Editorial

Obstetrics and Gynecology Intensive Care

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The intensive care unit (ICU) or critical care unit (CCU) is a specialized unit in a healthcare facility dedicated to the provision of care for patients who are ill from critical conditions from which there is potential for recovery. The goal of the ICU is to prevent morbidity and mortality among patients who are at high risk through the provision of critical care. Patients admitted to the ICU are offered more detailed observation, monitoring, and treatment as compared to the care available to patients admitted to the standard lying-in wards or departments.[1,2]

In Indonesia, there were 305 maternal deaths per 100,000 live births in 2015. In 2020, this figure dropped to 189 per 100,000 live births. Indonesia has a substantially higher Maternal Mortality Ratio (MMR) compared to other countries in Southeast Asia.[3] World Health Organization (WHO) declared in 2015 that the maternal mortality rate must continue to decrease or be lowered to 105 per 100,000 live births to meet the Sustainable Development Goals (SDGs).[4]

Obstetric and gynecologic intensive care demands a nuanced understanding of both the physiological complexities of pregnancy and the intricacies of gynecologic conditions. Obstetric medicine is different from the general medicine because of the various physiological changes occurring in pregnancy, and only an experienced obstetrician who has good knowledge of obstetric medicine can interpret and understand complex conditions in pregnancy. The percentage of obstetric and gynecologic population requiring admission to the ICU is different in different countries based on the socioeconomic status, criteria for ICU admission, availability of ICU beds, and availability of a high dependency unit. It ranges from 0.08 to 0.76 % of deliveries in developed countries and 0.13 to 4.6 % in developing countries. The mortality in these patients is high and ranges from 0 to 4.9 % of ICU admissions in developed and 2–43.63% in developing countries. Hypertensive disorders and obstetric hemorrhage are the two the commonest risk factors for ICU admission. The other risk factors are sepsis, cardiac disease, and severe anemia.[5,6]

Indication for Intensive Care Unit (ICU) admission may be elective, such as a planned admission for maternal congenital heart disease, or emergency, such as an admission for postpartum hemorrhage or acute respiratory failure. Women who become acutely unwell during pregnancy, labour and the postnatal period should have immediate access to critical care, of the same standard as other sick patients, irrespective of location. Admission to an ICU has recently been identified as: a marker of severe maternal morbidity by the American College of Obstetrics and Gynecology (ACOG). ICU admission remains rare for obstetric subjects in high-income countries, accounting for less than 1% of ICU admissions.[7,8]

A multidisciplinary team approach including obstetrician and intensivist is appropriate in obstetric and gynecologic critical care settings. Setting up of obstetric and gynecologic intermediate care units can lessen the burden. In addition to good antenatal care, timely referral, health education, training of health professionals, and investment in critical care infrastructure (e.g. oxygen supply chains, mechanical ventilation, blood banking) may improve clinical outcome and better obstetric practice, especially in developing country. In conclusion, "Obstetrics and Gynecology Intensive Care" represents a concerted effort to illuminate the complexities of intensive care in women's health.[9,10]

REFERENCES

1. Jayaratnam, S., Jacob-Rodgers, S., & De Costa, C. Characteristics and preventability of obstetric intensive care unit admissions in Far North Queensland. Au New Zealand J Obstet Gynaecol. 2020; 60(6): 871-6.
2. Kroelinger, C. D., Brantley, M. D., Fuller, T. R., Okoroh, E. M., Monsour, M. J., Cox, S., & Barfield, W. D. (2021). Geographic access to critical care obstetrics for women of reproductive age by race and ethnicity. Am J Obstet Gynecol (AJOG).2021; 224(3): 304-e1.
3. Indonesian Ministry of Health Research and Development Agency. Basic Health Research 2018. Jakarta. Indonesian Ministry of Health. 2018.
4. Government Agency Performance Accountability Report. Directorate of Nutrition and Maternal and Child Health for fiscal year 2022. Jakarta. Ministry of Health. 2022.
5. Rathod, A. T., & Malini, K. V. (2016). Study of obstetric admissions to the intensive care unit of a tertiary care hospital. J Obstet Gynecol Ind. 2016;66:12-7.
6. Yi, H. Y., Jeong, S. Y., Kim, S. H., Kim, Y., Choi, S. J., Oh, S. Y., & Kim, J. H. Indications and characteristics of obstetric patients admitted to the intensive care unit: a 22-year review in a tertiary care center. Obstet Gynecol Sci. 2018; 61(2): 209.
7. Gupta, H., Gandotra, N., & Mahajan, R. Profile of obstetric patients in intensive care unit: a retrospective study from a tertiary care center in North India. Indian journal of critical care medicine: peer-reviewed, official publication of Ind Soc Critical Care Med. 2021; 25(4): 388.
8. Prin, M., Kadyaudzu, C., Aagaard, K., & Charles, A. (2019). Obstetric admissions and outcomes in an intensive care unit in Malawi. Int J Obstet Anesth.2019; 39: 99-104.
9. Mahmood, M. A., Hendarto, H., Laksana, M. A. C., Damayanti, H. E., Suhargono, M. H., Pranadyan, R.,& Mufidah, I. Health system and quality of care factors contributing to maternal deaths in East Java, Indonesia. PLoS One. 2021; 16(2): e0247911.
10. Chavesdac, Cecatti JG, Carroli G, Lumbiganon P, Hogue CJ, Mori R, et al. Obstetric transition in the World Health Organization Multicountry Survey on Maternal and Newborn Health: exploring pathways for maternal mortality reduction. Rev Panam Salud Publica. 2015;37(4-5):203–10.

Research Article

Human Immunodeficiency Virus in Pregnancy a Retrospective Study on Maternal and Perinatal Outcomes

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Abstract

Objective: To assess the maternal and perinatal outcome in pregnant women with HIV infection and the role of Antiretroviral therapy in reducing complications of pregnancy.

Methods: A retrospective analysis was conducted on data from HIV-positive mothers receiving antenatal care at a tertiary care center between February 2015 and January 2020. The study examined various adverse pregnancy outcomes in relation to antiretroviral treatment. Statistical analysis employed chi-square and Fisher's exact tests to determine differences in distribution proportions of patients on ART versus those not on ART across various antenatal and neonatal complications, with significance attributed to p-values <0.05.

Results: A total of 155 patients were found to be HIV positive. Out of this 58 were diagnosed before pregnancy and 97 during pregnancy. Miscarriage was seen in one (0.6%) patient on ART and two (1.2%) not on treatment (p-value 0.6). Sixteen (10.3%) patients underwent medical termination of pregnancy (MTP); all were HIV-positive and they were all on ART (p-value <0.001). Anemia was seen in eighteen (11.6%) patients out of which 14(9%) were on ART (p-value 0.01). One (0.64%) woman had thrombocytopenia and she was on ART (p-value 1). Two (1.2%) patients on ART had diabetes mellitus (p-value 0.4). One (0.64%) patient who was on ART developed polyhydramnios (p-value 1). A total of 8 (5.16%) women had hypertensive disorders; out of which 4(2.58%) were on ART (p-value 1). 11(7.09%) patients who were on ART and 6(3.8%) not on ART had preterm labor (p-value 0.2). 12(7.74%) patients who were not on ART had intrauterine growth restriction (IUGR) and 2(1.29%) on ART had IUGR. A total of 6(3.87%) patients had Intrauterine fetal demise (IUFD), of which 3(1.93%) were on ART and 3(1.93%) were not (p-value 1). Pre-labour rupture of membranes (PROM) was observed in 2(1.29%) women on ART and 11(7.09%) patients not on ART (p-value 0.004). All women (100%) had CD4 counts more than 500. All (100%) babies delivered at our center received antiretroviral therapy with oral Nevirepine. Almost half the women (51.6%) had vaginal delivery. Almost one-fourth, 41(26.4%) had a cesarean section. All caesareans were done given obstetric indications. There were no instrumental deliveries. Our study had a total of 122 live births. All 122(100%) babies were exclusively breastfed. None of the babies delivered in our center developed HIV on follow up which was done at 6 weeks and 6 months. Nine (5.8%) patients had infections. None of these women were on ART(p-value < 0.001).

Conclusion: HIV infection during pregnancy is associated with various adverse outcomes, but ART plays a crucial role in mitigating these risks and preventing mother-to-child HIV transmission. Initiating ART in all HIV-positive mothers and their infants is essential regardless of HIV status.

Keywords: anemia, ART, HIV, MTP, Perinatal outcome, PROM.

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INTRODUCTION

Human immunodeficiency virus (HIV) in pregnancy is a topic of significant importance, affecting both the present and future generations. Globally, an estimated 1.3 million women living with HIV become pregnant each year[1]. The prevalence of HIV infection in pregnant women in India has ranged from 0.7% to 1.2% [2]. In India, the prevalence of HIV infection in pregnant women has ranged from 0.7% to 1.2%. In Karnataka, the estimated adult HIV prevalence declined from 0.81% in 2006 to 0.47% in 2017, with a corresponding decrease in prevalence among antenatal clinic attendees from 1.12% to 0.38% during the same period[3]. Often, the diagnosis of HIV is first made during pregnancy. Currently, there is no cure or vaccine for HIV. Women

with HIV who become pregnant or acquire the infection during pregnancy are at risk of both maternal and fetal complications, particularly if the virus is poorly controlled. There is also a risk of vertical transmission to the fetus during pregnancy, labor, and postpartum[4]. Without intervention, this risk ranges from 15% to 45%.1

Several studies have shown that untreated maternal HIV infection increases the risk of stillbirth, premature rupture of membranes and preterm delivery, low birth weight, and small-for-gestational-age infants[5]. These complications not only lead to poor obstetric outcomes but also increase the risk of mother-to-child transmission of HIV. Addressing this issue is crucial but challenging, requiring substantial efforts from policymakers, healthcare workers, and patients alike.

HIV remains a significant public health concern globally, affecting both developing and developed countries. HIV in pregnancy is particularly complex as it directly impacts the health of future generations. However, antiretroviral therapy has shown promise in reducing obstetric complications and the transmission of HIV from mother to child. Our study aims to assess maternal and perinatal outcomes in pregnant women with HIV infection and evaluate the role of antiretroviral therapy in reducing pregnancy complications.

