained 11 ul qPCR and 1 ul Siha 3 cell line as a positive control. After all, well were ready, the plate was covered with the Bio-rad RT-PCR shield, and short centrifugation (± 1 min) was performed. Then the thermal cycle PCR process was performed with an additional LIPA program in the PCR lab. Subsequently, electrophoresis is continued to determine HPV-positive samples.

Electrophoresis. First, the 3% agarose gel was prepared. Then the electrophoresis equipment and the samples were prepared. The PCR plate was removed from the freezer. Then the samples were melted and centrifuged. The gel was run at 120V for 45-60 minutes.

After data collection, the data were reviewed, edited, and coded. Data analysis was done using the Statistical Program for Social Sciences (SPSS) for Windows version 20.0. The data obtained were presented descriptively for categorical variables. The results of the analysis were presented in terms of number (n) and percentage (%) (proportion). The descriptive data presentation was in tabular form. Missing data were not calculated.

RESULTS

A total of 1,504 subjects met the inclusion criteria in the study period. Sixty-two subjects from FCP and PHC forty-two subjects from RSCM were excluded due to incomplete data (age), while three subjects were excluded due to duplicate subjects. Finally, 1,397 subjects were enrolled and processed (Figure 1).

As described in Table 1, the median subjects' age was 41, while the mean age of marriage was 22. The HPV-DNA test result were described in Table 2.

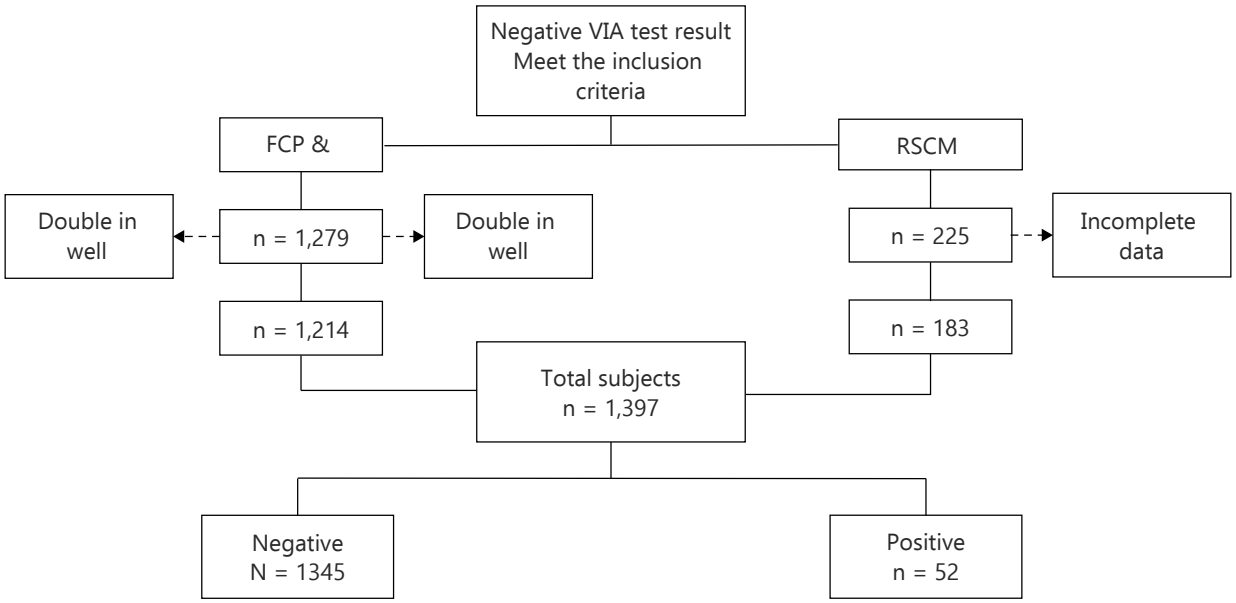

Figure 1. Subjects Flow Diagram FCP, Female Cancer Program; PHCs, Public Health Care; RSCM Clinics, Dr. Cipto Mangunkusumo General Hospital

Table 1. Demographic characteristics

	Description (n=1,397)	
Age (years old)		41 (19-84)
Age category	≤ 40 years old	681 (48.7)
	> 40 years old	716 (51.3)
Marital age (years old) *		22 (13-48)
First menstruation age (years old) **		13 (9-20)

Numeric variables for abnormal data distribution are presented with the median (minimum - maximum); Categorical variables are expressed as n (%).
*n = 1391, as many as six subjects missing data
**n = 543, as many as 854 subjects missing data

Table 2. HPV-DNA Test Results

Variabel	Category	n (%)
Status	Positive	52 (3.7)
	Negative	1.345(96.3)
Genotyping (n=52)	Hr-HPV	29 (55.7)
	Non Hr-HPV	7 (13.5)
	HPV X	16 (30.8)
Number of infections (n=52)	Single infection	35 (67.3)
	Multiple infection	17 (32.7)

Hr-HPV: High risk HPV

DISCUSSION

This study collected 1,397 subjects with negative VIA were collected. Fifty-two subjects (3.7%) had positive HPV-DNA results (95% CI 0.027-0.047). Of the 52 subjects, 55.7% were infected with high-risk HPV, regardless of single or multiple infections. These results showed that the false-negative of VIA testing could be considered low.

The sensitivity of VIA is lower in postmenopausal women than in premenopausal women but has higher specificity.[19-21] The cohort of the study consists of 51.3% women over 40 years. However, we did not have data on the menopausal status of the subjects. Therefore, the percentage of postmenopausal women cannot be presented. In this study, 228 out of 1397 subjects (16,32%) were > 50 years of age, while 8 of them (3.52%) were HPV positive. In Contrast, 1168 out of 1397 subjects (83,67%) were ≤ 50 years of age, while 44 subjects of this group (3,76%) were HPV positive. If we agrees that women aged > 50 years were considered postmenopausal, the false-negative VIA in premenopausal and postmenopausal women groups was very low, illustrating that it was an excellent screening method.

A similar study refers to HPV-DNA positive in VIA-hostile populations was limited. Instead, however, more studies refer to HPV-DNA positives in cytologically negative populations. In Pakistan, the study presented the same result (4.74%) with a similar total of subjects (1,011 subjects) compared to this study. The study assessed the prevalence of HPV infection in a population with normal cytologic results.[22] Then, the study reported that the prevalence of HPV in 1,859 subjects with normal cytology results in Colombia was 14.8%, with 9% of the women infected by hrHPV.[23] Worldwide HPV prevalence in women with normal cytology at any given point in time is approximately 10%.[24] The higher prevalence of HPV in normal cytology results was reported, which is 51%.[25]

The most comprehensive available data on HPV prevalence in women with normal cytology derives from a large, global meta-analysis of the literature published and compiled by the WHO. The study includes 157,897 women with normal cytology and specifically excludes data on women with any abnormality. Only women with reported normal cytology were included in the analysis. The results indicate that 10.4% (95% CI: 10.2–10.7) of the women worldwide are positive for HPV-DNA. HPV prevalence is higher in the less developed world (13.4%; 95% CI: 13.1–13.7) than in more developed regions (8.4%; 95% CI: 8.3–8.6). African women (22.1%; 95% CI: 20.9–23.4), in particular women in Eastern Africa, have the highest HPV prevalence rates (31.6%; 95% CI: 29.5–33.8), while the lowest estimates are identified in Southeast Asia (6.2%; 95% CI:

5.5–7.0).[26] Compared to these studies, if the HPV-DNA test is a gold standard, this study showed a very low false-negative VIA test in detecting HPV infection than using cytology with 5-40% false-negative rate. This allows us to conclude that the VIA test can provide results comparable to or maybe even better than the cytology test.

In this study, married individuals were considered sexually active because asking about sexual activity status was taboo in the Indonesian population. Therefore, we included married women as inclusion criteria instead of expanding the inclusion criteria to include sexually active womaen even though they are not yet married, which would lead to biased responses.

The VIA examination in this study was performed by a well-trained general practitioner or midwife. To avoid the subjectivity of VIA expertise, we use TeleDoVIA: teleconsultation to an experts panel on the Whatsapp Messenger telecommunication system of documented VIA with a specific technique that provides results comparable to colposcopy.

Of the 17 points of SDGs, cervical cancer elimination is in line with the third goal, specifically target 3.4, 3.7, and 3.8.[27-29] Target 3.4 aimed to reduce premature mortality from noncommunicable diseases, including cervical cancer, by one-third through prevention and treatment.[27] Target 3.7 ensures universal access to sexual and reproductive health services and the inclusion of reproductive health in national policies and programs.[29] Target 3.8 aimed to achieve universal health coverage. These targets are expected to be achieved by 2030.[30]

WHO defines premature NCDs-related death as the unconditional possibility of mortality from cardiovascular disease, cancer, diabetes, and chronic respiratory disease between 30 and 70 years old.[31]

Globally, 41 million people (71% of global mortality) die annually from non-communicable diseases. The number of annual premature deaths (between 30 and 69 years) due to NCDs is over 15 million worldwide. Unfortunately, low- and middle-income countries account for 77% and 85% of total NCD- and premature NCD-related mortality, respectively. Cancer is the second leading NCD-related mortality, which causes 9.3 million deaths annually.[32] Cervical cancer is the fourth most common cancer in women. In 2020, 341,831 women died from this disease (2.8% of total NCD-related mortality).[33] In Indonesia, 73% of deaths were caused by NCDs, while NCDs caused 26% of the probability of premature death.[34] Screening, early detection, treatment, and palliative care of NCDs are essential to the success of the 3.4 SDGs by 2030.[35]

The first SDG target, to end poverty in all its forms everywhere, is also in line with the cervical cancer elimination. Target 1.4 of this goal is to ensure that all men and women, in particular the poor and vulnerable, have equal rights to economic resources, as well as access to basic services, ownership and control over land and other forms of property, inheritance, natural resources, appropriate new technologies, and financial services, including microfinance. Basic services also include access to basic health services, including preventive health services. This goal is consistent with target 3.8.1 of the third goal.[36] Unfortunately, among the SDGs' various goals, targets, and indicators, there are several aspects that LMICs have not been able to implement due to several limitations. Therefore, LMICs need to focus on implementable goals.

The introduction of VIA as a national screening in Indonesia 7 has been in place since 2008. However, the screening coverage in Indonesia is still 21%.[37] Hence, achieving 70% HPV-DNA coverage in 9 years will considered difficult under Indonesian conditions. Although WHO has reduced the coverage for screening with high-performance testing from 80% to 70%, Indonesia, as a big island country with more than 17,000 islands[38], 34 provinces[39], and more than 40 million women between 30-50 years old[40] among more than 270 million of population[41], could not conduct HPV-DNA testing because laboratory facilities are not available throughout the Indonesian archipelago, except on the major islands, insufficient human resources, and lack of funding. In Indonesia, HPV DNA is currently proposed to be introduced as a national screening in 2022. In preparing for the introduction of the HPV-DNA screening program in Indonesia, based on the results of this study, the VIA can be used as an alternative "high-performance test". A screening method is called a gold standard if it has a sensitivity of 100% and a specificity of 100%.[42] False-negative VIA is considered low if HPV-DNA test is the gold standard. In several cases, we found positive VIA with histopathologic confirmed as high-grade lesions in negative HPV-DNA. Thus, we suggest a combination of HPV-DNA and TeleDoVIA test ("Co-testing") as the national cervical cancer screening program in Indonesia.

Although VIA is not a gold standard due to its subjectivity[43], the objectivity of VIA can be increased by introducing tiered supervision by an experienced oncologic-gynecologist. According to WHO, high-performance tests must have similar or better performance characteristics than the HPV-DNA test. However, new technologies may become available in the future.[4] In Indonesia, the tiered supervision is implemented using the DoVIA, which is the documentation of the cervix that underwent VIA using cell phone camera devices with special techniques (filling light mode, without backlight). The DoVIA is a material for communication and consultation between practitioners and the experts (gynecologic-oncologist). The expert panel consultation is called Portal Teleconsultation of DoVIA (TeleDoVIA). This photography-based consulting model utilizes a teleconsultation system via WhatsApp Messenger, which was immediately discussed with the experts and received feedback within 15 minutes, even less than 10 minutes.[17] DoVIA is effortless to implement, cost-effective and provides better images than colposcopy.[16] Hence, VIA, as well as DoVIA and TeleDoVIA, are reliable methods that could be considered as a "high-performance test" in Indonesia as a low-resource country to accelerate the achievement of the second pillar of the triple intervention to eliminate cervical cancer by 2030.[43]

This study has several strengths. First, it is recognized that the HPV-DNA testing was done in two different locations and at two different times, but the authors used standardized protocols, so these two results should be reliable. Second, all recruited patients were tested for HPV-DNA with no loss to follow-up. However, this study has a limitation; there was a random error in the collection of subjects. So that occurred three times in the whale data, but we excluded all of that to avoid bias.