METHODS

This was a retrospective study for which ethical committee clearance was obtained. Data concerning HIV-positive mothers receiving antenatal care at a tertiary care center in Mangaluru, India, from February 2015 to January 2020 (spanning 5 years) were collected from the medical records of both mothers and babies. The center was selected as the study setting due to its status as a referral center for high-risk pregnancies. Data collected included the status of HIV infection, whether diagnosed pre-conceptionally or antenatally, the status of antiretroviral therapy (ART), occurrences of medical termination of pregnancy (MTP), maternal and fetal complications during the antenatal period, mode of delivery, neonatal conditions at birth such as APGAR scores, the need for NICU admission, the status of HIV infection in babies at 6 weeks and 6 months, ART administration to babies, and breastfeeding status up to 6 months. All necessary measures were taken to maintain patient confidentiality. The collected data were entered into MS Excel and analyzed using SPSS V 22.0. Categorical variables, such as the presence of various antenatal complications like miscarriages, MTP, and polyhydramnios, along with current ART status, were expressed as frequency and percentage. The chi-square test of association and Fisher's exact test were employed to determine differences in the proportion of distribution between those currently on ART and those not across various antenatal and neonatal complications, with statistical significance attributed to a p-value <0.05.

RESULTS

A total of 155 patients were found to be HIV positive during the study duration and all of them were included in the study. Out of this 58 were diagnosed before pregnancy and 97 during pregnancy. Among them, 78(50.3%) were on antiretroviral therapy (ART) and 77(49%) were not on ART. Of these 78, 58(37%) were on ART before pregnancy and in 20 (12.9%) initiation of ART was done after pregnancy.

Miscarriage was seen in one (0.6%) patient on ART and two (1.2%) not on treatment (p-value 0.6). Sixteen (10.3%) patients underwent medical termination of pregnancy (MTP); all were given HIV-positive status and they were all on ART (p-value <0.001). Anemia was seen in eighteen (11.6%) patients out of which 14(9%) were on ART (p-value 0.01). One (0.64%) woman had thrombocytopenia and she was on ART (p-value 1). Two (1.2%) patients on ART had diabetes mellitus (p-value 0.4). One (0.64%) patient who was on ART developed polyhydramnios (p-value 1). A total of 8 (5.16%) women had hypertensive disorders; out of which 4(2.58%) were on ART (p-value 1). About 11(7.09%) patients who were on ART and 6(3.8%) not on ART had preterm labor (p-value 0.2). Almost 12(7.74%) patients who were not on ART had intrauterine growth restriction (IUGR) and 2(1.29%) on ART had IUGR. A total of 6(3.87%) patients had Intrauterine fetal demise (IUFD), of which 3(1.93%) were on ART and 3(1.93%) were not(p-value 1). Pre-labour rupture of membranes (PROM) was observed in 2(1.29%) women on ART and 11(7.09%) patients not on ART(p-value 0.004). All women (100%) had CD4 counts more than 500.

Twenty-four (15.48%) babies were low birth weight babies of these 10(66.6%) were on ART. Of these 24 low birth weight babies, 14(9.03%) had

IUGR. It was found that, [6] (3.87 %) babies had low APGAR at 1 minute, of which 4(2.58%) mothers were not on ART(p value 0.4). One (0.64%) baby was admitted to NICU given low APGAR and later died on the 5th post-natal day. There were no maternal deaths reported during the study period. All (100%) babies delivered at our center received antiretroviral therapy either with oral Nevirepine. None of the babies delivered in our center developed HIV on follow up which was done at 6 weeks and 6 months. Almost half the women (51.6%) had vaginal delivery. Almost 41(26.4%) had a cesarean section. All caesareans were done given obstetric indications. There were no instrumental deliveries. Our study had a total of 122 live births. All the 122(100%) babies were breastfed. As per the medical records, all were exclusively breastfed without any reports of mixed feeding.

Nine (5.8%) patients had infections. 4 women had tuberculosis, One had chicken pox and Vaginal infection and malaria were seen in three and one patient respectively. None of these women were on ART (p-value < 0.001).

Table 1. Antenatal Complications in Pregnancies with HIV (N=155)

Complications	On ART (n=78) (%)	Not on ART (n=77) (%)	Total (%)	P value(by Chi-square / Fisher exact test)
Miscarriage	1(0.6)	2(1.2)	3(1.8)	0.6
MTP*	16(10.3)	0	16(10.3)	<0.001
Anemia*	14(9)	4(2.5)	18(11.6)	0.01
Thrombocytopenia	1(0.64)	0	1(0.64)	1
Gestational diabetes	2(1.2)	0	2(1.2)	0.4
Polyhydramnios	1(0.64)	0	1(0.64)	1
Hypertension	4(2.58)	4(2.58)	8(5.16)	1
Preterm labour	11(7.09)	6(0.38)	17(10.8)	0.2
Pre-labour rupture of membranes*	2(1.29)	12(7.7)	14(9)	0.004
Intrauterine fetal demise	3(1.9)	3(1.9)	6(3.8)	1
Various maternal Infections*	0	9(5.8)	9(5.8)	<0.001
CD4 count less than 500	0	0	0	

MTP: Medical termination of pregnancy, *statistically significant. Figures in parenthesis indicate the percentage of N

Table 2. Neonatal Parameters (N=155)

Complications	On ART (n=78)(%)	Not on ART (n=77)(%)	Total (%)	P value(by Chi-square / Fischer exact test)
Low APGAR	2(1.2)	4(2.58)	6(3.8)	0.4
NICU admission	0	1(0.6)	1(0.6)	0.4
Neonatal death	0	1(0.6)	1(0.6)	0.4
Babies developing HIV	0	0	0	

Figures in parenthesis indicate the percentage of N

DISCUSSION

HIV continues to be a significant health burden in many countries, impacting both developed and developing nations alike. Our study was retrospective in nature, aiming to analyze maternal and perinatal outcomes in pregnancies among mothers with HIV. Of the patients included in our study, 98 (63.3%) were first diagnosed during pregnancy. While an opt-out method is typically employed when offering diagnosis during the antenatal period, it is important to recognize that actively providing counseling sessions at the time of diagnosis can encourage patients to undergo testing. This proactive approach can significantly reduce the number of undiagnosed and missed cases. This finding aligns with previous studies on the subject..[6] Undiagnosed cases pose a huge threat to public health including neonatal health as it may lead to HIV propagation [7].

In this study, approximately half (50.3%) of the HIV-positive women were already on ART. These women maintained good general health and quality of life. Conversely, an equal number of women commenced ART only after pregnancy diagnosis. ART plays a crucial role in preventing mother-to-child transmission of HIV, although this was not statistically significant in our findings. Nevertheless, our results are supported by recommendations from the Panel on Treatment

of HIV During Pregnancy and Prevention of Perinatal Transmission[8]. In contrast to the old recommendation, all pregnant women are now advised to start ART as soon as pregnancy is diagnosed, and should be done irrespective of CD4 or viral RNA levels.

HIV exerts a significant psychosocial impact on women, as evidenced by the observation that 16 women (10.3%) opted for termination of pregnancy due to HIV, all of whom were on ART. This finding was statistically significant (p-value <0.001). Similar findings and suggestions were reported in a previous study.[9] Personalized psychological counseling sessions could potentially reduce these numbers by assisting women in overcoming their fears of transmitting HIV to their babies.

HIV is not a common etiological factor for miscarriage. It has been observed in various studies such as those, that miscarriages are more common than the general population in women with HIV[10]. Our study made similar observations. However, infection in general is an established cause for miscarriage. So HIV as an infection could cause miscarriage. In our study three women had miscarriages. Among these, two women were not on ART and the remaining woman was on ART. Although these findings are not statistically significant, this hints about the protective role of ART in preventing miscarriages and stresses the significance of starting ART at the earliest.

In our study 17(10.9%) women had preterm labour, mostly late preterm labour. Among these 11(7%) patients were not on ART and 6(3%) on ART. HIV is known to cause preterm labor. Most of these are iatrogenic preterm labor. About 13(8.3%) women had Pre labor rupture of membranes (PROM). Almost 11 (7%) of these women were not on ART whereas 2 (1.2%) women developed PROM despite being on ART. Our study had more preterm labor and pre-labour rupture of membranes in women who did not receive ART. This finding was statistically significant (p-value 0.01). Similar observations were made in their study on pregnancy complications in HIV-positive women[11]. These findings in our study go a long way in emphasising the importance of ART in women with HIV.

Twenty-four babies (15.4%) were born with low birth weight. Among these, 14 (9%) had mothers who did not receive ART, while 10 (6.4%) had mothers who were on ART and still delivered low birth weight babies. However, these findings did not reach statistical significance. Intrauterine growth restriction was identified as the cause of low birth weight in all babies whose mothers did not receive ART. These findings suggest a potential protective effect of ART against low birth weight and intrauterine growth restriction. Similar conclusions were drawn in another study on HIV in pregnancy. Conducting further research could provide more precise data on this matter [12].

Low APGAR at 1 minute was seen in about 6 babies, out of which 4 mothers were not on ART. Among this one baby was admitted to NICU in view of low APGAR and later died on 5th postnatal day. As this finding did not show statistical significance, it may be difficult to draw a conclusion that ART would help preventing babies with low APGAR. However it is highly probable that ART is efficacious in preventing babies with low APGAR and further studies would be required to establish this. These findings were similar to a study conducted by Yang et al about impact of maternal HIV infection on pregnancy outcomes in China [13].

A total of 6 patients had intrauterine fetal demise. Out of which 3(1%) were on ART and 3(1%) were not on ART. Although, this finding is not statistically significant, it is possible that ART doesn't have an impact on reducing intrauterine fetal demise. A study made similar observations [14]. It is also important to keep in mind that intrauterine fetal demise is multifactorial. This confounding effect by other factors might skew the data and make it difficult to draw a conclusion. Further research in this area is warranted.

In our study, eight (5%) women had hypertensive disorders. Out of this, 4(2.5%) were on ART and remaining 4 (2.5%) were not on ART. Women with HIV in pregnancy were found to have slightly higher incidence of hypertensive disorders of pregnancy[15]. Similar to the aforementioned discussion, our data indicates that ART does not appear to impact the occurrence of hypertensive disorders in pregnancy. Although this finding did not reach statistical significance (p-value 1), it is important to note that most ART medications have adverse effects on liver function, which could potentially lead to hypertension and elevated liver enzymes. Consequently, there may be an increased likelihood of misdiagnosing severe forms of hypertension in pregnancy such as HELLP syndrome and preeclampsia. This is in contradiction to a study which concluded long term ART could be protective in hypertensive disorders in pregnancy[16]. Unfortunately, there is a lack of data on the type of hypertension and

severity because of the retrospective nature of study. Further studies on this are strongly recommended.