In this study, the results can be generalized to the Indonesian population. The objectivity of the VIA test can be increased with TeleDoVIA. Further diagnostic studies are needed for "co-testing" of HPV-DNA and VIA test as a more accurate screening test with a lower cost than the existing co-testing.

CONCLUSION

VIA has a low false-negativity rate. TeleDoVIA could be recommended as a reliable cervical cancer screening method in low-resource settings, which is in line with the third SDG, paticularly targets 3.4, 3.7, and 3.8. However, further diagnostic studies on "co-testing" of HPV-DNA and VIA tests are needed.

DATA AVAILABILITY

The data supporting this study's finding are available from the corresponding author upon reasonable request.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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Research Article

Impact of Freeze-Dried Amnion Membrane and Human Amnion Stem Cell Seeding on TGF-β and Collagen Type III in Vesicovaginal Fistula

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Abstract

Objective: To analize how freeze-dried amniotic membrane and human amniotic stem cell seeding affect TGF β and type III collagen expression in suturing a New Zealand rabbit vesicovaginal fistula model.

Metode: This experimental study employed New Zealand rabbits and a vesicovaginal fistula model with a post-test only control group design. The rabbits were divided into 3 groups: vesicovaginal fistula suturing alone, suturing with freeze-dried amniotic membrane, and suturing with freeze-dried amnion-seeded stem cells. After 7 days of treatment, specimens near the repaired vesicovaginal fistula were collected for immunohistochemical analysis of TGF β and collagen type III expression.

Result: TGF β expression was significantly higher in the freeze-dried amniotic membrane with stem cell seeding group (p=0.001) compared to the freeze-dried amniotic membrane without stem cell seeding group (p=0.017) and the suturing-only group (p=0.049). Additionally, type III collagen expression was significantly elevated in the freeze-dried amnion membrane and stem cell seeding group (p=0.001) compared to the freeze-dried amnion group without stem cell seeding (p=0.09) and the suturing-only group (p=0.026).

Conclusion: The expression of TGF β and type III collagen was higher in rabbits with vesicovaginal fistulas treated using freeze-dried amnion and amniotic stem cell seeding compared to those without amniotic stem cell seeding and vesicovaginal fistula suturing alone.

Keywords: freeze-dried amnion, stem cell, vesicovaginal fistula.

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INTRODUCTION

Vesicovaginal fistula is a health concern within the field of urogynecology that can significantly impact patients' psychosocial and sexual well-being. Its symptoms often manifest as continuous urinary leakage from the vesicovaginal opening. A case report from the Urogynecology Reconstruction Division of dr. Soetomo Hospital for the years 2016 to 2018 revealed a notable recurrence rate of 31.2% for repeated fistula suturing. Gynecologic fistula cases resulting from iatrogenic causes remain a significant challenge, yet advancements in labor monitoring have led to a substantial decrease in obstetric fistula cases following childbirth.[1]

While surgical intervention through the transvaginal approach is favored over the transabdominal method in the treatment of vesicovaginal fistulas, the current scenario presents a challenge due to persistently high failure rates.[2-4] The dissatisfaction with this surgical procedure has led to the exploration of alternative approaches involving biomaterial application to replace the lost biological structure resulting from the defect. This is expected to enhance the repair process and sustain the normal functionality of the compromised tissue through tissue engineering. An emerging biomaterial in the field of urogynecology is the amnion membrane, chosen for its abundant presence in obstetrics and its non-invasive nature.[5,6] The amnion membrane

can be directly applied to the wound defect or in the form of stem cells. Human amnion stem cells have also been extensively researched and hold significant potential for transplantation and tissue engineering applications. They possess immunomodulatory properties, self-renewal capabilities, and the ability to differentiate through the production of growth factors such as TGF β, which is commonly found in the bladder's stromal layer.[7-9] The role of TGF β can stimulate the formation of type III collagen during the initial phases of wound healing. Thus, the application of amnion membrane stem cells is expected to reinstate the function and form of the layer to its original state or near-original state in vesicovaginal fistula cases.[10,11]

METHODS

An experimental study was conducted using New Zealand rabbits as the animal model for vesicovaginal fistula. The research design followed a post-test only control group approach. The study comprised three groups: a control group, which underwent vesicovaginal fistula suturing alone; treatment group 1, which underwent fistula suturing and received freeze-dried amnion membrane without amnion stem cell seeding; and treatment group 2, which underwent fistula suturing and received freeze-dried amnion membrane with amnion stem cell seeding. On the seventh day post-treatment, all rabbits were euthanized, and specimens were collected. These specimens were subjected to immunohistochemical staining to determine the expression of TGF β and type III collagen. The measurements of the rabbits' body weights were analyzed using the Shapiro-Wilk normality test, revealing normally distributed data ($p > 0.05$). The test for homogeneity of variance yielded a p-value greater than 0.05, indicating no significant variation in the body weights of rabbits among the different groups.

RESULTS

The analysis of the comparison of TGF β expression revealed significant differences among the groups, with the most significant difference observed between treatment group 2 and the control group, exhibiting the largest mean difference of 2.4.

Table 1. The Results of the Rabbit's Body Weight Analysis

Groups	Mean + SD	Normality	Homogenity test
Control	3.37 ± 0.16	0.103	
Intervention 1	3.37 ± 0.14	0.2	0.668
Intervention 2	3.47 ± 0.14	0.065	

Table 2. The Outcomes of the Statistical Analysis of Immunohistochemical TGF β Expression

Groups	Mean	Min	Max	*P-value*
Control	2.57	1.2	4.1	
Intervention 1	3.6	2.4	6.0	0.001
Intervention 2	5.0	2.9	7.4	

One Way Anova test

Table 3. The Results of the intergroup comparison analysis of TGF β expression

Groups	Mean	*P-value*
Intervention 1 VS Control	1.09	0.049
Intervention 2 VS Control	2.4	0.001
Intervention 2 VS Intervention 1	1.3	0.017

Kruskal-Wallis test

The analysis of the comparison of type III collagen expression revealed significant differences among the groups, with the most notable distinction observed between treatment group 2 and the control group, demonstrating the largest mean difference.

DISCUSSION

The research findings revealed statistically significant differences in TGF β expression among all groups. The treatment group that underwent vesicovaginal fistula suturing along with the application of freeze-dried amnion and amnion stem cell seeding exhibited higher TGF β expression compared to both the group treated with only freeze-dried amnion application and the group that underwent fistula suturing alone.

This study is in line with the work conducted who investigated the effectiveness of amnion stem cells in inducing wound healing processes from the epithelial to the urothelial muscle layer in a rat model of hemisystectomy, followed by suturing. Liu et al. examined the immunohistochemical expression of TGF β, as well as the number of muscle layers and fibroblasts within the urothelial layer through histological analysis (Hematoxylin and Eosin-H&E staining) in rat models with wounds extending to

the dermal layer. The research findings showed enhanced epithelialization of the rat's skin layer by the seventh day, along with a significantly increased TGF β expression after amnion stem cell administration compared to the control group without stem cells. The level of fibroblasts was also quantified, revealing an increase in the number of mature fibroblasts histologically. It is suggested that fibroblast cells constitute the dominant layer within the urothelial layer, and TGF β acts as the most potent stimulator capable of synthesizing myofibroblasts from fibroblast differentiation to produce collagen during the proliferative phase of wound healing.[12]

The study conducted highlights that reducing inflammatory conditions can lead to a decrease in the formation of fibrosis or scar tissue, thereby accelerating wound healing. This research focused on the effectiveness of mesenchymal stem cells applied to the injured bladder of mammalian rat models. The study reported a significant increase in TGF β expression within the damaged bladder layers through immunohistochemical analysis ($p < 0.05$), indicating an upregulation that expedited tissue regeneration.[13]

The research findings also indicate that the treatment group, which underwent primary suturing and received freeze-dried amnion without stem cell seeding, exhibited higher TGF β expression compared to the group that only underwent primary suturing. Macroscopically, the surface layer of the vesicovaginal fistula that had been sutured in treatment group 1 showed improved results when compared to the group that underwent suturing alone. A study demonstrated an increase in TGF β expression in oral mucosal ulcers in rats when treated with TGF β growth factor, as opposed to the control group.[14]

Similar findings were obtained from the study by Jepsen et al. on a group of pigs with a vesicovaginal fistula model, where stem cells were injected into the formed vesicovaginal fistula area. This randomized study involved seven pig models with vesicovaginal fistula, wherein the results showed an increase in smooth muscle α-actin content by 98.8% ± 0.6% in immunoreactive cells in the group that received stem cell injections. These findings closely mirror those from a human study, which involved patients with Crohn's Disease and perianal fistulas treated with autologous stem cell injections into the fistula defect. This study, classified as a phase 1 clinical trial, achieved a fistula closure success rate of 82% (27 out of 33).15 Another research effort demonstrated successful wound healing after using freeze-dried amnion membrane in a case of vesicovaginal fistula in a 64-year-old human patient. The study reported positive outcomes from applying an amnion patch to the vesicovaginal fistula suturing performed through an open abdominal surgery approach (transabdominal). By the 7th day post-operation, there were no reports of urinary leakage through the vagina (incontinence), and a cystography examination showed robust epithelial layer healing.[16]

The research outcomes also reveal that the group subjected to fistula suturing and the application of freeze-dried amnion with amnion stem cell seeding exhibited significantly higher expression of type III collagen compared to both the group that received freeze-dried amnion without stem cell seeding and the group that underwent suturing alone. The results of TGF β in this study are consistent with the increased products it generates, namely collagen deposits and other ECM components, serving as biomarkers for successful wound healing. It is well understood that TGF β acts as a key stimulator of collagen production during wound healing processes.[16] Collagen and fibronectin are essential for patching tissue defects, enhancing tensile strength, and preparing the tissue for neovascularization. The primary biological function of collagen is to form an extracellular matrix that strengthens, binds, fills, and maintains the structure of tissues. Without collagen, cellular tissues would become fragile. The organized deposition of collagen matrix is a pivotal indicator of the remodeling or maturation phase.[17]

This study aligns with the research on primary intestinal anastomosis in Wistar rat models with intraperitoneal infection, where the application of Freeze-Dried Amnion Membrane (FDAM) resulted in increased collagen density histopathologically at 400x magnification in the FDAM-treated group compared to the control group. The study reported a significant difference between the groups with a p-value of 0.001 ($p < 0.05$).[18,19] Amnion membrane contains a variety of growth factors such as bFGF, EGF, and TGF-β that stimulate fibroblast growth, neovascularization, and ultimately the formation of collagen deposits. The role of amnion also involves inducing the migration of epithelial cells towards the wound site, thereby triggering cell proliferation and differentiation. Another study

by Robles et al. found similar outcomes. In their research, they reported the successful repair of vesicovaginal fistula in two patients through the application of collagen (Pelvicol) from porcine dermal layers (xenograft) as an interposition during vesicovaginal fistula repair. The study achieved satisfactory repair outcomes within 1-2 weeks post-treatment, and at the 12-month follow-up, optimal results in the urinary vesicle structure were noted from the increased collagen deposition observed through histological analysis.[20] The study also reported similar findings to this research. In their study, they observed an increased TGF β expression in the urinary bladder of rats treated with mesenchymal stem cells along with a scaffold using Bladder Acellular Matrices (BAM) in a rat urinary bladder reconstruction model. The study demonstrated optimal regeneration of the detrusor layer of the urinary bladder through the increased collagen composition. This research concluded that the absence of inflammation reduces scar tissue formation and accelerates wound healing. Conversely, prolonged expression of inflammatory factors is associated with the formation of fibrotic tissue, which worsens the reconstruction of the urinary bladder's layers.[21]

CONCLUSION

The expression of TGF β and type III collagen in New Zealand rabbit models of vesicovaginal fistula, treated with freeze-dried amnion combined with human amnion stem cell seeding, was found to be higher compared to both the group treated solely with freeze-dried amnion and the group that underwent vesicovaginal fistula suturing alone.