Anemia was seen in eighteen (11.6%) patients. Out of this, 14(9%) were on ART. This observation is statistically significant(p value 0.01). Thrombocytopenia developed in One (0.6%) woman. She was on ART. Both anemia and thrombocytopenia could be due to the myelosuppressive effect of ART. This was in accordance with a study by Dennis El Jacobson where he suggested that anemia could be a complication of ART[17]. Hemoglobin levels of pregnant women on ART should be maintained well above normalcy during pregnancy. Stringent monitoring of hemoglobin and cell counts are advised.

Diabetes was diagnosed in two (1.2%) women on ART. A patient who had diabetes and was on ART developed polyhydramnios. These findings were not statistically significant. Nonetheless a study came up with similar findings[18]. This could be attributed to the fact that ART drugs lead to deranged glucose metabolism. Another aspect to consider is the occurance of gestational diabetes due to the hyperglycemic effect of human placental lactogen (HPL). However, management of diabetes irrespective of its etiology remains the same. At the same time we must give due consideration to multidisciplinary management with special stress on ART dose modification.

Almost half the women (51.6%) had vaginal delivery. Caesarean section was done in 41(26.4%). All caesareans were done in view of obstetric indications. None of the caesareans were performed to prevent mother to baby transmission. Similar observations were made in a study at Finland [19]. However it is advisable to make a conscious effort to avoid emergency caesarean sections as it may accentuate mother to baby transmission as well as put the staffs at risk of blood splashes and needle stick injuries. This shows that route of delivery was mainly determined by obstetric condition of the mother and fetus. There were no instrumental deliveries. This is in accordance with the latest guidelines stating to avoid instrumental deliveries to prevent accelerated mother to baby spread of HIV infection [1].

All babies delivered in our study were exclusively breastfed. The practice of discontinuing breastfeeding to prevent mother-to-baby transmission is considered outdated. Breastfeeding should be encouraged, particularly when both babies and mothers are receiving ART coverage. However, mixed feeding, involving episodes of formula feeding alongside breastfeeding, should be strongly discouraged. An article strongly advocates for counseling HIV-positive mothers to breastfeed their babies, provided they are on AR. [20].

All babies delivered in our center received antiretroviral therapy with oral Nevirepine. None of the babies delivered in our center developed HIV on follow-up at 6 weeks and 6 months. This explains the importance of administering ART to all babies without fail. An article also reiterates the same [20].

Various infections were reported in 9 (5.8%) mothers, including tuberculosis, chickenpox, malaria, and vaginal infections. An intriguing observation is that none of these women were on ART. This finding was statistically significant (p-value 0.001), clearly indicating that ART improves immunity in pregnant women with HIV and helps prevent maternal infections. Opportunistic infections have been shown to increase the risk of mother-to-child transmission, as found in previous studies.[21]. However in our study, none of the babies were found to be HIV positive at 6 weeks and 6 months follow up; prime reason for this being the administration of ART. Studies such as those emphasise on the role of ART in preventing maternal infections and mother-to-child transmission of HIV; and this is in agreement with our findings[22,23]. This strategy is further emphasized in other studies and editorials advocating for universal screening for HIV and the use of ART to prevent vertical transmission[25].

STRENGTH of the STUDY

The study spanned a period of five years, affording a substantial follow-up duration. The meticulous maintenance of medical records facilitated comprehensive data collection concerning HIV-positive mothers and their babies.

LIMITATIONS of the STUDY

It was a retrospective study.

CONCLUSION

HIV during pregnancy can lead to various adverse pregnancy outcomes. However, antiretroviral therapy (ART) is highly effective in mitigating

these complications and reducing the risk of mother-to-child transmission of HIV. ART should be initiated in all mothers with HIV and their babies irrespective of their HIV status.

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REFERENCES

1. WHO global HIV program, mother-to-child transmission of HIV, World health Organization .2023
2. Shah I, Lala M, Damania K. Prevalence of HIV infection in pregnant women in Mumbai, India. Experience from 1993-2004 and 2008. J Fam Med Prim Care. 2017;6(2):240-2.
3. Aridoss Santhakumar, Balasubramanian Ganesh, Malathi Mathiyazhakan, et al. Confined vulnerability of HIV infection among pregnant women attending antenatal care clinics in Karnataka, India: Analysis of data from the HIV sentinel surveillance 2017. Clin Epidemiol Global Health.2020;8(4):1127-33
4. Chilaka VN, Konje JC. HIV in pregnancy - An update. Eur J Obstet Gynecol Reprod Biol. 2021 Jan;256:484-491. doi: 10.1016/j.ejogrb.2020.11.034. Epub 2020 Nov 12. PMID: 33246666; PMCID: PMC7659513.
5. Tukei, Vincent J. MMed et al. Adverse Pregnancy Outcomes Among HIV-positive Women in the Era of Universal Antiretroviral Therapy Remain Elevated Compared With HIV-negative Women. Pediatr Infectious Dis J. 2021; 40(9): 821-6.
6. Soh, Q.R., Oh, L.Y.J., Chow, E.P.F. et al. HIV Testing Uptake According to Opt-In, Opt-Out or Risk-Based Testing Approaches: a Systematic Review and Meta-Analysis. Curr HIV/AIDS Rep. 2022; 19: 375–83 . https://doi.org/10.1007/s11904-022-00614-0
7. Mammone, Alessia, Patrizio Pezzotti, Vincenza Regine, et al. How many people are living with undiagnosed HIV infection? An estimate for Italy, based on surveillance data. AIDS .2016 April 24. 30(7): 1131-6.
8. Panel on Treatment of HIV during Pregnancy and Prevention of Perinatal Transmission Recommendations for the Use of Antiretroviral Drugs During Pregnancy and Interventions to Reduce Perinatal HIV Transmission in the United States. Department of Health and Human Services. Guidelines. 2023: 10-12.
9. de Bruyn M. Safe abortion for HIV-positive women with unwanted pregnancy: a reproductive right. Reprod Health Matters. 2003 Nov;11(22):152-61. doi: 10.1016/s0968-8080(03)02297-3. PMID: 14708406.
10. Finocchario-Kessler S, Goggin K, Staggs V, Wanyenze RK, Beyeza-Kashesya J, Mindry D, Birungi J, Wagner GJ. High report of miscarriage among women living with HIV who want to conceive in Uganda. BMC Res Notes. 2018 Oct 22;11(1):753. doi: 10.1186/s13104-018-3857-9. PMID: 30348201; PMCID: PMC6198531
11. Reitter, A., Stücker, A., Linde, R., Königs, C., Knecht, G., Herrmann, E., Schlößer, R., Louwen, F. and Haberl, A. Pregnancy complications in HIV-positive women. HIV Med. 2014; 15: 525-56. https://doi.org/10.1111/hiv.12142
12. Twabi, H.S., Manda, S.O. & Small, D.S. Assessing the effects of maternal HIV infection on pregnancy outcomes using cross-sectional data in Malawi. BMC Public Health.2020; 20: 974 . https://doi.org/10.1186/s12889-020-09046-0
13. Yang, M., Wang, Y., Chen, Y., Zhou, Y., & Jiang, Q. Impact of maternal HIV infection on pregnancy outcomes in southwestern China – a hospital registry based study. Epidemiol Infect. 2019; 147: E124. doi:10.1017/S0950268818003345
14. Vincent J. Tukei , MMed, * Heather J. Hoffman, PhD,† Lauren Greenberg et Al. Adverse Pregnancy Outcomes Among HIV-positive Women in the Era of Universal Antiretroviral Therapy Remain Elevated Compared With HIV-negative Women, Pediatr Infect Dis J. 2021 Sep; 40(9): 821–6
15. Saums MK, King CC, Adams JC, Sheth AN, Badell ML, Young M, Yee LM, Chadwick EG, Jamieson DJ, Haddad LB. Combination Antiretroviral Therapy and Hypertensive Disorders of Pregnancy. Obstet Gynecol. 2019 Dec;134(6):1205-14. doi: 10.1097/AOG.0000000000003584. PMID: 31764730; PMCID: PMC7036166.
16. Slogrove AL, Davies MA, Phelanyane F, et al. Hypertensive disorders of pregnancy and HIV: analysis of a province-wide cohort during 2018 and 2019. AIDS. 2023;37(7):1047-55.
17. Denise L Jacobson, Daniela Neri, Audrey Gaskin, et al. Maternal anemia and preterm birth among women living with HIV in the United States. Am J Clin Nutr.2021;113(6) 1402–10. https://doi.org/10.1093/ajcn/nqaa441.
18. Phoswa WN. The Role of HIV Infection in the Pathophysiology of Gestational Diabetes Mellitus and Hypertensive Disorders of Pregnancy. Front Cardiovascul Med. 2021 May 12;8:613930. doi: 10.3389/fcvm.2021.613930. PMID: 34055923; PMCID: PMC8149620.
19. Aho I, Kaijomaa M, Kivelä P, Surcel H-M, Sutinen J, Heikinheimo O, et al. (2018) Most women living with HIV can deliver vaginally National data from Finland 1993–2013. PLoS ONE.2018; 13(3): e0194370. https://doi.org/10.1371/journal.pone.0194370
20. Njom Nlend AE. Mother-to-Child Transmission of HIV Through Breastfeeding Improving Awareness and Education: A Short Narrative Review. Int J Womens Health. 2022 May 13;14:697-703. doi: 10.2147/IJWH.S330715. PMID: 35601795; PMCID: PMC9114103.
21. Lazenby GB. Opportunistic infections in women with HIV AIDS. Clin Obstet Gynecol. 2012 Dec;55(4):927-37. doi: 10.1097/GRF.0b013e3182718e0d. PMID: 23090461.
22. Gupta A, Verma A, Kashyap M, Gautam P. ART in Prevention of Mother-to-Child Transmission of HIV. J Obstet Gynecol Ind. 2020;70(1):18-22. doi:10.1007/s13224-019-01263-x
23. Mugwaneza, P., Lyambabaje, A., Umubyeyi, A. et al. Impact of maternal ART on mother-to-child transmission (MTCT) of HIV at six weeks postpartum in Rwanda. BMC Public Health.2018; 18; 1248 . https://doi.org/10.1186/s12889-018-6154-6

24. Sitti Fausihar, Yudianto B Saroyo. Difference of Vertical Transmission in HIV-Infected Women with Complete and Incomplete PMTCT. Indones J Obstet Gynecol .2015 ;3(2): 69-75. DOI: https://doi.org/10.32771/inajog.v3i2.30.

25. Yudianto B Saroyo. Is it time for "Universal Screening" of HIV infection in pregnant women?. Indones J Obstet Gynecol .2017;5(1): 2. DOI: https://doi.org/10.32771/inajog.v5i1.455.

Research Article

Prevalence of Gestational Diabetes and its Related Risk Factors among Rural Pregnant Women

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Abstract

Objectives: To estimate the prevalence of gestational diabetes among rural pregnant women and to assess the related risk factors among gestational diabetes mothers in rural areas.

Methods: A community-based cross-sectional study was conducted among antenatal mothers between 24 to 28 weeks of gestation in rural areas of Kancheepuram district, Tamil Nadu, for a period of one year from January 2017 to December 2017. Data were collected using a semi-structured questionnaire through face-to-face interviews with antenatal mothers regarding their demographic profile, obstetric history, nutrition, and lifestyle. The level of stress was assessed using the Perceived Stress Scale. Oral glucose tolerance tests (OGTT) were performed after an overnight fast of at least 12 hours, with a 75 g glucose load administered, and venous samples were drawn after 2 hours. GDM was diagnosed using specific criteria.

Results: Out of 244 antenatal mothers, 36 (14.8%) were found to have gestational diabetes. The majority of gestational diabetes mothers were housewives (p=0.02). In the current study, most of the GDM mothers were from lower-middle-class families (p=0.04). GDM mothers with a family history of chronic diseases like diabetes, hypertension, and heart disease (p=0.009), as well as those with an increased number of pregnancies, had a higher risk of gestational diabetes (47.6%), which was statistically significant (p=0.001). Patients with hypertension and thyroid disorders were also at an increased risk of developing diabetes during pregnancy (p=0.04). Past history of surgery (p=0.03), low calorie intake, and nutritional deficiencies in their diet (p=0.02) were other identified risk factors.

Conclusion: This study suggests that the prevalence of gestational diabetes is high among rural antenatal mothers. Therefore, these risk factors should be identified and managed through a risk-based approach to minimize the complications of GDM in both the mother and fetus.

Keywords: diabetes, pregnancy, stress.

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INTRODUCTION

There is a significant burden of non-communicable diseases, particularly diabetes, which needs to be addressed by our public health facilities. Diabetes poses a major public health challenge globally, including in India. The prevalence of diabetes is increasing worldwide, and these figures include women with gestational diabetes mellitus[1].The prevalence of diabetes mellitus (DM) is increasing worldwide and more so in developing countries such as India[2]. Gestational Diabetes Mellitus (GDM) is defined as 'Carbohydrate intolerance with recognition or onset during pregnancy', irrespective of the treatment with diet or insulin[3].

In highly developed countries like the United States, approximately 135,000 cases of GDM are diagnosed annually, representing 3–8% of all pregnancies[4]. "Gestational Diabetes Mellitus (GDM)"has been identified as a potential risk factor for poor health outcomes in pregnant women, contributing to various complications during pregnancy and childbirth, which directly in.

Diabetes complicating pregnancy is associated with adverse maternal and perinatal outcomes, including an increased risk of pre-eclampsia

during the antepartum period, as well as a higher risk of macrosomia, hypoglycemia, jaundice, respiratory distress syndrome, polycythemia, and hypocalcemia in infants,[5,6].

Despite glucose levels returning to normal after delivery, mothers with a history of GDM are at a higher risk of developing type 2 diabetes, while children born to mothers with GDM are at increased risk of developing metabolic syndrome.[7] In India, one of the world's most populous countries, GDM rates are estimated to be between 10-14.3%, significantly higher than in the West. The prevalence of gestational diabetes among rural pregnant women is 9.9%, compared to 16.6% among urban pregnant women. This suggests a significant underdiagnosis of GDM cases, particularly in rural populations. [8,9] This study aims to investigate the prevalence and risk factors of gestational diabetes among rural pregnant women.

METHODS

A cross-sectional, community-based study was conducted in the rural field practice area of the Rural Health and Training Center, Poonjeri, Chettinad Hospital and Research Institute, over a period of one year from January 2017 to December 2017. All pregnant women with an estimated gestational age between 24th and 28th weeks during the study period were included, while women who were known diabetics, critically ill, or refused to participate were excluded. Based on a prevalence rate of 9.9% from previous studies3 and assuming a 95% confidence level with an allowable error of 5%, the sample size was calculated using the formula N= 4pq/E^2, where p represents prevalence, q is 1-p, and E is the allowable error of P. The sample size was calculated to be 244. Institutional Ethical Committee approval from Chettinad Hospital and Research Institute was obtained prior to commencing the study.

The total population of 12 villages, as recorded in the household family register maintained at RHTC, Poonjeri, was 39,545. Out of these 12 villages, 6 were selected using a simple random sampling method (lottery method). The population of pregnant mothers with a gestational age between 24 to 28 weeks in these 6 villages was 815, as obtained from the respective primary health centers, sub-centers, and ICDS of the villages. A total of 514 pregnant mothers were identified from this population. Systematic random sampling was then employed to select every 2nd antenatal mother until the required sample size of 244 was achieved.

After obtaining consent from the pregnant women, they were interviewed using a semi-structured questionnaire containing demographic profile, obstetric history, nutrition and lifestyle history, and the Perceived Stress Scale. The following day, each mother was given a 75-gram oral glucose load, and at a 2-hour interval, a 2 ml venous blood sample was collected. Plasma glucose was measured using the Glucose Peroxidase method in our institutional laboratory, which is certified by the National Accreditation Board for Laboratories. If the plasma glucose level was ≥140mg/dl, the participant was diagnosed with GDM according to the DIPSI guidelines. The collected data were checked for completeness before being entered into a Microsoft Excel spreadsheet. The entered data were analyzed using the Statistical Package for Social Sciences (SPSS IBM) 21.

RESULTS

The majority of the participants belonged to the age group of 26-30 years (39.6%), followed by those aged 21-25 years (31.8%). Most of the participants were not employed (56.3%), followed by unskilled (15.9%) and semi-skilled workers (12.2%). In terms of education, 40.4% had completed secondary education, with high school (25.7%) and graduate school (22.4%) being the next most common levels. Regarding socioeconomic status, 48.2% belonged to the lower middle class, 23.3% to the middle class, and 22.9% to the lower class, based on the modified BG Prasad classification 2018.

Table 1. Distribution of Antenatal Mothers According to GDM

GDM	Frequency	%
Yes	36	14.8
No	208	85.2

Frequency Data used for prevalence of Gestational Diabetes.

Table 1 shows prevalence of the Gestational diabetes, among 244 antenatal mothers 36 (14.8%) were diagnoses as GDM.

Table 2. Demographic Variable Associated with Gestational Diabetes Mellitus

Variable		GDM		*P-value*
Socioeconomic status		**Yes**	**No**	
	Upper	1	1	0.045
	Upper middle	3	8	
	Middle	13	44	
	Lower middle	16	102	
	Lower	3	53	
Occupation	Professional	0	4	0.029
	Semi professional	2	7	
	Clerk/shop owner/Farmer	2	0	
	Skilled	5	17	
	Semi skilled	4	26	
	Unskilled	3	36	
	Unemployed	20	118	
Education	illiterate	0	0	0.436
	Primary	2	6	
	Middle	2	15	
	High	13	50	
	Secondary	10	89	
	Graduate	9	46	
	Post graduate	0	2	
Age	Below 20	0	8	0.002
	21-25	3	75	
	26-30	18	79	
	31-35	11	40	
	Above 35	4	6	

The Frequency data was mentioned. Chi square test was applied for quantitative variables in which p value<0.05 was taken as significant.

Among the antenatal mothers in the study, the majority were primigravida (48.2%), followed by 42% who were gravid with their second child. The highest proportion of participants had no history of abortions (82.4%), while 15.9% had experienced one abortion, and only 1.2% had experienced two abortions. Regarding parity, 43.3% had a parity index of one, and only 0.8% had a parity index of two. Most participants did not have any living children (56.7%), while 42.9% had one living child. The majority of participants had blood pressure below 130/90 mmHg (84.5%), while 15.5% had blood pressure above 140/90 mmHg. In terms of weight, the majority of participants fell within the 51-60 kg range (56.7%), followed by 32.2% in the 41-50 kg range. Regarding BMI, the majority had a normal BMI (57.6%), while 39% were overweight before pregnancy. Family history showed that the majority had a history of diabetes mellitus (41.2%), followed by systemic hypertension (31.4%) ($p<0.05$). Only 10% had a history of complications in a previous pregnancy.

Majority of the participants had family history of diabetes mellitus (41.2%), followed by systemic hypertension (31.4%).It was seen that majority of the participants with GDM had diabetes. The association between family history and GDM was statistically significant ($p=0.009$).Majority of the participants that is 57.1% consumed 1800-1999 calories per day, followed by 2000- 2500. Statistically significant association between calories and GDM participants was seen with ($p= 0.02$). Among the participants 48.2% were primi, 42% were gravid status 2, 8.6% percentage of the mother third time got pregnant and remaining 0.8% participants got 4^{th} time got pregnant. Most of the participants with GDM had gravida status of 2. Association between gravida and GDM between participants was found to be statistically significant ($p<0.05$).Association between past surgery and GDM between participants was found to be statistically significant. ($p=0.03$)

Table 3. Obstetric History Associated with Gestational Diabetes Mellitus

Variable		GDM		*P-value*
Gravida		**Yes**	**No**	
	1	9	109	
	2	17	86	0.001
	3	10	11	
	4	0	2	
Abortions	0	25	17	
	1	10	29	0.087
	2	1	2	
Parity	0	11	125	
	1	25	81	0.019
	2	0	2	

Chi square test was applied for quantitative variables in which p value <0.05 was taken as significant.