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Case Report

Atypical Findings of Suspect Twin to Twin Transfusion Syndrome Quintero V

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Abstract

Objective: To report atypical findings in a case of suspected twin-to-twin transfusion syndrome Quintero V.

Methods: Case report

Case: A-38-year old woman, G3P2AO presented at 34–35 weeks of gestation with a diagnosis of a diamniotic monochorionic twin pregnancy. The first fetus was in breech position with polyhydramnios and intrauterine fetal demise (IUFD) in the second fetus, raising suspicion for TTTS Quintero stage V. However, this case presented an atypical finding: the single deepest pocket (SDP) of the donor fetus was normal (without oligohydramnios), a rarity in TTTS cases that should be noted. The diagnosis of TTTS Quintero V was based on maternal-fetal ultrasound findings. No additional complications were observed in this case upon further examination.

Conclusions: Several therapies are available for TTTS including amnioreuction, laser ablation of the vascular placental anastomosis, selective feticide, and septostomy. Timing of delivery after management of singleton fetal death in the late second or early third trimester is still debatable. Delivery method is determined based on obstetric indications.

Keywords: atypical, case report, TTTS, Twins.

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INTRODUCTION

A multiple pregnancy is defined as a pregnancy with more than one fetus, a pregnancy with two fetuses is called multiplet.[1] A pregnancy with three fetuses is called a triplet, four are quadruplets and five are quintets.[2] The multiple birth rate has increased from 18.9 to 32 .1 per 1000 live births in the United States from 1980 to 2005. This increase occurred due to fertility therapy and the application of assisted reproductive techniques (ART) as well as the increasing number of women who gave birth over the age of 35 years.[1] In Taiwan, between 1983-1995, only 12% of 34 triplet pregnancies resulted from natural conception, with the remaining 88% were the result of ovulation induction (including in vitro fertilization or IVF). Simirlarly, in Japan, 73.2% of multifetal pregnancies with more than two fetuses result from IVF, 22.1% by ovulation induction, and only 4.3% by spontaneous pregnancy. In Indonesia, as many as 30% of ART produce multifetal pregnancies.[1]

The prevalence of dizygotic (fraternal) twins is higher than that of monozygotic twins, with rates of 70% and 30%, respectively. Population-specific variations exist in the prevalence of dizygotic twins, whereas the global prevalence of monozygotic twins remains relatively constant at 3-5 per 1000 births. Several factors influence the occurrence of multiple births such as race, heredity, parity, age, and maternal BMI. The timing of delivery, position, and presentation of the fetus during delivery, along with its chorionicity and amniocity, need to be taken into account when managing multiple pregnancy

labor.[3,4] However, there is also evidence that multiple pregnancies increases the likelihood of pregnancy complications, particularly in cases of monochorionic multiple pregnancies.[5]

In monochorionic multiple pregnancies, twin-to-twin transfusion syndrome (TTTS) is a serious complication. The diagnosis of TTTS is made using antenatal sonography and is characterized by the presence of oligohydramnios (deepest vertical pocket <2 cm) in the donor with a small or empty bladder and polyhydramnios (single deepest pocket (SDP) >8 cm) with bladder distension in the recipient. [5-7] TTTS affects 10–15% of monochorionic multiple pregnancies and typically manifests between gestation weeks 16 and 26. For optimal perinatal outcomes, TTTS must be identified early and accurately. A notable variation in amniotic fluid (polyhydramnios in recipient babies, oligo or an hydramnios in donor babies) is the primary finding of prenatal ultrasound. Multiple pregnancies of the same sex, monochorionic, weight differences between twins of more than 20%, hydramnios in large fetuses, oligohydramnios and stuck twins in small fetuses, and hemoglobin differences of more than 5 g/dL are the typical findings and diagnostic criteria for TTTS. [1,5-7]

The placentas of the multiple fetuses in a monochorionic pregnancy are shared. The two fetal umbilical circulations are connected by a vascular anastomosis in the chorion in nearly all instances. Deep arteriovenous anastomoses that allow unidirectional blood flow are the foundation of the TTTS mechanism.[5] Following identification, the Quintero staging system typically classifies TTTS. As in this instance, Quintero V TTTS is TTTS with the death of one or both fetuses.[6,8] There are several ways to treat TTTS, ranging from close observation and termination to intervention techniques like selective and non-selective laser fetoscope coagulation.[6,9] Based on the stage of TTTS, a treatment plan can be selected.

However, atypical findings of TTTS are rarely found and should be taken as special notes, asin this case. We will report a rare finding of TTTS in which the SDP level of the donor fetus was normal (not oligohydramnios). Therefore, this paper will also discuss the clinical aspects of multiple pregnancies (gemelli) with suspected TTTS Quintero V, which is characterized by intrauterine fetal death (IUFD) in one of the fetuses. The goal of this study is to examine and assess the diagnostic approach, pathogenesis, pathophysiology, and treatment of TTTS to determine whether they align with theoretical expectations.

CASE

A-38-year-old woman, G3P2AO presented at 34–35 weeks of gestation with a diagnosis of a diamniotic monochorionic twin pregnancy. The first fetus was in breech position with polyhydramnios and intrauterine fetal demise (IUFD) in the second fetus, raising suspicion for TTTS Quintero stage V. She reported no complaints of increasing labor pain or profuse vaginal discharge. She first learned of her twin pregnancy at her 2-month follow-up with an obstetrician. She denied any family history of multiple pregnancies or congenital abnormalities, as well as any history of medication use, herbal intake, industrial area exposure, or chronic illnesses such as hypertension, diabetes, asthma, or heart Due to her symptoms, she initially sought care at a local hospital and was then referred to a tertiary Maternal-Fetal Medicine Polyclinic. This is her third pregnancy, and she has been married once. Her antenatal care (ANC) included six visits, split between a midwife (three visits) and an obstetrician (three visits).

Her physical examination results were within normal limits. On obstetric examination, two fetuses were found with the first fetus in 5/5 breech position and the second fetus in 5/5 head position. Fetal heart rate (FHR) were found in the first fetus and no FHR was found in the second fetus. The first fetus was estimated to weigh 2759 grams and the second fetus was 512 grams. Speculo examination and internal examination were not performed on this patient (Figure 1).

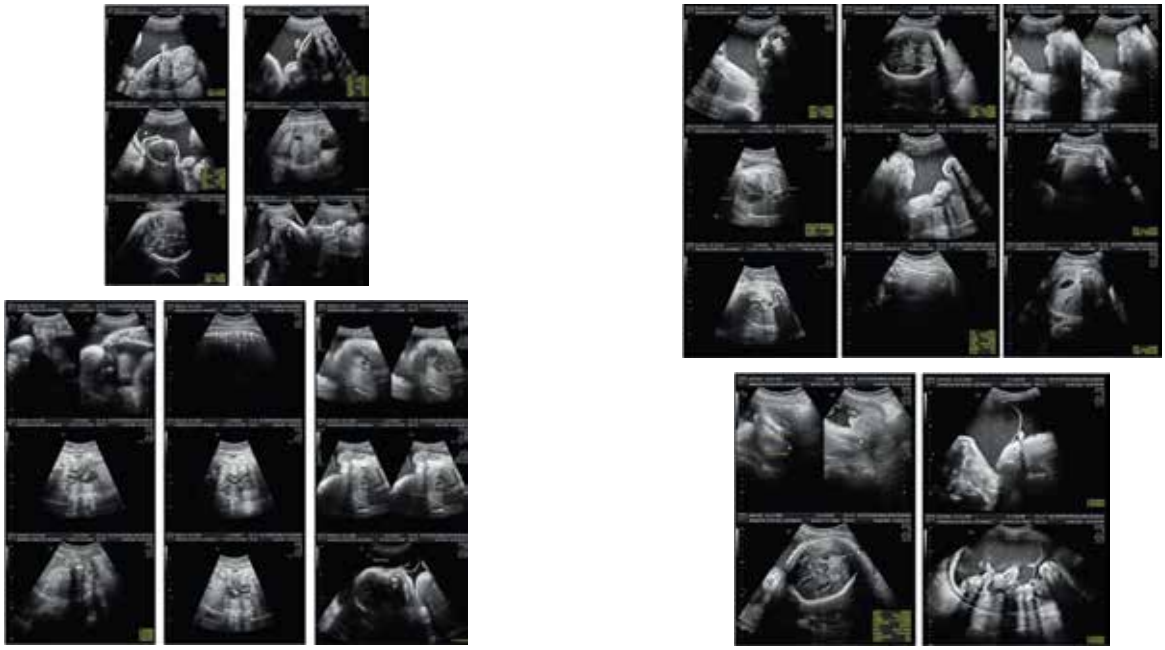

Figure 1. Maternal-Fetal Ultrasound of This Patient's Babies

Laboratory tests showed elevated quantitative D-Dimer levels. Cardiotocography (CTG) for the first twin indicated a category I status. Maternal-fetal ultrasound revealed a 35–36 weeks' gestation with twins, with the first twin in breech position, and the second twin showing polyhydramnios and intrauterine fetal death (IUFD), consistent with Twin-to-Twin Transfusion Syndrome (TTTS) Quintero stage V. Fetal MRI of the head showed no evidence of periventricular leukomalacia or congenital anomalies (Figure 2).

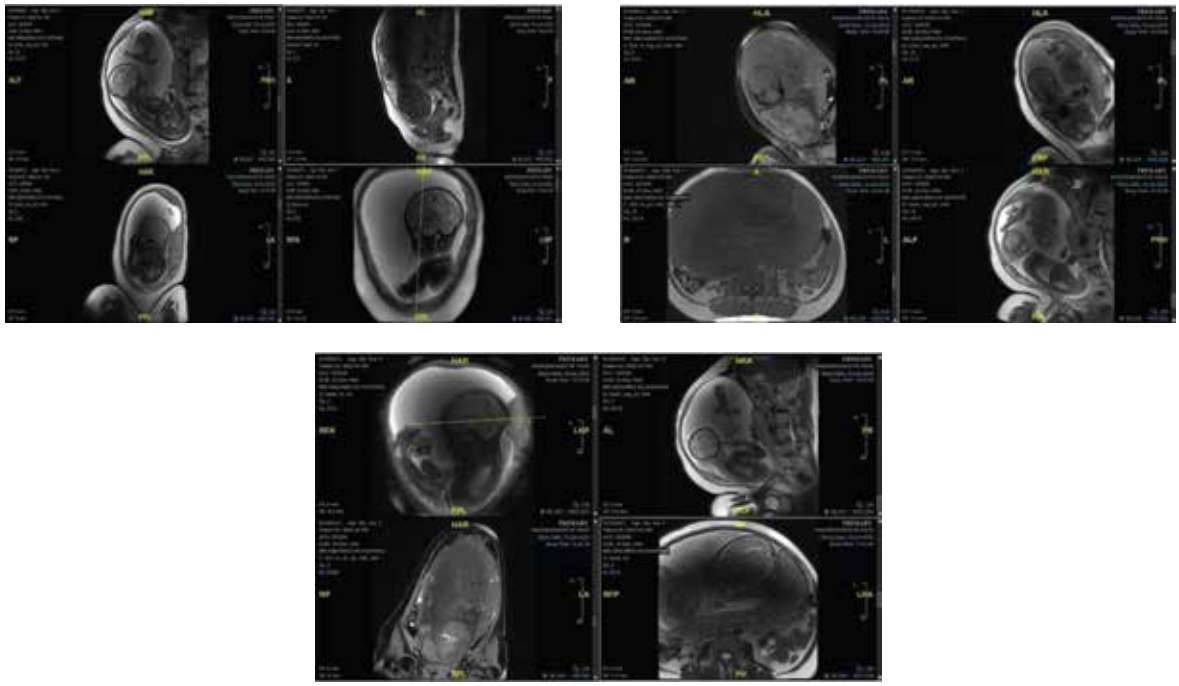

Figure 2. Maternal Abdominal MRI for The First Baby

The initial working diagnosis was G3P2A0 35-36 weeks of gestational age; Gemelli (Multiple Pregnancy); Baby I was in breech position; Baby II was in head position; Polyhydramnios, IUFD; Suspected Twin-to-Twin Transfusion Syndrome (TTTS Quintero V). Conservative treatment was

initially planned. At the following weeks, the patient was admitted to the Obstetrics emergency ward with a diagnosis of G3P2A0 gravida 35-36 weeks; Gemelli; Baby I was in breech position; Baby II was in head position, polyhydramnios, IUFD; suspected Twin to Twin Transfusion Syndrome (TTTS Quintero V). Then conservative management was carried out, until the two following days, an irregular FHR was found in the first baby with a CTG impression of category III. Intrauterine resuscitation was attempted, but as fetal distress in Baby I persisted, an emergency caesarean section was decided. Informed consent and close monitoring of patients were performed. The first baby boy was born weighing 2610 grams and APGAR Score 1'/5' was 7/9. The second baby was born without signs of life, weighing 510 grams, already experiencing grade III maceration. The placenta was born after active management in the third stage with a size of 500 grams (monochorionic diamniotic) (Figure 3).

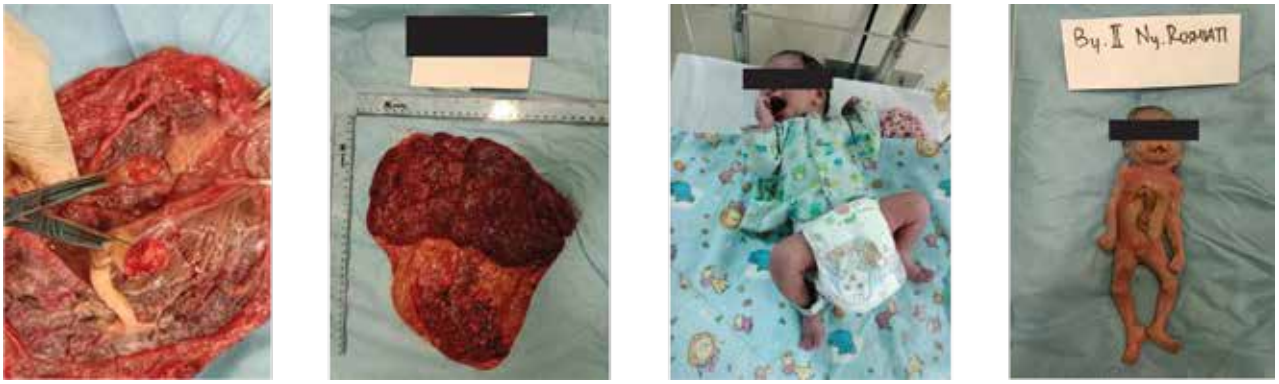

Figure 3. Babies and Placenta

The histopathological examination of the placenta revealed chorangiosis in the larger placenta. The smaller placenta was noted to be within normal limits, as were both umbilical cords.

DISCUSSIONS

In this case, the patient was a G3P2A0 gravida 35-36 weeks; Gemelli; Baby I was in breech position; Baby II was in head position, polyhydramnios, IUFD; suspected Twin-to-Twin Transfusion Syndrome (TTTS Quintero V). Multiple pregnancies are associated with an increased risk of complications during pregnancy, such as hypertension, spontaneous abortion, placenta previa, and fetal malformations. They are also associated with an increased risk of preterm labor, premature rupture of membranes, low birth weight of the baby, and low APGAR scores, which in turn It is also associated with an increased risk of perinatal death in multiple pregnancies. [1-4]

Twin-to-twin transfusion syndrome (TTTS) is a significant issue in many monochorionic pregnancies. In these pregnancies, the fetuses share a single placenta, and nearly all cases involve vascular anastomoses within the chorion that connect the two fetal umbilical circulations. The TTTS mechanism is based on unidirectional blood flow through deep arteriovenous anastomoses. Deoxygenated blood from the placental arteries of both the recipient and donor twins is pumped toward their respective cotyledons. Once oxygen exchange occurs in the chorionic villi, the oxygenated blood is transported out of the cotyledons via the placental vein of the recipient twin. [1,2,5-7.]

Multiple anastomotic connections are probably present in all monochorionic placentas. Monochorionic twins' placentas form a special anastomosis. Three types of anastomoses are possible; arterio-arterial, arteriovenous, and veno-venous (25%). Anastomoses between the veno-venous and arterio-arterial veins are "superficial" and "bidirectional." Anastomosing blood vessel connections, which enable connections between arteries and veins, are referred to as superficial connections because they show up on the chorion's surface. According to the pressure differential between the twins, the flow in this region is bidirectional. Veno-venous anastomosis can be fatal and is linked to lower rates of perinatal survival. Similar to arteriovenous anastomoses, arterio-arterial anastomoses are flexible and can compensate for imbalances that arise in unidirectional flow. There is typically only one type of arterio-arterial

anastomosis in monochorionic placentas. Veno-venous anastomoses in twins with monochorionic placentas cause the venous drainage, which carries oxygen-rich blood, to become rigid and divided. [5,7]

Arteriovenous anastomoses, on the other hand, are "deep" and "unidirectional." While "unidirectional" anastomosis only permits flow in one direction, "deep" anastomosis takes place in the placental lobule's deeper capillary layers. One twin can receive an arterial supply from the other child through an arteriovenal anastomosis, while the other twin receives venous (oxygen-rich) drainage. The monochorionic placenta in arteriovenous anastomosis is actually composed of three parts: two that belong to each twin separately and a third that is supplied by the arteriovenous anastomosis and belongs to the twins collectively.[5,7] Because arteriovenous anastomoses are unidirectional, transfusion imbalance may result unless other deep anastomoses or superficial anastomoses provide sufficient compensation through oppositely directed transfusions. Multiple arteriovenous and venoarterial anastomoses, in addition to arterio-arterial and/or veno-venous anastomoses, are present in about 90% of monochorionic placentas. [4,6] Arterioarterial anastomoses are more effective at compensating for flow imbalances than opposite-direction arteriovenous anastomoses, according to mathematical computer models that simulate TTTS. This is because the arterio-arterial anastomosis, which takes place at the capillary level, has substantially less resistance than the arteriovenous anastomosis.[4,6]

Despite the fact that vascular anastomosis is a necessary anatomical prerequisite for TTTS pathogenesis, the actual mechanism is more intricate than a simple net transfer of red blood cells because hemoglobin levels are typically similar in both twins. As previously stated, TTTS is not a hemoglobin incompatibility issue with polycythemia recipients or anemic donors; rather, it is an amniotic fluid incompatibility issue with volume-overloaded recipients and volume-deficient donors. As a result, hormones associated with maintaining fluid and pressure homeostasis may also be at play. Keep in mind that when two twins exchange blood, one of them naturally becomes exposed to the other's hormonal environment. Consequently, the recipient's hypertensive cardiomyopathy and volume overload may be partially explained by the transfer of renin-angiotensin-aldosterone effectors from the donor. [1,2,5-7]

Acute hypotension may cause cerebral pathology in the victim if one of the affected pregnancy's twins passes away. An embolism of thromboplastic material derived from a deceased fetus is a less common cause. When one twin dies (fetal demise/IUFD), brain damage and rapid antenatal hypovolemia and ischemia are caused by transfusion of the twin anastomosis from the surviving twin's high-pressure blood vessels to the dead twin's low-resistance blood vessels. According to one piece of research, there is a 26% higher chance of neurodevelopmental morbidity and complications from single fetal death in monochorionic twins than in dichorionic twins. The gestational age at the time of the infant's death is correlated with this morbidity. Monochorionic twins have nearly eight times the risk of neurodevelopmental morbidity compared to dichorionic twins at the same gestational age if death occurs between 28 and 33 weeks of gestation (OR=1.48).[5]

Data showed that in this instance, the first baby in a monochorionic diamniotic pregnancy had a different amnion size (normal), while the second baby had polyhydramnios and intrauterine fetal death (single fetal demise). As a result, Quintero V's finding was deemed atypical as it did not fit the criteria for Twin-to-Twin Transfusion Syndrome (TTTS). The Cincinnati Modification of Quintero Staging System could not be applied in this instance since fetal cardiomyopathy was not evaluated. Due to a normal middle cerebral artery-peak systolic velocity (MCA-PSV), there were no Twin Anemic-Polycythemic Syndrome (TAPS) sequelae. According to the mother's abdominal MRI results, the recipient child did not have any neurological complications (which typically occur in 18% of cases of single fetal demise in the cotwin). No congenital abnormalities were found in the recipient, such as acardiac twins and there were no sequelae of Twin Reversed Arterial Perfusion Sequence (TRAP). However, a finding in this case did not align with the underlying theory: the SDP level of the donor fetus (the smaller fetus) was normal (no oligohydramnios), and the larger fetus did not exhibit polyhydramnios; in fact, the opposite was observed. This finding should be noted carefully, as it raises the question of whether atypical presentations of TTTS are common or if this finding may result from other conditions that warrant further investigation.

For TTTS, several treatment options are available, including selective feticide, amnioreduction, laser

ablation of the vascular placental anastomosis, and septostomy. Amnioreduction involves needle drainage to remove excess amniotic fluid. Although septostomy, which creates an intentional hole in the dividing amniotic membrane, was once used, it has largely been discontinued as a medical intervention (Society for Maternal-Fetal Medicine, 2013). Randomized trials indicate that laser therapy offers better neonatal outcomes compared to selective amnioreduction. A trial involving 42 women found comparable 30-day survival rates of one or both twins treated with either selective fetoscopic laser ablation or amnioreduction (75% vs. 65%, respectively). Further assessment of twins from the Eurofetus trial up to six years of age showed no significant improvement in neurologic outcomes or additional survival benefit after six months. For severe TTTS (stages II–IV), laser ablation of the anastomosis is currently the recommended treatment. However, the optimal approach for stage I TTTS remains debated. Close and continuous monitoring is essential after laser therapy.[6,7-10]

If growth disturbances and excessive amniotic fluid occur before 20 weeks, fetal therapy has typically been considered. In most cases, no action is necessary, and both fetuses will die. Because the twins' circulatory systems are shared, any drug injected into one can have an impact on the other. Feticide techniques (also known as fetal therapy) for fetuses that are chosen for reduction thus comprise techniques such as radiofrequency ablation, fetoscopic ligation, or coagulation using laser or monopolar energy to obstruct the umbilical vein or cord. The remaining fetus is still at significant risk, though, even after this surgery.[1,5-9]

Management decisions should be based on the surviving fetus's risk, cause of death, and gestational age. For this specific indication, further monitoring is not necessary in the case of fetal loss occurring in the first trimester. However, if fetal loss occurs after the first trimester in monochorionic twin pregnancies, there is a significant risk of morbidity or mortality for the surviving twin. This is often due to vascular anastomoses, which can cause one twin's death and lead to sudden hypotension in the other. In cases where one twin in a monochorionic pregnancy dies after the first trimester but before viability, pregnancy termination may be considered due to the increased risks associated with continued pregnancy. Occasionally, maternal complications may result in the death of one fetus but not all of them. Since delivery usually takes place three weeks after fetal death is diagnosed. The goal in managing these cases is to prolong the pregnancy while minimizing risks, regardless of whether the intrauterine environment is compromised.[4-6,11,12]

It is controversial to schedule an elective delivery for the late second or early third trimester following conservative management of a singleton fetal death. Twin pregnancies that are monochorionic are more challenging to handle and typically end between 34 and 37 weeks of gestation. Most choose delivery over gravid management when there is a single fetal death at term, particularly if the cause is unknown. In cases like these, the American College of Obstetricians and Gynecologists, or ACOG (2016), advocates for a customized approach.4-6 In this instance, fetal lung maturation was the goal of the patient's conservative treatment plan. Later, while the patient was being observed, it was found that baby I was experiencing fetal distress. This led to the decision to perform a cesarean section to end the patient's pregnancy.

CONCLUSIONS

The existence of "unidirectional blood flow from deep arteriovenous anastomoses" could cause of suspected TTTS Quintero V. Based on ultrasonography results, TTTS is diagnosed. A diamniotic monochorionic gemelli pregnancy with IUFD in the second fetus II led to the clinical suspicion of TTTS Quintero V in this instance. In this instance, there were aberrant results, though, as the donor fetus's SDP level was normal (not oligohydramnios). In this instance, no additional follow-up issues were discovered. For TTTS, a number of treatments are available, such as selective feticide, amnioreduction, laser ablation of the vascular placental anastomosis, and septostomy. It is controversial to deliver a baby in the late second or early third trimester following management of a singleton fetal death. The mode of delivery is chosen based on obstetrics indications.

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Systematic Review

Actinomycin-D vs Methotrexate in Low-Risk Gestational Tropoblastic Neoplasia: Which is the better Option?

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Abstract

Objective: To compare the efficacy and safety of the ACT-based regimen and MTX-based regimen for LRGTN treatment.