Among the pregnant mothers, 10.2% had a history of hypertension, 13.9% had thyroid disorders, and 2.8% had PCOD ($p<0.05$). The majority of participants (96%) did not engage in any form of exercise. Most participants were involved in inactive work for approximately 3 hours every day. Among the study participants, 22% were engaged in inactive work for around 4 hours, while only 14% were inactive for less than 2 hours, such as watching TV, reading newspapers, or simply sitting. Regarding sleep duration, 78% of participants slept for 8-10 hours per day, followed by 17% who slept for 10-12 hours per day. The majority of participants (57%) consumed fewer calories than required for a pregnant mother ($p<0.05$). Upon evaluating stress scores using the PSS stress scale, 50.8% had moderate stress, while 49.2% had low stress.

DISCUSSION

The majority of the participants belonged to the age group of 26-30 years, followed by those aged 21-25 years, indicating an increased likelihood of gestational diabetes with age. Most participants were not employed (56%), with smaller percentages being unskilled (15.9%) and semi-skilled (12.2%), suggesting lower activity levels. In terms of education, 40% completed secondary education, while 25.7% attended high school and 22.4% graduated. Among the study participants, 48% belonged to the lower middle class, 23% to the middle class, and 22.9% to the lower class.

In our study, the majority of participants were primigravida (48%), followed by 42% gravid with their second child. The highest proportion of participants had no history of abortions (82%), while 15.9% had one abortion in the past, and only 1.2% had two abortions. Among the study participants, 43% had a parity of one, followed by just 0.8% with a parity index of two. The majority of participants did not have any living children (56.7%), while 42.9% had one living child. These findings were somewhat similar to those reported in previous studies.[10]

In this study, the majority of participants had blood pressure below 130/90 mmHg (84%), while 15.5% had blood pressure above 140/90 mmHg. Additionally, the majority had a normal BMI (57.6%), while 39% were overweight. Body mass index (BMI) ≥ 25 was significantly higher in cases than controls (37.9% vs. 14.3%).

Regarding chronic diseases, hypertension was found in 10.2% of participants, followed by 13% with thyroid disease, and approximately 2.5% with PCOD. Only 10% had a history of complications in a previous pregnancy, with the majority experiencing stillbirths (92.3%), followed by large babies (7.7%). In terms of physical activity, 96% of participants did not engage in any form of exercise. Most were involved in inactive work for approximately 3 hours every day, with 22% engaged in inactive work for around 4 hours, and only 14% inactive for less than 2 hours. Regarding sleep duration, 78% slept for 8-10 hours per day, followed by 17% who slept for 10-12 hours per day. In terms of calorie intake, 57% consumed 1800-1999 calories per day, followed by 2000-2500 calories. None of the participants had insomnia, and 98% did not have eating disorders. Among the study participants, 71% had fasting blood sugar levels less than 100mg/dL, while 25% had impaired fasting glucose. On evaluation of postprandial blood sugar levels, 85.2% had levels less than 140 mg/dL.

The mean age of these pregnant women was 23 +/- 4 years. There was a significant increase in the prevalence of GDM in relation to gravidity. Out of the 1251 women who underwent the 50 gm oral glucose challenge test, 670 (53.55%) had one-hour plasma glucose levels greater than or equal to 130 mg/dl. Among the 891 pregnant women who underwent the 75 gm OGTT, 168 (18.9%) were diagnosed with GDM, with both fasting plasma glucose levels greater than or equal to 126 mg/dl and/or 2-hour postprandial glucose levels greater than or equal to 140 mg/dl used as cut-off values. Considering only the 2-hour plasma glucose for analysis, 144 (16.2%) had values greater than or equal to 140 mg/dl.

Stillbirth and the number of premature babies were higher in women with GDM.[11]. Women with GDM had a history of PCOS more frequently than the control group, but regarding body mass index, a history of PCOS did not show a significant relationship with GDM.[12]

In this study, we utilized the Perceived Stress Scale to assess mental stress among pregnant women, particularly those in rural areas. There has been no previous research exploring the relationship between mental stress and gestational diabetes, thus prompting the use of the Perceived Stress Scale in this study. Among the 244 antenatal mothers surveyed, 120 (49.2%) reported low stress levels, while 124 (50.8%) reported moderate stress levels. Interestingly, the study also revealed that 51% of antenatal mothers were unaware of gestational diabetes. This highlights the importance of raising awareness about gestational diabetes among pregnant women.

Previous studies have primarily been conducted in tertiary care centers, which may not accurately reflect the prevalence rate of gestational diabetes. Tertiary care centers often report higher prevalence rates due to referred cases from other healthcare facilities such as subcenters, primary health centers, and secondary care centers. This study, however, was conducted as a community-based study to accurately estimate the prevalence rate of gestational diabetes.

CONCLUSION

This study reaffirms previous findings of a high prevalence rate of gestational diabetes in rural areas of Tamil Nadu, estimated at 14.8%. The identified risk factors include an increased number of pregnancies, being a skilled worker, belonging to the lower middle class, having a family history of diabetes, and chronic diseases such as hypertension, coronary artery disease, thyroid disorders, and polycystic ovary syndrome. Additionally, a history of previous surgery, both obstetric and non-obstetric, and low-calorie intake were identified as risk factors. To mitigate the risk of gestational diabetes, it is crucial to emphasize the importance of nutritional supplements, healthy food choices, balanced diets, and high protein intake before pregnancy, particularly during the prenatal period.

It is imperative to prioritize raising awareness about gestational diabetes among antenatal mothers, given that many are unaware of the condition. Despite the existence of a well-established healthcare system in rural areas, the nutritional status of pregnant women in these regions remains poor. Consequently, interventions aimed at enhancing nutritional intake and providing supplements through primary health centers (PHCs) and Integrated Child Development Services (ICDS) centers are essential. Additionally, promoting increased physical activity in conjunction with regular antenatal care can assist in regulating blood glucose levels and enhancing overall maternal well-being. These strategies should be given precedence to improve the health outcomes of pregnant women residing in rural areas.

LIMITATIONS

Certain risk factors, such as familial structure and religious affiliation, were not accounted for in this study. Furthermore, there was a lack of continuous monitoring during both the antenatal and postnatal periods to evaluate potential fetal complications.

REFERENCES

1. O'Sullivan JB. Gestational diabetes and its significance. In: Camerini – Davalos R, Cole HS (eds) Early Diabetes. New York. Academic Press. 1970:339-44.
2. Interrnational diabetes federation.IDF diabetes Atlas. 6th ed. Brussels,Belgium: Int Diabet Fed. 2013.
3. Seshiah V, Balaji V, Balaji MS, et al,. Prevalence of geatational diabetes mellitus in South India (Tamil Nadu) a community based study. J.Assoc Physi Ind. 2008;56;329-33.
4. American College of Obstetricians and Gynecologists: ACOG Practice Bulletin: Assessment of risk factors for preterm birth: clinical management guidelines for obstetrician-gynecologists. Obstet Gynecol. 2001; 98(31): 709–16.
5. Kjos SL, Buchanan TA. Gestational diabetes mellitus. N Engl J Med 1999;341:1749-56.
6. DeSisto CL, Kim SY, Sharma AJ. Peer reviewed: Prevalence estimates of gestational diabetes mellitus in the United States, pregnancy risk assessment monitoring system (prams) 2007–2010. Preventing chronic disease. 2014:11.
7. American Diabetes Association Gestational diabetes mellitus Diabetes care 2004;27(1) S88-90
8. Seshiah V, Balaji V, Balaji MS, Sanjeevi CB, Green A. Gestational diabetes mellitus in India. J Assoc Phys Ind. 2004;52:707–11.
9. Rajput M, Bairwa M, Rajput R. Prevalence of gestational diabetes mellitus in rural Haryana: A community-based study. Indian J Endocrinol Metab. 2014 May;18(3):350.
10. Bhatt AA, Dhore PB, Purandare VB, Sayyad MG, Mandal MK, Unnikrishnan AG. Gestational Diabetes Mellitus in rural population of Western India–Results of a community survey. Indian J Endocrinol Metab. 2015 Jul;19(4):507.

11. Ramachandran A, Snehalatha C, Clementina M, Sasikala R, Vijay V. Foetal outcome in gestational diabetes in south Indians. Diabet Res Clin Pract. 1998;41:185–9
12. Kashanian M, Fazy Z, Pirak A. Evaluation of the relationship between gestational diabetes and a history of polycystic ovarian syndrome. Diabet Res Clin Pract. 2008;80:289–92.

Research Article

Clinicopathological of Pre-Operative Thrombocytosis in Epithelial Ovarian Cancer

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Abstract

Objective: To investigate the clinicopathological of preoperative thrombocytosis in patients with epithelial ovarian cancer at dr. Soedarso Regional General Hospital Pontianak.

Methods: A cross-sectional retrospective study was conducted over three months from January 2022 to March 2022, and bivariate analysis was performed using the Chi-Square test.

Results: A total of 28 subjects met the inclusion criteria, with 19 subjects had thrombocytosis (67.9%) and 9 subjects did not experience thrombocytosis (32.1%). Meanwhile, the results of the Chi Square Test showed a relationship between thrombocytosis and histopathological type in the subjects (p=0.036).

Conclusion: Preoperative thrombocytosis is associated with the histopathological type of epithelial ovarian cancer at dr. Soedarso Regional General Hospital Pontianak.