Methods: Electronic databases were systematically searched for Randomized Controlled Trials (RCTs) and High-Quality Non-Randomized Controlled Trials (Non-RCTs) comparing ACT with MTX for patients with LRGTN. Studies without Complete Remission (CR) were excluded. The meta-analysis was carried out to quantify the efficacy and safety of each regimen based on odds ratios (ORs) and 95% confidence intervals (95% CIs).

Results: Eight RCTs and 14 non-RCTs were included (2203 patients). Our study concludes that ACT has a higher CR than MTX (79.4% [716/902] vs 66.9%[871/1301]; OR 2.13; 95% CI 1.46-3.10, in the random-effects model). Furthermore, ACT is better in terms of efficacy compared to MTX in both the RCTs [81.2% (259/319) vs 66.1% (199/301); OR 2.17; 95% CI 1.49-3.16, in the fixed-effects model] and non-RCTs group [457/583 (78.4%) vs 672/1000(67.2%); OR 2.10; 95% CI 1.28-3.45, in the random-effects model]. Safety-wise, the use of ACT has a higher incidence of alopecia (OR 3.52, 95% CI: 1.27-9.75, in the random-effects model) compared to MTX, while MTX has a higher risk of developing liver toxicity (OR 0.54, 95% CI: 0.32-0.91, in the fixed-effects model) compared to ACT. Other side effects are not significantly different between the two groups.

Conclusion: Our meta-analysis concluded that ACT has a better efficacy compared to MTX for LRGTN patients. In terms of safety, ACT-based regimens have a higher chance of suffering from alopecia and a lower chance of suffering from liver toxicity. Future clinical studies on single-drug regimens for LRGTN should be conducted in order to produce higher-quality data.

Keywords: act, actinomycin-D, dactinomycin, methotrexate, mtx, low-risk gestational trophoblastic neoplasia, LRGTN.

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INTRODUCTION

Gestational Trophoblastic Neoplasia (GTN) is a malignant transformation of the placental villous trophoblast in sequelae with any type of prior pregnancy. GTN includes choriocarcinoma, invasive mole, placental site tumors, and epithelioid trophoblastic tumors. GTN has a cure rate of around 80-100% with the effective treatment regimen[1,2] GTNs' clinical presentations differ depending on the previous pregnancy type, disease progression, and histopathological classifications.[3,4]

The International Federation of Gynecology and Obstetrics (FIGO), together with the World Health Organization (WHO), developed a scoring system to assess the risk of chemotherapy resistance in gestational trophoblastic neoplasia (GTN). This system classifies GTNs into low-risk and high-risk categories. Low-risk GTNs (LRGTNs) include patients with stage I GTN according to FIGO staging, or patients with stage II–III GTN with a WHO score of 0–6. Meanwhile, GTN patients diagnosed with FIGO stage IV or FIGO stage II–III with a WHO score of ≥7 are classified as high-risk GTNs (HRGTNs). LRGTNs are often treated with single-drug regimens, whereas HRGTNs are typically treated with multidrug regimens[1,3]

Although several regimens are available for LRGTNs, single-drug regimens with either ACT or MTX remain the first choice. However, there is currently No. clear consensus on the best single-drug regimen for LRGTNs thus the choice is often made based on the institutional preference [5-7]. This meta-analysis was conducted to compare the safety and efficacy of each regimen.

METHODS

The study was done in accordance with the PRISMA.

Data Searches and Information Sources

Two investigators independently searched the electronic medical databases including PubMed/ Medline, Google Scholar, and Embase for articles written in English from January 2003 to January 2023. The keywords used were "Methotrexate, Actinomycin-D, Low-risk gestational trophoblastic neoplasia)". After the database search, the most recent studies were reviwed to identify potentially relevant publications. Full texts of these studies were then assessed for eligibility based on criteria for data synthesis.

Studies Eligibility Criteria

Eligible studies included both RCTs and non-RCTs that directly compared ACT and MTX in patients with LRGTN. Studies were if they provided detailed information on each regimens' outcomes and adverse events. Non-RCTs were included due to the rarity of RCTs on LRGTN. We excluded brief data such as abstracts, case reports, posters, and presentations of ongoing RCTs, as these lack detailed case information.

Data Extraction and Definition

The authors reviewed the main texts of each article to extract data regarding first author, publication year, region of publication, study design, total patients, chemotherapy regimens, and the number of the CR and adverse events. Complete Remission rate (CR) was made as the main inclusion criteria which is the number of patients who reached CR compared to the number of patients receiving regimens as the first line treatment. Few studies that did not include the adverse events were still included. The adverse events that show specific toxicities are used as the safety quantifier of each agent. We use Odds Ratio (OR) and 95% CI (95% Confidence Interval) to quantify the safety and efficacy of each agent.

Data Synthesis

ORs with 95% CIs were used to assess the safety and efficacy of MTX and ACT. Studies were pooled based on RCTs and non-RCTs. We applied fixed-effect models for homogeneous studies and random-effect models for studies with significant heterogeneity. Heterogeneity was assessed using the I^2 inconsistency test, with heterogeneity considered significant if the I^2 value was >50%. Forest plots were generated to provide graphical representations of the results. IBM SPSS Statistics V22.0 was used to analyze ORs, with statistical significance set at a p-value of 0.05.

RESULTS

Studies Selection and Characteristics

Results were taken from 8 RCTs [15-22] and 14 non-RCTs [23-36]. Out of all the studies there was only 1 multi-nation RCT, this may be due to the rarity of the case and the variety of regimens in different centers. FIGO/WHO 2000 Scoring System or Hammond Criteria was used as the basis of LRGTN diagnosis. The efficacy and toxicity comparison between MTX and ACT was taken from 22 papers. a total of 2203 patients were analyzed, 902 patients were given ACT while 1301 patients were given MTX.

Meta-analysis of Efficacy Profile

The regimen-based meta-analysis was done to compare the ratio of CR achieved in ACT-based regimen compared to MTX-based regimen. The final analysis shows that overall ACT-based regimen's efficacy was found higher than MTX-based regimen in complete remission (79.4% [716/902]) vs 66.9% [871/1,301]; OR 1.83, 95% CI: 1.49-2.26; I2 = 59%, P = 0.0002). The random-effects model was applied due to substantial heterogeneity, the complete remission event remained superior in the ACT-based group compared to the MTX-based group (OR: 2.13, 95% CI: 1.46-3.10). Furthermore, in the stratified analysis, we divided the studies into RCTs and non-RCTs separately. In RCT studies included, ACT-based regimen was found superior in complete remission (81.2% [259/319] vs 66.1% [199/301]; OR 2.17, 95% CI: 1.49-3.16; I2 = 41%, P = 0.10). For patients in non-RCTs, there was also a better complete remission seen in the ACT-based group (78.4% [457/583] vs 67.2% [672/1,000]; OR 1.70, 95% CI: 1.32-2.19; I2 = 66%, P = 0.0003), the results were not significantly different compared to when the random-effects model was applied due to the substantial heterogeneity (OR 2.10, 95% CI: 1.28-3.45). The meta-analysis of efficacy

profile in ACT-based group and MTX-based group can be seen in Figure 1

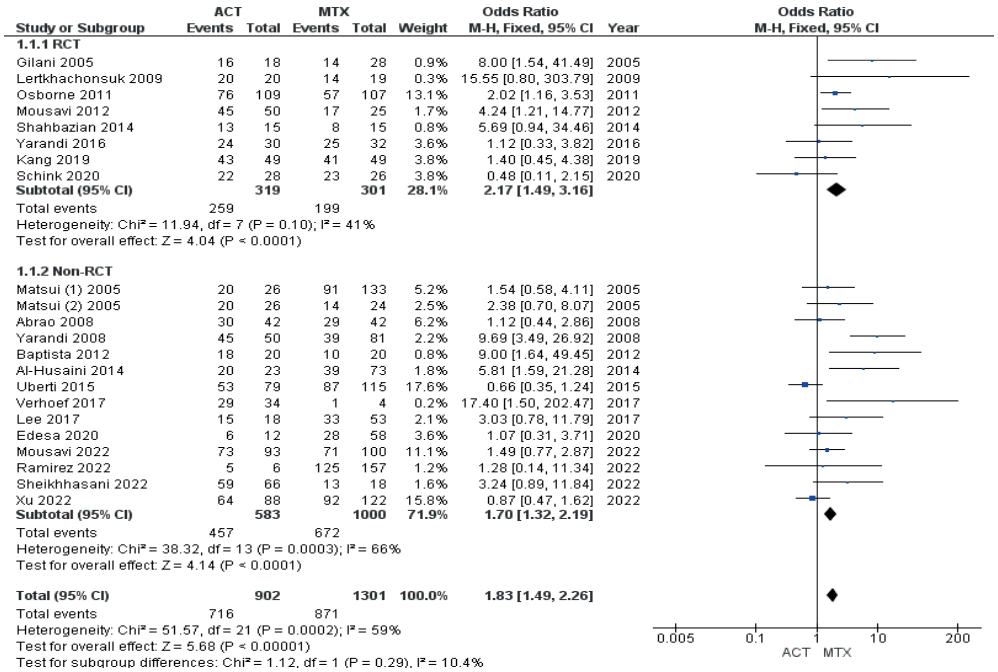

Figure 1. Meta-analysis of efficacy profile based on regimens and study type (fixed-effects model).

Meta-analysis for Toxicities

The toxicities of the regimen used were categorized into the side effects in hematological system, gastrointestinal system, reproductive system, and others.

Side Effects in Hematological System

Figure 2 demonstrated the comparison of hematological toxicities in ACT-based regimen and MTX-based regimen. The analysis shows that the patients who received ACT-based regimen have a significant lower risk of suffering leucopenia (OR 0.47, 95% CI 0.30-0.73; I2 = 83%, P = 0.0005). However, the risk of suffering leucopenia became insignificant upon the application of the random-effects model due to substantial heterogeneity (OR 0.55, 95% CI: 0.14-2.07). On the other side effects in hematological system, the final analysis showed no significant difference in anemia (OR 1.36, 95% CI 0.80-2.34; I2 = 0%, P = 0.36), neutropenia (OR 1.14, 95% CI: 0.65-2.01; I2 = 25%, P = 0.25), and thrombocytopenia (OR 1.52, 95% CI: 0.71-3.26; I2 = 32%, P = 0.21) between the two groups.

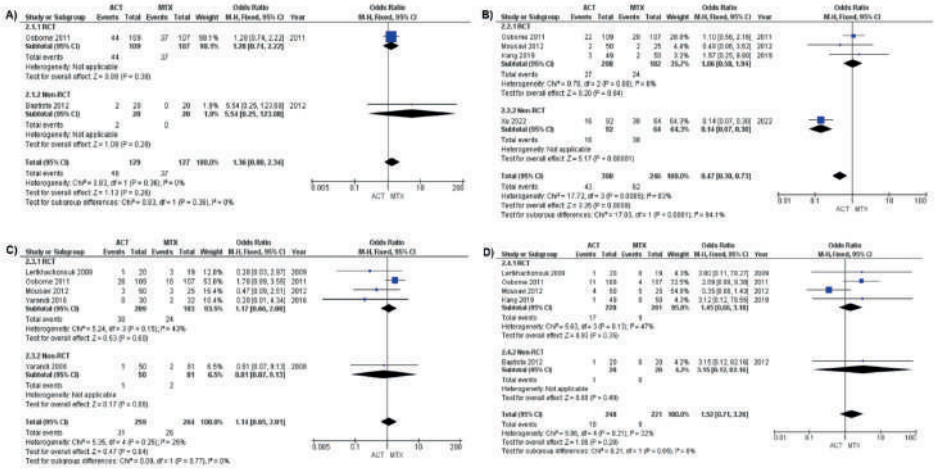

Figure 2. Meta-analysis of side effects in hematological system based on regimens and study type (fixed-effects model): (A) anemia, (B) leucopenia, (C) neutropenia, and (D) thrombocytopenia

Side Effects in Gastrointestinal (GI) System

Figure 3 shows the comparison of gastrointestinal toxicities in ACT-based regimen and MTX-based regimen. The analysis shows that the patients who received ACT-based regimen have a significantly higher risk of suffering nausea (OR 2.41, 95% CI: 1.73-3.35; I2 = 74%, P<0.0001). However, the risk of suffering nausea became insignificant upon the random-effects model application (OR 0.87, 95% CI: 0.38-1.96). The analysis also showed that ACT-based regimen increased the vomiting (OR 2.48, 95% CI: 1.69-3.65; I2 = 60%, P = 0.007). The risk of vomiting was insignificant upon the random-effect model application (OR 2.13, 95% CI: 0.97-4.67). The other pooled analysis showed that no significant difference in constipation (OR 0.92, 95% CI: 0.44-1.90; I2 = 0%, P = 0.81), diarrhea (OR 0.82, 95% CI: 0.49-1.38, I2 = 49%, P = 0.10), anorexia (OR 1.38, 95% CI: 0.58-3.29, I2 = 40%, P = 0.19), oral mucosa problem (OR 1.23, 95% CI: 0.35-4.26, I2 = 23%, P = 0.25), and other unspecified problems (OR 0.87, 95% CI: 0.40-1.90, heterogeneity test: not applicable) between the two regimen groups.

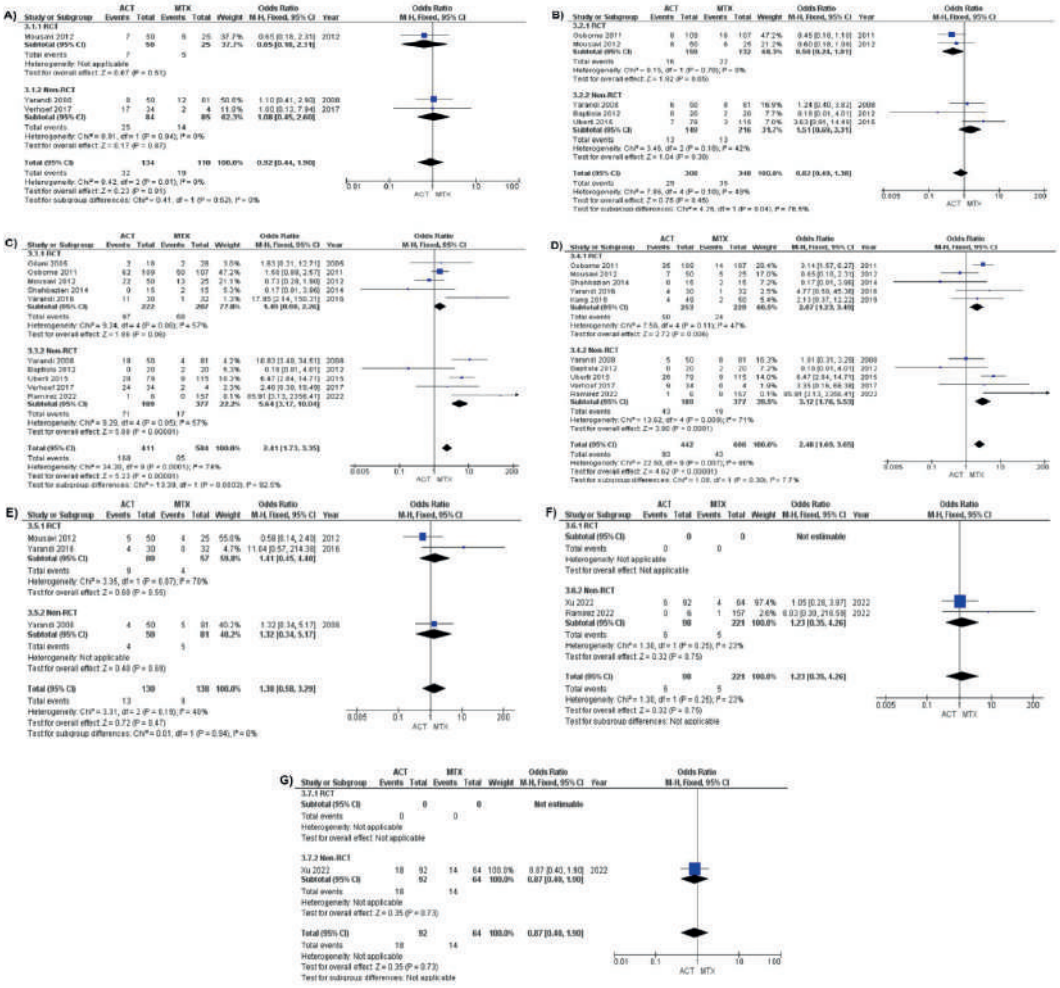

Figure 3. Meta-analysis of side effects in gastrointestinal system based on regimens and study type (fixed-effects model): (A) constipation, (B) diarrhea, (C) nausea, (D) vomiting, (E) anorexia, (F) oral mucosa problem, and (G) other unspecified disorder

Side Effects in Reproductive System

Figure 4 depicts the comparison of reproductive system toxicities in ACT-based groups with MTX-based groups. The analysis shows no significant difference of abnormalities in reproductive system, including abnormal menstrual cycle (OR 0.87, 95% CI: 0.49-1.52), change in menstrual period (OR 1.28, 95% CI: 0.68-2.42), change in menstrual volume (OR 0.78, 95% CI: 0.44-1.38), change in sexual desire (OR 1.29, 95% CI: 0.74-2.26), vaginal dryness (OR 1.06, 95% CI: 0.61-1.85), reduced sexual satisfaction (OR 0.84, 95% CI: 0.48-1.48), and sexual pain (OR 0.74, 95% CI: 0.38-1.45).

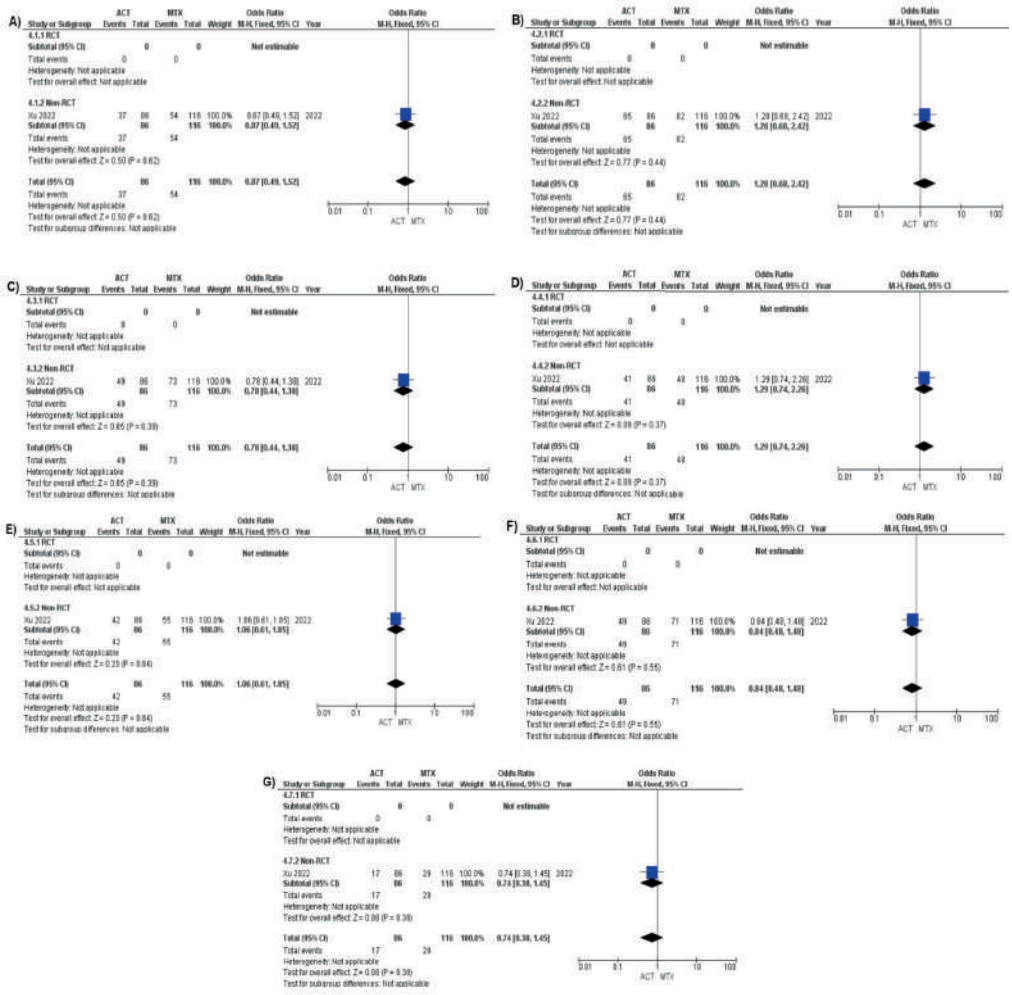

Figure 4. Meta-analysis of side effects of regimen in reproductive system abnormalities according to the regimens and study type (fixed-effects model): (A) abnormal menstrual cycle, (B) change in menstrual period, (C) change in menstrual volume, (D) change in sexual desire, (E) vaginal dryness, (F) reduced sexual satisfaction, and (G) sexual pain

Side Effects in Other System

The analysis shows that ACT-based regimen increased the risk of suffering alopecia (OR 3.21, 95% CI: 1.89-5.44; I2 = 58%, P = 0.003). The results remain significant after the application of the random-effects model due to substantial heterogeneity (OR 3.52, 95% CI: 1.27-9.75). In the stratified analysis of the total studies, the risk of suffering alopecia remained the same in RCTs (OR 2,92, 95% CI: 1.48-5.77; I2 = 63%, p = 0.05) and non-RCTs (OR 3.70, 95% CI: 1.60-8.53; I2 = 68%, p = 0.05). However, the results of each study became insignificant upon the application of random-effects model (RCTs: OR 2.81, 95% CI: 0.73-10.76 and Non-RCTs: OR 7.39, 95% CI: 0.71-77.25). Besides, the use of ACT-based regimen has a lower risk of developing liver toxicity compared to MTX (OR 0.54, 95% CI: 0.32-0.91; I2 = 0%, P = 0.43). In the stratified analysis, the risks of liver toxicity was found lower in RCTs studies (OR 0.38, 95% CI: 0.19-0.76; I2 = 0%, P = 0.55). However, the difference between the risk of liver toxicities in non-RCTs studies was found insignificant (OR 0.91, 95% CI: 0.40-2.09). Last, there is no significant difference reported in malaise symptoms between the two groups (OR 1.04, 95% CI: 0.59-1.82).

DISCUSSION

GTNs are malignant trophoblastic tumors with high sensitivity to chemotherapy. A single drug regimen of either MTX or ACT is commonly given as first-line treatment to women with LRGTN who wish to preserve fertility during chemotherapy, and the prognosis is favorable even in cases with metastasis. LRGTNs are highly curable, a CR rate approaching 100% (10-12). Currently there are still no definitive guidelines regarding the use of

single drug regimen for LRGTN. Our study which include 8 RCTs and 14 non-RCTs (2203 patients) was conducted in order to determine the safety and efficacy of each regimen. We disregarded each regimen's cycles and dosages to objectively assess the efficacy and safety of each regimens more objectively. Future studies may be needed in order to compare specific cycles and dosages of each regimen. In terms of efficacy, our findings are similar to the previous studies [8-12] confirming that ACT has a higher rate of CR compared to MTX as a single drug regimen for LRGTN patients[10-16]. Both RCTs and non-RCTs were pooled in the data analysis. In terms of safety, our study indicates that haematological and hepatic adverse events are more common in patients treated with ACT than with MTX. This finding was supported by other studies, which report that ACT may cause mild to moderate myelosuppression potentially leading to anaemia, leukopenia, neutropenia, thrombocytopaenia, or even pancytopaenia[13-18]. Conversely, LRGTN patients treated with MTX have shown more GI, reproductive system, and alopecia adverse events. Our safety analysis wa consistent with previous studies, though with slight variations. One study found that MTX-based regimen showed a higher incidence of hepatological side effects and ACT-based regimen showed a higher incidence of dermatological side effects including alopecia. Additionally, another study reported a higher incidence of nausea and vomiting with MTC regiments.[8,9,10]. Our findings also showed that MTX regiments were associated with more reproductive system adverse compared to ACT regimens. After the final analysis of the data, we concluded that ACT-based regimens have a higher chance of causing alopecia and a lower risk of liver toxicity.

This meta-analysis has some notable limitations. First, the limited number of studies required us to comnbine RCTs and non-RCT data. Although we performed pooled analyses for both RCTs and non-RCTs, the heterogeneity of the data could introduce bias. Second, there was a lack of standardization in the treatments across studies; we included all studies involving single-drug regimens of ACT and MTX regardless of dosage or regimen type. Third, there was a lack of uniform criteria for defining adverse events and CR rates among studies. Adverse events were graded using various criteria, such as WHO, Gynecologic Oncology Group Criteria, and CTCAE; some studies did not report any adverse events, which limited the sample size available for toxicity analysis and affected the assessment of drug safety.