Keywords: epithelial ovarian cancer, histopathology, stage, thrombocytosis

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INTRODUCTION

Ovarian cancer remains a leading cause of death among gynecological malignancies. According to GLOBOCAN 2020, the global incidence of ovarian cancer reached 313,959 cases, resulting in 207,252 deaths. For instance, in the US, the risk of developing ovarian cancer stands at 1 in 70 women.[1] Especially in Indonesia, ovarian cancer ranks third in terms of cancer prevalence among women. This number is projected to increase significantly, with ovarian cancer accounting for 64.5% of deaths from cancer.[2,3] This number is predicted to increase significantly to 64.5% of deaths from ovarian cancer.[1]

Epithelial ovarian cancer (EOC) constitutes approximately 90% of all ovarian cancer cases across different races and ethnicities.[4] The most common and lethal type of EOC is High Grade Serous Carcinoma (HGSC).[5] In this case, the heterogeneity and rapid progression of ovarian cancer resulted in delayed and complex diagnosis cause poorer prognosis. As the stage increases, the 5-year-survival rate also deteriorates to below 30%.[6]

One of the studies regarding the detection of ovarian cancer that has been widely carried out is an increase in the number of platelets or thrombocytosis. This phenomenon can be seen in a variety of solid tumors, where the amount of elevated platelet may precede the diagnosis of malignancy. Malignant cells invade the physiological process of thrombopoiesis that promote the growth and survival of tumor, up to its metastasis. Cancer-associated thrombocytosis is correlated with reduced progression-free survival (PFS).[7,8]

Several studies have shown an association between pre-treatment thrombocytosis and the prognosis of ovarian cancer showing that pre-treatment thrombocytosis is an independent risk factor for the prognosis of patients with EOC or advanced ovarian cancer. In addition, pre-treatment thrombocytosis is also associated with

poor overall survival (OS) and PFS. In particular, pre-treatment thrombocytosis that increases tas the FIGO stage progresses has a very poor prognosis in stage III-IV patients compared to those in stage I-IV. Furthermore, a cohort study found that thrombocytosis is experienced in patients over 40 years of age, and is detected to be closely associated with carcinosarcoma and *clear cell* malignancies.[7-9]

Our hospital serves as the sole referral facility for patients suspected of cancer, including ovarian cancer, in West Borneo. Annually, the incidence of ovarian cancer is on the rise, presenting increasingly complex cases. This study was initiated in response to the necessity for a rapid, accessible, and cost-effective diagnostic and treatment approach for ovarian cancer.

METHODS

This cross sectional with retrospective study was conducted at dr. Soedarso Regional General Hospital Pontianak. We reviewed medical records of patients with suspected ovarian cancer at the Gynecology Oncology Polyclinic of dr. Soedarso RSUD hospital from 2017 to 2021, considering the number of cases and data limitations on hospital. The following criteria for our study were patients who have had their complete blood count checked before surgery, diagnosed with ovarian cancer after surgery, histopathological examination of the mass, and undergoing treatment at dr. Soedarso Regional General Hospital Pontianak. We excluded patient who were not examined or no complete blood results, multiple malignancies or infections from our study. We collect the data included age, parity, stadium, histopathology, CA-125, and platelet count. Meanwhile, this study was conducted for 3 months from January 2022 to March 2022. Of the 70 patients suspected of having ovarian cancer, 28 patients met the inclusion criteria set out in this study.

Thrombocytosis was an increase in the number of platelets exceeding 400,000 u/dL before treatment. The stages were classified according to the International Federation of Gynecology and Obstetrics System (FIGO) 2018. We divided the stages based on the severity, namely early stages (I and II) and advanced stages (III and IV). The division of histopathological type is based on WHO standard reference. After that, the Chi Square test was performed to find the relationship between thrombocytosis and the stage, as well as the type of histopathology. The test was carried out using SPSS *(Statistical Package for the Social Sciences)* 25. P values less than 0.05 were considered to be statistically significant. This study was approved by the ethical committee of dr. Soedarso Regional General Hospital Pontianak.

RESULTS

The baseline characteristics of the subjects are detailed in Table 1. We observed a predominance of subjects in advanced stages of FIGO (stages III and IV). High Grade Serous Carcinoma (HGSC) emerged as the most prevalent histopathological type, with 10 individuals (35.7%) diagnosed with this subtype. Moreover, the group of subjects exhibiting pre-operative thrombocytosis comprised 19 individuals (67.9%).

Table 1. Baseline Characteristics of Subjects

Variable	n (=28)	(%)
Age		
< 50	16	57.1
≥ 50	12	42.9
Parity		
0	5	17.9
1	16	57.1
>1	7	25.0
Stadium		
Early	7	25
Advanced	21	75
Histopathology		
High Grade Serous Carcinoma	10	35.7
Low Grade Serous Carcinoma	4	14,3
Mucinous	6	21.4
Clear cell	5	17.9
Endometrioid	3	10.7
CA-125 (U/mL)		
< 250	8	28.6
≥ 250	20	71.4
PLT (x103 u/dL)*		
≤400	9	32.1
>400	19	67.9

*PLT, platelet

Bivariate analysis was conducted on the variables of the preoperative platelet group using the Chi-Square test (refer to Table 2). The age variable showed a p-value of 1.000, while the parity variable resulted in a p-value of 0.258. Similarly, the stage variable showed a p-value of 0.646, and the CA-125 variable showed a p-value of 0.214. Notably, the histopathological variable demonstrated a significant association

with pre-operative thrombocytosis, with a p-value of 0.036 ($p<0.05$). The clinical implications of these findings are substantial, as higher histopathological findings correlate with increased pre-operative thrombocytosis. Moreover, pre-treatment thrombocytosis is indicative of disease progression, leading to higher FIGO stage advancements and ultimately indicating a very poor prognosis for patients with epithelial ovarian cancer.

Table 2. Result of Fisher Exact Test

Variable	Group		*P-Value*	Odds Ratio (95% CI)
	PLT >400 x 10^3 u/dL (n=19)	PLT ≤400 x 10^3 u/dL (n=9)		
Age				
< 50	11	5	1.000	0.909 (0.184-4.500)
≥ 50	8	4		
Parity				
0	5	0	0.258	
1	10	6		
>1	4	3		
Stages				
Early	3	4	0.646	1.875 (0.319-11.021)
Advanced	6	15		
Histopathology				
High Grade Serous Carcinoma	10	0	0.036	
Low Grade Serous Carcinoma	2	2		
Mucinous	3	3		
CC	3	2		
Endometrioid	1	2		
CA-125 (U/mL)				
< 250	7	1	0.214	0.214 (0.022-2.091)
≥ 250	12	8		

DISCUSSION

This study showed that subjects with pre-operative thrombocytosis were patients with advanced stages and types of HGSC. Bivariate analysis indicated that while the stages did not reach statistical significance ($p>0.05$), the histopathological test did ($p<0.05$). In addition, the results of this study did not show that preoperative thrombocytosis was correlated with other variables such as age, parity and CA-125 ($p>0.05$). Previous studies found same result.[10] Two studies showed that HGSC is the most common type, and its association with preoperative thrombocytosis exacerbates the disease and influences treatment outcomes.[8,11] However, a review of the literature studied from Ye et al stated that very few studies on the histopathological type of epithelial ovarian cancer with preoperative thrombocytosis were carried out, especially in the lethal type of HGSC.[7]

The results of this study indicate that the age group most affected by ovarian cancer is <50 years old. A study showed that age group is a predisposing factor in the growth of ovarian cancer. It is argued that age is associated with an increased number of ovulatory cycles, which are influenced by factors such as early menarche and late menopause. This is because the more the number of ovulation cycles, the more the process of cell mitosis that causes the inflammatory process due to the ovulation cycle. Therefore, it predisposes to the development of neoplastic cells.[12] Epithelial ovarian cancer (EOC) is considered a postmenopausal disease. Accordingly, previous studies have examined mean ages ranging from 50 to 79 years. However, age is not an independent prognostic factor.[13] The results of the research at Sanglah Hospital Denpasar, Dr. Cipto Mangunkusumo Hospital, and dr. Sardjito Hospital found an increase in the incidence of ovarian cancer as one gets older, with a peak incidence at the age of 41-50 years. However, the incidence decreases again at thereafter age. This is because the ethnic of Asian descent has a tendency to suffer from ovarian cancer at a younger age.[14,15]

The results in this study showed that parity presents the largest percentage of ovarian cancer cases. A study at RSUD H. Abdul Moeloek Bandar

Lampung showed the same results as this study.[16] Likewise, cohort studies in Europe and Sweden suggest that parity is not associated with survival in epithelial ovarian cancer (EOC).[17,18]

Advanced stages such as stages III and IV dominate in this study compared to the early stages, which is stage I. Some research at Sanglah Hospital Denpasar, H Adam Malik Hospital Medan, dr. Sardjito Hospital and Wahidin Sudirohusodo Hospital showed the same results, where stage III was the most common.[14,15,19,20] Correspondingly, conducted a study to examine the clinicopathological patterns and outcomes in patients with epithelial ovarian cancer (EOC) over a 35-year period (1985-2015) and showed the same results that stage III continues to be the most prevalent stage and shows a tendency to increase, although other stages also exhibit an upward trend.[5] Overall survival in patients with ovarian cancer tends to be low because 70% of patients are diagnosed first with an advanced stage, thus creating chemotherapy resistance.[21] In addition, high mortality is associated with epithelial and advanced ovarian cancer.[7]

More than half of the cases in this study had histopathological High Grade Serous Cancer (HGSC). In line with this study findings, a study Chang et al showed that the serous type was the most common around 43.3% and diagnosed at a more advanced stage about 82.8% of the total cases of the serous type.[22] A cohort study conducted from 1985 to 2015 showed that HGSC still ranks first as the most common histopathology, while mucinous decreased significantly over 35 years. Although, HGSC showed the lowest outcome at 5-year disease-specific survival (DSS), there was an increase in HGSC with an advanced stage resulting from a lack of awareness of the symptoms of ovarian cancer.[21]

CA-125 serum (cancer antigen 125) is one of the tumor markers used in initial screening of patients with a diagnosis of solid ovarian tumor which serves to monitor the outcome of chemotherapy in patients with epithelial ovarian cancer. Seventy-one percent (71%) of all study subjects had a CA-125 of 250 u/mL. CA-125 elevates in 50% of patients with stage I ovarian cancer and in 80-90% of patients with advanced ovarian cancer.[23] Hence, this finding is in line with this research.

Theoretically, preoperative thrombocytosis has a relationship with staging where the incidence of thrombocytosis increases as the FIGO stage progresses.[8] This theory may be related to the stage found in this study, where patients with pre-operative thrombocytosis were found to have stage III cases on average. Thus, the findings of this study confirmed the findings of studies stating that thrombocytosis was associated with metastases from ovarian cancer, and the incidence of thrombocytosis increases as the FIGO stage progresses.[7,8] Despite our study not demonstrating a direct relationship between stage and pre-operative thrombocytosis, the results of univariate analysis indicated a higher prevalence of advanced stages compared to early stages.