CONCLUSION

In this study comparing the efficacy and safety of MTX and ACT in LRGTN patients, we concluded that the ACT regimen is more effective in terms of achieving complete remission, while there are no significant safety differences between the two groups. This article may serve as a valuable resource for establishing a more effective regimen for LRGTN and as a reference for future studies on LRGTN treatment.

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Literature Review

Anxiety in Pregnant Women During the Covid-19 Pandemic

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Abstract

Objective: This study systematically reviewed and meta-analysis the prevalence and factors associated with anxiety in pregnant women during the pandemic.

Methods: We searched PubMed MEDLINE, Web of Science, Scopus, ProQuest, EBSCO, Science Direct, and Garuda journal databases in July 2021 and updated them in October 2021. All articles from December 2019 and the English and Bahasa Journal articles were included in the search. We included studies that investigate factors affecting anxiety exclusively in pregnant women. The primary outcome was the prevalence ratio. The secondary outcome was the risk and protective factors as the independent variable. Joanna Briggs Institute Critical Appraisal Tools and RevMan 5.4 were used for the analysis.

Results: After screening 2082 articles, we included 21 studies with 42.177 pregnant women. The pooled prevalence of anxiety was estimated at 28% (95% CI, 23-33.3). We found that 12 of the 21 studies contributed to 8 risks and one protective factor in the meta-analysis. Not married/divorced/widowed, monthly income < 780 USD, screen time > 3 hours/day, history of exposure to COVID-19, complications in the current pregnancy, sleep less than 7 hours per day, subjective poor sleep quality, and high perception of vulnerability were risk factors. Meanwhile, the protective factor was trust in the government's official media.

Conclusion: There is a significant increase in the prevalence of maternal anxiety during the pandemic. Mental health screening during the antenatal visit must be carried out, and interventions to lower the anxiety level must be planned to prevent further harm.

Keywords: anxiety, COVID-19, mental health, pandemics, pregnancy.

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INTRODUCTION

The COVID-19 pandemic causes psychological distress and fear in some individuals due to periods of isolation, quarantine, and hospitalization[1]. As one of the vulnerable populations, pregnant women experience increased anxiety, which has been reported in various countries[2–4]. Anxiety is a normal response to threats and is an attempt to save oneself [5]. However, there will be interference if the response is excessive.

In pregnant women, anxiety is associated with an increased risk of obstetrics problems, cesarean delivery, increased chances of preterm birth, small for gestational age, and smaller infant head circumference[6], including premature rupture of the membrane[7] If not prevented, anxiety during pregnancy could lead to more extensive harm. Therefore, knowing the risk and protective factors for anxiety during pregnancy is essential, especially during a pandemic.

Several systematic literature reviews discussed the psychological impact of COVID-19 on pregnant and postpartum women during the pandemic[8,9]. However, none specifically discusses the prevalence of anxiety during pregnancy and its determinants using a systematic review accompanied by a meta-analysis method. Therefore, this study aims to systematically review risk and protective factors, estimate the

pooled effect size of risk and protective factors, and estimate the pooled prevalence of anxiety in pregnant women during the COVID-19 pandemic.

METHODS

The organization of this manuscript followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) guidelines[10], and the research protocol was registered in PROSPERO CRD42021270107. The literature search was carried out in July – August 2021 and updated in October 2021. We used PUBMED Medline, Web of Science, Scopus, Science Direct, ProQuest, EBSCOhost, and the Garuda journal database to conduct our search.

The search for titles and abstracts was carried out systematically using the thesaurus and MeSH. Combination of keywords: pregnant*, antenatal, prenatal, perinatal, maternal, gravid, prepartum, peripartum, antepartum, expectant mother, anxiety, worry, mental health, stress, distress, COVID, Coronavirus, Wuhan virus, Wuhan Pneumonia, SARS-CoV-2, 2019-nCOV, and pandemic both in English and Bahasa were used. All English and Bahasa journal articles from December 2019 to July 2021 were included in the search.

The Population, Intervention/Exposure, Comparison, Outcome, and Study design frameworks were used to clarify the inclusion and exclusion criteria (see Table 1).

Table 1. PICOS Design Frameworks

	Inclusion	Exclusion
Population	Studies on pregnant women only or pregnant and postpartum women	Studies that did not dissociate pregnant and postpartum women in the analysis.
Intervention/ Exsposure	COVID-19 pandemic	
Comparator		
Outcome	The primary outcome was the prevalence of anxiety among pregnant women. The secondary outcome was the risk and protective factors as the independent variable and anxiety as the dependent variable with Odds Ratio/ Prevalence Odds Ratio, p <0.05, and 95% CI.	Studies only mention descriptive analysis for the outcome and studies that combine anxiety and other mental health problems as the dependent variable.
Study Design	Cross-sectional, case-control. cohort	Reviews, editorials, letters, opinions, purely qualitative studies, conferences, and proceedings.

The articles obtained from the initial search were then imported into Rayyan[11], checked for duplication and screened. Preliminary screening through titles and abstracts was carried out independently by CPP. After selecting the title and abstract, the full text of potential articles was screened based on the data extraction compared to inclusion and exclusion criteria. Articles that did not meet the requirements were removed with a description of the reason. In case of doubt, CPP consulted with other reviewers (BAT, MS). Articles that did not provide access to the full text were excluded.

According to the study design, articles passed the title, abstract, and full-text screening process, then assessed for quality using the Joanna Briggs Institute (JBI) Critical Appraisal Tools[12] and scored. Articles that did not meet the minimum cut-off value of included studies (50%) were excluded to prevent bias due to study quality.

Th data extraction process is carried out by CPP independently. Studies containing statistical data were synthesized quantitatively during the meta-analysis. The combined prevalence was calculated using the proportion formula for pregnant women with anxiety disorders based on the cut-off score of the anxiety measurement instrument provided in the article. Because one article can contain more than one risk factor, each risk factor was analyzed separately. Articles that did not report odds ratio but had 2x2 table data were included in the meta-analysis.

Revman 5.4 software was used to analyze the data. The heterogeneity assessment used the I2 test (I2 > 50%) and the Q test.[13] The random-effects model was used in heterogeneous study conditions otherwise, the fix-effects model was used. The prevalence and 95% confidence intervals for each study were presented in a forest plot. Jamovi 2.0 software was used to assess publication bias by using the Egger and Begg test and the Fail Safe-N test result.[14] The significance of publication bias was obtained when the $P < .05$.

RESULTS

In the initial search, 2802 articles were obtained from 7 journal databases, leaving 21 articles to synthesize the narrative quantitatively after screening. All 21 articles were cross-sectional, with China being the most studied country (66.67%). The two most widely used measuring instruments were GAD-7 (Generalized Anxiety Disorder-7) and SAS (Zung Self-rating Anxiety Scale), with 38.09% each. There were nine articles with a sample size of >1000 pregnant women. Based on the JBI assessment, two studies scored 100%, and five scored 62.5%. The summary of the characteristics of the study is provided in Table 2.

Table 2. Summary of Study Characteristics of 21 Articles Included in the Analysis

Characteristics	n (%)
Study design	
Cross-Sectional	21 (100)
Country of origin	
China	14 (66.67)
Turkey	2 (9.52)
Poland	1 (4.76)
Canada	1 (4.76)
United States	1 (4.76)
Iran	2 (9.52)
Publication year	
2020	2 (9.52)
2021	19 (90.48)
Time of data collection	
The first 6 months of pandemic	19 (90.48)
After 6 months of pandemic	2 (9.52)
Methods of data collection	
Online questionnaire	16 (76.19)
Physical questionnaire	5 (23.81)
Anxiety measurement instruments	
GAD-7	8 (38.09)
SAS	8 (38.09)
HADS-A	1 (4.76)
PROMIS	1 (4.76)
PRAQ	1 (4.76)
DASS-A	2 (9.52)
Sample size	
< 500	5 (23.81)
501 – 1000	7 (33.33)
> 1000	9 (42.86)

Abbreviations: DASS-A, The Depression Anxiety and Stress Scale-Anxiety subscale; GAD-7, Generalized Anxiety Disorder-7; HADS-A, Hospital Anxiety and Depression Scale-Anxiety subscale; PRAQ, Pregnancy Related Anxiety Questionnaire; PROMIS, Patient Reported Outcomes Measurement System; SAS, Self-report Anxiety Scale

Narrative Synthesis

Sociodemographic factors

There were four modifiable sociodemographic factors associated with anxiety. The first was a residential area. Living in a pandemic epicentre location[15,16] or experiencing a lockdown in the place of residence[17] was associated with increased anxiety. The second was socioeconomic status. During the pandemic, the decline in income was associated with anxiety, with higher declines leading to higher anxiety.[18] Lower income (< 780 USD/ month or 7000 USD/ year) was associated with increased anxiety.[19,20]

On the other hand, higher income and a better economic level were protective factors for anxiety.[15,21] The third factor was education, but this study's findings indicate inconsistencies in the variables and outcomes of the effect of education on anxiety. The fourth factor related to anxiety was marital status. Unmarried/divorced/ widowed had a higher risk of anxiety.[18] Age was positively correlated with anxiety in some studies, but the results were inconsistent across studies.

Environmental Exposure Factors

The time spent watching television and cell phones, more than 3 hours per day, was associated with high anxiety in pregnant women. The longer the time spent, the higher the risk of anxiety,[20] especially when watching the news about COVID-19.[22] Increased use of social media was also associated with anxiety.[23] On the other hand, less than 2 hours of screen time was a protective factor even when accompanied by lack of sleep.[20] The presence of COVID-19 infection in close relatives was associated with anxiety.[17] The presence of suspected or confirmed cases around[22], family members who died from COVID-19,[15] and COVID-19 infection during pregnancy was associated with increased anxiety.[2,23]

Occupational Factor

Not working or losing a job during the pandemic was associated with increased anxiety in 4 studies[18,21,23,24] but not in one study.[25] Working as farmers[26] and civil servants[20] was a protective factor for anxiety.

Lifestyle Factor

Physical inactivity was associated with anxiety.[24] On the other hand, being physically active was a protective factor.[20,22,27] In particular, [22]

the interaction between lack of time for physical exercise (< 30 minutes per day) and sleep (< 7 hours per day), and spending more than one hour per day on social media increased the prevalence of anxiety in pregnant women. Sitting more than 10 hours per day and drinking alcohol were also associated with increased anxiety.[18]

Physiological Factors

Nine articles discussed pregnancy complications and comorbidities and their association with anxiety. The result was consistent. Pregnancy complications and comorbidities were associated with increased anxiety in pregnant women.[2,15–18,20,21,25,26,28,29] Planning for vaginal delivery is a protective factor for anxiety.[18]

Sleep time of more than 6 hours per day was a protective factor for anxiety. The longer sleep time, the lower the anxiety,[20] and the lower the sleep time (< 7 hours per day), the higher the anxiety.[30] Further, inconsistent time to sleep, sleep after 00:00, and difficulty initiating sleep was associated with anxiety.[30] Subjective poor sleep quality was also associated with anxiety.[30,31] Research[29] stated that obesity was protective against anxiety. However, this finding was not consistent with [15] research, which stated that obesity and overweight were risk factors for anxiety.

Psychological Factor

Three articles consistently stated that a previous history of anxiety and depression was associated with high anxiety in pregnant women during the pandemic.[23,32,33] Good knowledge of COVID-19[28,34] and its prevention,[15] the simplicity of mothers accessing antenatal information from hospitals,[28] and trust in official government media as sources of information were protective factors for anxiety.[2,34]

On the other hand, mothers who did not receive information about the impact of COVID-19 on pregnancy and mothers who did not receive information from doctors/nurses/midwives about the impact of COVID-19 on the baby's health experienced increased anxiety.[24]

Response to Trauma

The perception of COVID-19's severe impact on their lives [17,26,27,30] or their psychological well-being[30] was associated with increased anxiety. In addition, the perceived susceptibility was also associated with anxiety, both concern for oneself[22,34] and the baby.[31,35]

Mothers who felt uncomfortable during antenatal visits and mothers who did not delay/reduce the number of antenatal visits were associated with higher anxiety.[24,34] In addition, worrying about pandemic control and being afraid to leave the house were also associated with increased anxiety.[2] In contrast, self-efficacy was associated with lower anxiety.[26]

High levels of stress during the pandemic and its relationship with high anxiety levels in pregnant women were described in three articles.[16,31,32] Worrying about the baby, family, friends, and financial adequacy was also associated with higher anxiety levels.[15,26,27] Conversely, not worrying about contracting COVID-19 was associated with less anxiety in pregnant women.[34]

Relational Factor

Social restrictions due to the pandemic increased the anxiety level of pregnant women.[23,35] Family dysfunction, tension with partners, and lack of support from others during the pandemic were associated with increased anxiety.[16,18] On the other hand, high support from a spouse,[30,35] family,[22,33] and generally, was associated with lower anxiety levels.[17,21,35]

Quantitative Synthesis

Anxiety Prevalence

The range of anxiety prevalence was 10 to 65% (k=21), and the pooled prevalence of anxiety was 28% (95%CI; 23-33; N=42,177). There was a significant study heterogeneity (Q = 3150.66; P < .001 and I2 = 99.62%); hence, the most appropriate model used to analyze was a random-effect model. The meta-regression results showed that the country of origin variable significantly moderated the existing heterogeneity (P < .001). There were significant differences in anxiety prevalence between groups from China, Turkey, Iran, and others. The prevalence of anxiety was higher in Turkiye (63%, 95% CI, 60-66), followed by other countries (39%, 95% CI, 15-62), China (22%, 95% CI, 17-27), and finally Iran (20%, 95% CI, 17-23). The forest plot of prevalence is presented in Figure 1.

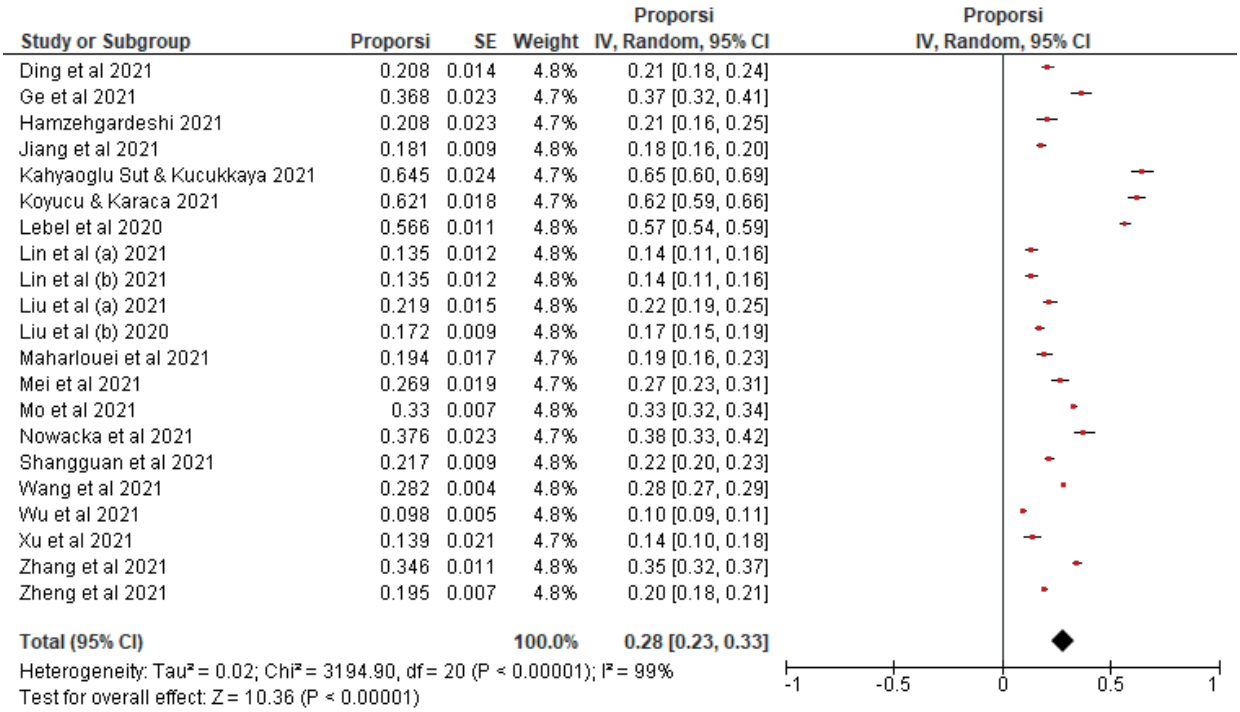

Figure 1. Forest Plot of Prevalence of the Anxiety

In this study, the pooled prevalence of anxiety in pregnant women during the pandemic from 21 studies was 28%. This result is higher than the systematic review of antenatal anxiety before the pandemic (1950 - 2016) [36], which is 22.9% (95% CI; 20.25-25.2; N = 142,833) but not much different from the prevalence of anxiety in the general population during the COVID-19 pandemic of 27.3% (95% CI: 23.7-31.2; N = 140.732)[37] and 31.9% (95%CI; 27.5-36.7; N = 63.439).[38]

However, the prevalence of anxiety in pregnant women in this study was lower than other systematic reviews during the pandemic [8] (37%, 95%CI; 25-49; N = 20.569). One of the possible causes was the origin of the study. In this study, most studies came from China (k = 14, 67%). Cross-cultural aspects influence this difference in anxiety levels. Contextual factors, how one perceives one's body, and dependence on others influence anxiety. Considering this condition, people in Asia generally have lower anxiety levels than other races in the world.[39] This finding is consistent with data from WHO which shows that the prevalence of anxiety in the Asian region is relatively low compared to other countries in the world.[40]

Factors Associated with Anxiety

Twelve studies contribute to 18 factors of anxiety in pregnant women during the pandemic. Of the 18 factors, only nine gave significant results (8 risk factors and one protective factor). They were marital status, monthly income, screen time, history of COVID-19 exposure, pregnancy complications, sleep duration, sleep quality, perceived susceptibility, and trust in the official government social media. Heterogeneity in each study varied, ranging from 0% to 93%. A summary of the combined effects can be seen in Table 3.

Table 3. Summary of 18 Articles Included in the Meta-Analysis of Risk and Protective Factors

Factors	No of studies (k)	Combined sample size	Pooled OR (95% CI)	*P-value*	I^2 (%)
High school education/ lower	6	9107	1.18 (0.82-1.69)	.37	89
Not married/ divorced/ widowed	2	4185	2,20 (1.59-3.04)	<.001	0
Age > 35 y.o	4	5717	1.11 (0.64-1.94)	.70	77
Monthly income < 5000 CNY (~780 USD)	2	2545	1.31 (1.09-1.57)	.004	0
Screen time > 3 hours/ day	2	2545	1.89 (1.43-2.51)	<.001	0
History of exposure to COVID-19	3	3137	1.96 (1.39-2.76)	<.001	0
Not working during pandemic	6	9260	1.20 (0.97-1.50)	.10	58
Work as civil servant	2	2160	1.67 (0.75-3.75)	.21	90
Daily physical exercise	2	4185	0.59 (0.23-1.53)	.28	93
3rd Trimester	9	10,960	1.14 (0.93-1.40)	.22	68
Multipara	7	9924	0.92 (0.74-1.14)	.45	73
Complications and Comorbidities	8	11,394	1.77 (1.39-2.24)	<.001	66
Sleep duration <7 hours/ day	2	2545	1.51 (1.12-2.02)	.007	27
Subjective poor sleep quality	2	1025	7.35 (2.11-25.57)	.002	87
Prepregnancy Overweight/ obesity	2	3509	1.71 (0.59-5.01)	.33	93
Official media trust	2	2764	0.65 (0.52-0.81)	<.001	0
High risk of susceptibility	3	1842	3.91 (2.37-6.45)	<.001	0
Live in the city	2	2081	0.89 (0.40-1.99)	.78	84

Abbreviations: CNY, Chinese Yuan; OR, Odds Ratio; USD, United States Dollar.

The risk of cross-study bias was assessed using an Egger and Begg regression test and the Fail-Safe N value. The p-value in the regression test is .14, which means that there was no bias in the publication of the meta-analysis study. The Fail Safe-N score in this study was 92,340 with P < .001. Because the value of 5k+10 (115) is less than the Fail Safe-N value, it can be concluded that there was no publication bias problem in this study.

DISCUSSION

In this study, the pooled prevalence of anxiety in pregnant women during the pandemic from 21 studies was 28%. This result is higher than the systematic review of antenatal anxiety before the pandemic (1950 - 2016)[36], which is 22.9% (95% CI; 20.25-25.2; N = 142.833) but not much different from the prevalence of anxiety in the general population during the COVID-19 pandemic of 27.3% (95% CI: 23.7-31.2; N = 140,732) [37] and 31.9% (95%CI; 27.5-36.7; N = 63,439) [38].

However, the prevalence of anxiety in pregnant women in this study was lower than other systematic reviews during the pandemic.[8], (37%, 95%CI; 25-49; N = 20,569). One of the possible causes was the origin of the study. In this study, most studies came from China (k = 14, 67%). The results of the sub-group analysis showed that in the group of studies from China, the prevalence of anxiety tended to be lower (22%), while in the study by Yan et al., only four studies were from China. Another nine studies were from Canada, Italy, and other countries. Consistently, the results of studies [8], also show that the prevalence of anxiety in China is lower (33%) than in other countries (Canada 37%, Italy 49%). Cross-cultural aspects influence this difference in anxiety levels. Contextual factors, how one perceives one's body, and dependence on others influence anxiety. Considering this condition, people in Asia generally have lower anxiety levels than other races in the world [39]. This finding is consistent with data from WHO, which shows that the prevalence of anxiety in the Asian region is relatively low compared to other countries in the world [40].

Based on the results of the narrative synthesis, sociodemographic factors that are consistently associated with increased anxiety are living near the pandemic's epicentre, experiencing lockdown, low income, poor economic level, and unmarried/divorced/widow status. However, only marital status and low-income factors are supported by data from the meta-analysis. This can happen because not all studies use the same variables to assess anxiety risk factors, and not all studies provide sociodemographic data.

From the narrative and quantitative synthesis results, the protective factor for pregnant women's anxiety is public trust in the official government media. It was explained before that obtaining too much information from various media during the

pandemic led to increased anxiety.[22,23,41] However, if the duration can be controlled and social media is used to get information about COVID-19 from the government and hospitals, it could reduce anxiety.[2,28,34] Public trust in the national media has a protective effect on anxiety.[42] On the other hand, the perception of COVID-19 politicization and the number of confusing news sources related to COVID-19 are related to anxiety.[43] Hence, it is hoped that public health messages announced by the government must also provide solutions with one consistent message and from one source to increase trust.

Strengths and Limitations of the Study

This study involved quite a lot of articles (k=21). However, there is high heterogeneity between studies. In addition, the definition of a variable as a risk factor is also inconsistent between studies. These differences make it challenging to compare age, education level, parity, and trimester from one study to another.

Another limitation of this study is the use of various measuring instruments and differences in the cut-off value of anxiety even with the same measuring instrument. Almost all studies use a self-report questionnaire that can increase the possibility of bias in answering and is not a standard in determining the diagnosis of anxiety. However, this method is still acceptable for use as an initial screening. Another limitation of this study lies in the design of the articles included in the study. All studies used a cross-sectional design, so we can not conclude the causal relationship.

Most studies use online questionnaires with potential selection bias that limits the possibility of subjects with no internet connection being involved in the study. As a result, we must not generalize the findings without caution.[44,45] However, several authors have described the methods used to reduce bias in using this online questionnaire, including telephone contact for willingness to fill out a questionnaire and the use of previously validated questionnaires. However, given that in the context of a pandemic and physical contact restrictions, online questionnaires are the best option to collect data without the risk of contracting the disease.

The strength of this study lies in the size of the combined sample and the comprehensive discussion of anxiety during pregnancy exclusively, which, to the best of our knowledge, is the first systematic review to address anxiety and specific risk factors during pregnancy with a meta-analysis. In addition, the exclusion of low-quality articles also minimizes the possibility of bias towards the study results.

CONCLUSIONS

Our findings from this research can emphasize that pregnant women's services at Public Health Centers, Hospitals, Clinics, Private Practice Midwives, and other service places must consider the anxiety factor. This research can also serve as a guideline to identify pregnant women at risk of experiencing anxiety, which is essential during this pandemic, given the high level of anxiety and the magnitude of the impact. Screening for anxiety is recommended when the mother has an antenatal visit as it was shown a good result in a previous study.46 This study's limited source of articles with only a cross-sectional design indicates the need for a better design, such as a cohort or case-control, to better assess anxiety and conclude causality. Future research designs should also pay attention to and minimize bias when forced to use online questionnaires.

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