On the other hand, considering this research marks the first investigation conducted in West Kalimantan, it inevitably carries certain limitations. For instance, the small number of subjects or samples and the inadequate clinical data present limitations in this study. Therefore, future research focusing on the development of epithelial ovarian cancer should consider incorporating additional parameters that are easy to collect and obtain. Such studies could potentially be conducted even within primary health facilities, enabling early treatment initiation and thereby improving prognosis.

CONCLUSIONS

Pre-operative thrombocytosis have a relationship with histopathological type in epithelial ovarian cancer in Dr. Soedarso Regional General Hospital Pontianak.

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REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021: 71: 209- 49.
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Indonesia's Fact Sheet 2020. CA Cancer J Clin. 2021 May;71(3):209-49. doi: 10.3322/caac.21660. Epub 2021 Feb 4. PMID: 33538338.

3. Jogja Cancer Registry. Laporan Registrasi Kanker Berbasis Rumah Sakit periode Januari 2020. 2020. Diakses dari : https://canreg.fk.ugm.ac.id/laporan-data/registrasi-kanker-berbasis-rumah-sakit-dr-sardjito-fkkmk-ugm/januari-2020/
4. Berek JS, Kehoe ST, Kumar L, Friedlander M. Cancer of the ovary, fallopian tube, and peritoneum. Int J Gynecol Obstet. 2018 Oct;143:59-78.
5. Irodi A, Rye T, Herbert K, Churchman M, Bartos C, Mackean M, Nussey F, Herrington CS, Gourley C, Hollis RL. Patterns of clinicopathological features and outcome in epithelial ovarian cancer patients: 35 years of prospectively collected data. BJOG. 2020 Oct;127(11):1409-20. doi: 10.1111/1471-0528.16264. Epub 2020 May 7.
6. Torre LA, Trabert B, DeSantis CE, Miller KD, Samimi G, Runowicz CD, Gaudet MM, Jemal A, Siegel RL. Ovarian cancer statistics, 2018. CA Cancer J Clin. 2018 Jul;68(4):284-96.
7. Ye Q, Cheng J, Ye M, Liu D, Zhang Y. Association of pretreatment thrombocytosis with prognosis in ovarian cancer: a systematic review and meta-analysis. J Gynecol Oncol. 2019 Jan 1;30(1):e5. doi: 10.3802/jgo.2019.30.e5. Epub 2018 Sep 10.
8. Nakao S, Minaguchi T, Itagaki H, Hosokawa Y, Shikama A, Tasaka N, Akiyama A, Ochi H, Matsumoto K, Satoh T. Pretreatment thrombocytosis as an independent predictive factor for chemoresistance and poor survival in epithelial ovarian cancer. J Ovarian Res. 2020 Dec;13(1):1-9.
9. Abdulrahman GO, Das N, Lutchman Singh K. The predictive role of thrombocytosis in benign, borderline and malignant ovarian tumors. Platelets. 2020 Aug 17;31(6):795-800.
10. Andrijono, Heru Prasetyo, Gunardi ER, Purwoto G, Winarto H. The Role of Thrombocytosis as a Prognostic Factor for Epithelial Ovarian Cancer. Indones J Obstet Gynecol. 2021;9(3):153–6
11. Feng Z, Wen H, Bi R, Duan Y, Yang W, Wu X. Thrombocytosis and hyperfibrinogenemia are predictive factors of clinical outcomes in high-grade serous ovarian cancer patients. BMC cancer. 2016 Dec;16(1):1-7
12. Gaona-Luviano P, Medina-Gaona LA, Magaña-Pérez K. Epidemiology of ovarian cancer. Chin Clin Oncol. 2020 Aug;9(4):47.
13. Momenimovahed Z, Tiznobaik A, Taheri S, Salehiniya H. Ovarian cancer in the world: epidemiology and risk factors. Int J Women's Health. 2019;11:287.
14. Dhitayoni IA, Budiana IN. Profil pasien kanker ovarium di Rumah Sakit Umum Pusat Sanglah Denpasar-Bali Periode Juli 2013-Juni 2014. E-jurnal Med. 2017;6(3):1-9.
15. Jogja Cancer Registry. Laporan Registrasi Kanker Berbasis Rumah Sakit periode Maret 2021. 2021. Diakses dari : https://canreg.fk.ugm.ac.id/laporan-data/registrasi-kanker-berbasis-rumah-sakit-dr-sardjito-fkkmk-ugm/rkbr-maret-2021/
16. Simamora RP, Hanriko R, Sari RD. Hubungan Usia, Jumlah Paritas, dan Usia Menarche terhadap Derajat Histopatologi Kanker Ovarium di RSUD Dr. H. Abdul Moeloek Bandar Lampung Tahun 2015-2016. Jur Majority. 2018 Mar 5;7(2):7-13.
17. Beęvić J, Gunter MJ, Fortner RT, Tsilidis KK, Weiderpass E, Onland-Moret NC, et al. Reproductive factors and epithelial ovarian cancer survival in the EPIC cohort study. Br J Cancer 2015;113(11):1622-31.
18. Sköld, C, Koliadi, A, Enblad, G, Stålberg, K, Glimelius, I. Parity is associated with better prognosis in ovarian germ cell tumors, but not in other ovarian cancer subtypes. Int. J. Cancer. 2022; 150(5): 773-81.
19. Dwilestari, Ayu. Karakteristik Penderita Kanker Ovarium Di RS Wahidin Sudirohusodo Makasar Periode 1 Januari 2015–31 Desember 2016. 2017.
20. Syam DM. Analisis Faktor-Faktor yang Berhubungan dengan Kejadian Rekurensi Kanker Ovarium Epitel di RSUP H. Adam Malik Medan Tahun 2018. 2019.
21. Tao Y, Li H, Huang R, Mo D, Zeng T, Fang M, Li M. Clinicopathological and prognostic significance of cancer stem cell markers in ovarian cancer patients: Evidence from 52 studies. Cell Physiol Biochemist. 2018;46(4):1716-26.
22. Chang LC, Huang CF, Lai MS, Shen LJ, Wu FL, Cheng WF. Prognostic factors in epithelial ovarian cancer: a population-based study. PLoS One. 2018 Mar 26;13(3):e0194993.
23. Harsono AB. Kanker Ovarium:"The Silent Killer". Indones J Obstet Gynecol Sci. 2020 Mar 29;3(1):1-6.

Research Article

Higher HIF-1α Level in Cervical Cancer Worsen the Outcome of Radiotherapy in Stage IIIB Squamous Cell Carcinoma of the Cervix

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Abstract

Objective: To assess and evaluate HIF-1α levels as predictors of radiotherapy outcomes for patients with stage IIIB cervical cancer.

Methods: This retrospective cohort study was conducted in the Gynecology Oncology Division, Department of Obstetrics and Gynecology at FKUI, RSCM Jakarta. Biopsy data from 76 patients were analyzed to investigate HIF-1α levels using ELISA. Subsequently, these patients underwent complete radiotherapy, and outcomes were assessed using magnetic resonance imaging (MRI). Outcomes were categorized as positive response (disappearance of all lesions or a ≥30% decrease in the sum of the longest diameter compared to before radiotherapy) or negative response (lack of positive response criteria and a ≥20% increase in the smallest sum or new lesions). The one-year survival rate according to HIF-1α levels was also calculated. Data were analyzed accordingly.

Results: Among the 76 samples, 49 (61.8%) patients exhibited positive (complete and partial) responses, while 27 (38.2%) exhibited negative (progressive and stable disease) responses. The HIF-1α cut-off level ranged from 0.001 to 0.297 pg/mg, with the cut-off set at 0.019 pg/mg. We observed that higher HIF-1α levels worsened the outcomes of radiotherapy in patients with stage IIIB squamous cell carcinoma (SCC) cervical cancer (p = 0.044, RR= 1.909, 1.07- 3.75, 95% CI). A low HIF-1α level was associated with a better one-year survival rate (p=0.011).

Conclusion: Patients with stage IIIB squamous cell carcinoma cervical cancer and higher HIF-1α levels are at a 1.909-fold increased risk of experiencing negative radiotherapy responses compared to those with lower HIF-1α levels.

Keywords: cervical cancer, HIF-1α, radiotherapy response, stage IIIB SCC.

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INTRODUCTION

Cervical cancer is one of most prevalent cancers in women worldwide. During 2018, the number of cervical cancer cases worldwide has unalterable, namely 570.000 new cases and 311.000 death on 2018. The average age at which cervical cancer claimed a life was 59 worldwide; the range was 45 years (Vanuatu) to 76 years (Martinique). Out of 185 nations evaluated, 146 (79%) had cervical cancer in the top three malignancies affecting women under 45 years of age.[1] Due to the absence of symptoms in the early stages, cervical cancer is often diagnosed at an advanced stage. A study conducted at Dr. Cipto Mangunkusumo National General Hospital revealed that 41.6% of cervical cancer patients were diagnosed at stage III, with 71.6% of cases being of the squamous cell type.[2,3]

A common characteristic of cervical cancer, akin to other solid cancers, is oxygen deprivation (hypoxia) induced by aberrant tumor vasculature[4]. During the hypoxic state of the tumor, the tumor-specific immune response is modulated by the activation of Hypoxia-Inducible Factors (HIFs) and their downstream signaling pathways, including

CXCR4, M-CSFR, and CD47. Consequently, various immunosuppressive cytokines and growth factors are produced, promoting immune evasion and accelerating tumor progression.[5] Additionally, hypoxic tumor cells exhibit increased resistance to radiation. These cells initiate stress response mechanisms to adapt to low oxygen concentrations. Certain cells within the tumor respond adaptively to hypoxic stress by modifying their gene expression, leading to an aggressive phenotype and therapeutic resistance.[6] Cancer cells alter their metabolism in order to increase growth, survival, proliferation, and long-term survival. The unifying hallmark of this altered metabolism is enhanced glucose absorption and lactate fermentation. This process is observed even in the presence of fully functional mitochondria, and it is referred to as the Warburg Effect. Monocarboxylate transporter 4 exports lactate from cells as an end product of lactic acid fermentation following glycolysis. The lactate is subsequently taken up by cancer cells via the monocarboxylate transporter 1 (MCT1) and converted to pyruvate via the enzymatic activity of lactate dehydrogenase-B (LDH-B). Increased intracellular pyruvate levels limit the formation of alpha ketoglutarate, which stabilises and activates HIF-1, resulting in the activation of VEGF-dependent tumour angiogenesis and the acceleration of tumour growth.[7] Radioresistance caused by HIF-1-induced Warburg effect results in cancer cells that are difficult to treat and may result in tumour recurrence. HIF-1 activators represent a promising group of targets that could lead to the development of novel therapies.[7] As the consequences, the sensitivity of hypoxic tumor cells to radiation therapy will be decreased. In aggressive tumors, HIF-1α is generally more pronounced. It can be independent predictor of poor prognosis in certain types of cancer.[8]

The treatment method required is determined by the stage and extent of cervical cancer progression, which may comprise one or a combination of surgery, radiation, and chemotherapy.[9] However, failure rate of radiotherapy in cervical cancer patients is still unsettling, about 42% for stage III patients and 74% for stage IVA patients.[3] Prognostic factors of outcome for radiotherapy are related to patient age, stage of the disease, tumor size, histopathology of tumor, differentiation rate, and lymph node metastasis.[3] It is also affected by apoptosis factors and various biomarkers such as ki67, cell division cycle 6 (CDC6), maintenance protein 5 (MCM5), and c-myc.[3]

HIF-1 is the biomarker that need further study in order to determine their relation to radiotherapy outcome. It is one of the biomarkers regulated during hypoxia state of cells. Solid tumors must have experienced hypoxic state during its growth along with angiogenesis process.[10] As the consequences, it would also regulated HIF-1 during growth. HIF-1 consists of 2 subunits, HIF-1α and HIF-1ß. HIF-1α is a subunit which regulated by oxygen level and stimulates angiogenesis, erythropoiesis, and eventually apoptosis.[11] As a result, tumour therapies, such as radiotherapy, chemotherapy, and immunotherapy, can be less successful in a hypoxic tumour microenvironment (TME).[12]

Based on statements mentioned above, this study aims to assess and evaluate HIF-1α as a radiotherapy outcome predictor for stage IIIB cervical cancer patients.

METHODS

This cohort study included 76 patients with stage IIIB cervical cancer, all of whom had squamous cell carcinoma type and had not received any prior treatment before undergoing HIF-1α examination. Consecutive sampling was employed during patient selection. Exclusion criteria for the study encompassed patients with any other type of cancer besides cervical cancer and those who had undergone alternative forms of therapy such as surgery or chemotherapy. All patients received treatment at the Department of Radiotherapy, Faculty of Medicine, Universitas Indonesia. The study was conducted within the Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, at Dr. Cipto Mangunkusumo National General Hospital.

HIF-1α level as independent variable was assessed using lab study before radiotherapy was initiated. Biopsy of cancer tissue was performed. Level of HIF-1α was assessed using ELISA method in Biochemical Laboratory Department of Biochemistry Faculty of Medicine, Universitas Indonesia. Due to absence of cutoff for significant HIF-1α level category for cervical cancer patients, HIF-1α level was assessed quantitatively and categorized into two groups, namely high and low level of HIF-1α using ROC (Receiver Operating Characteristic Curve) into two study groups in consideration of highest sensitivity, specificity, positive likelihood ratio, and accuracy possible.

Meanwhile, radiotherapy outcome was

determined using magnetic resonance imaging (MRI) which was assessed by radiologists in Dr. Cipto Mangunkusumo National General Hospital. Complete and partial response was then categorized as positive response while stable or progressive response was categorized as negative response.

The study was approved by the Faculty of Medicine, Universitas Indonesia. All human studies had been approved by the Research Ethics Committee (ethical number: 0944/UN2.F1/ETIK/2018). All patients who were included in this study had given their informed consent prior to their inclusion in the study.

This study use 5% error bound and 95% confidence interval limit, power of the test considered to be 90%. Collected data were then analyzed using SPSS for Macintosh version 22 software. The data was analyzed using chi square test.

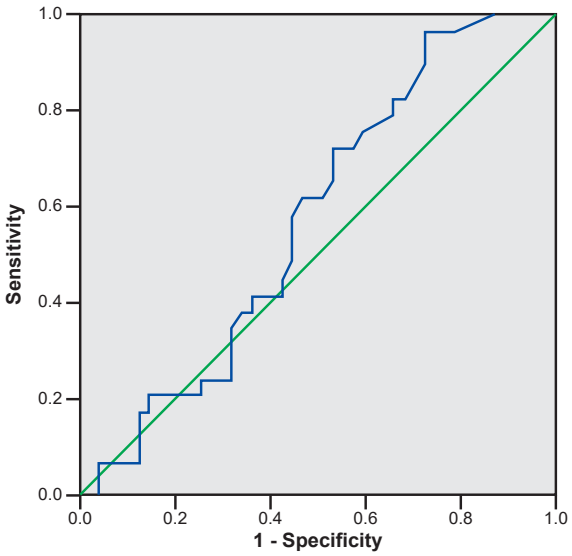
RESULTS

Seventy-six samples met the inclusion criteria for this study. Univariate tests were conducted to assess the characteristics of the study group subjects. The authors did not differentiate age since a normal distribution was observed, with a mean of 50.29 and a standard deviation of 8.6. Consequently, similarities were found in sociodemographic and clinicopathological characteristics among the study subjects, allowing for comparisons between groups. The characteristics among the groups in this study are presented in Table 1.

Table 1. Sociodemographic and Clinical Pathological Characteristics of Stage IIIB Cervical Cancer Patients

Subjects characteristics	n (%) (N = 76)
Age (years), mean (SD)	50.3 (8.6)
30–40	13 (17.1)
41–50	23 (30.3)
51–60	31 (40.8)
61–70	9 (11.8)
First sexual intercourse age (years)	
<20	33 (43.4)
≥20	43 (56.6)
Parity, mean (SD)	3 (0–8)
Diameter of tumor (cm), mean (SD)	5.35 (1.9–15.0)
Tumor differentiation	
Grade I	19 (25.0)
Grade II–III	57 (75.0)
Radiation therapy response	
Positive	49 (61.8)
Negative	27 (38.2)

In order to classify subjects into two study groups, HIF-1α level was categorized using ROC (Receiver Operating Characteristics) curve analysis based on HIF-1α positive response and negative response. Considering highest LR+ value of 1.36, sensitivity of 72.4%, specificity of 46.8%, and accuracy of 59.60%, cutoff point for HIF-1α was determined at 0.019 pg/mg. ROC curve of HIF-1α level can be seen in figure 1.



Afterwards, clinicopathologic and sociodemographic characteristics for each of the study groups were analyzed as shown in table 2.

Table 2. Clinicopathologic and Sociodemographic Characteristics Based on HIF-1α Level

Subjects characteristics	Positive response n (%)	Negative response n (%)	Total	*P-value*
Age (years), mean (SD)	50.45 (8.74)	50.03 (8.52)		0.059
First sexual intercourse age				0.449
<20	21 (63.6)	12 (36.4)	33 (43.4)	
≥20	26 (39.5)	26 (60.5)	43 (61.4)	
Diameter of tumor (cm)	5 (1.9–10.2)	6 (3.1–15.0)		0.337
Differentiation degree				0.054
Grade I	31 (66.6)	26 (89.7)	57 (75.0)	
Grade II–III	16 (34)	3 (10.3)	19 (25.0)	
Types of cancer				0.278
SCC	44 (63.8)	25 (36.2)	69 (90.8)	
Ceratinized SCC	3 (42.9)	4 (57.1)	7 (9.2)	
HIF-1α level (pg/mg)				0.044
≥0.019	23 (52.3)	21 (47.7)	44 (57.9)	
<0.019	24 (75)	8 (25)	32 (42.1)	

HIF-1α=hypoxia inducible factor-1 alpha; SD=standard deviation; SCC=squamous cell carcinoma

Chi square analysis was done to determine relationship between HIF-1α level and radiation therapy response. P value of the study was 0.044 which showed there was a significant relationship between HIF-1α level and radiation therapy response in stage IIIB SCC cervical cancer patients. Relative risk of this study was 1.909 (1.07 – 3.75, CI 95%).

DISCUSSION

In this study, a high level of HIF-1α was identified as one of the risk factors associated with a poorer outcome following radiotherapy, with a relative risk (RR) of 1.909 (95% CI = 1.07–3.75). This finding aligns with the pathophysiology of cervical cancer, as tumors with elevated HIF-1α levels face challenges in maintaining ATP levels during radiation therapy. Conversely, tumors in a hypoxic state tend to exhibit increased bioenergetic processes mediated by HIF-1α, leading to sustained tumor proliferation.[6,13]

The study concludes that a high level of HIF-1α is a risk factor for a poorer radiotherapy outcome, with a relative risk of 1.909 (95% CI: 1.07–3.75). This finding is consistent with the tumor's pathophysiology, as tumors with elevated HIF-1α levels struggle to maintain ATP levels during radiation therapy. Conversely, tumors in a hypoxic state tend to exhibit increased bioenergetic processes mediated by HIF-1α, which contributes to sustained tumor proliferation.[14]

In general, stage IIIB SCC cervical cancer patients in Dr. Cipto Mangunkusumo National General Hospital were about 50 years old, had born 3 children and had bad differentiation degree. This result is similar to a comparable study which shown that women aged more than 50 years is more prone to suffer from cervical cancer.[3] As mentioned before, we found that there are similarities in sociodemography and clinicopathology between subjects' characteristics.

Based on the bivariate analysis of each sample's characteristics, no significant relationship was found between patient characteristics and radiation therapy outcome in patients with stage IIIB squamous cell carcinoma (SCC) of the cervix. This result aligns with a previous study that demonstrated no significant association between patient age, overall survival, and relapse-free survival in cervical cancer patients.[10]

From this study, it can be concluded that HIF-1α promotes radioresistance in tumors. Three out of four functions of HIF-1α—apoptosis, metabolism, and proliferation—promote radiosensitization, while the fourth function, vascular protection, paradoxically promotes radioresistance for tumors. Despite having more functions that promote radiosensitization, it is concluded that the radioresistance function of HIF-1α is significantly more dominant. This is because an increase in HIF-1α levels leads to an upregulation of the transcription factor nrf2, which in turn regulates the production of enzymatic (endogenous) antioxidants such as superoxide dismutase (SOD), glutathione peroxidase, and catalase. The increase in enzymatic antioxidants can exacerbate radiation therapy responses.[15]

As the result, HIF-1α level influenced the

outcome of radiotherapy in cervical cancer patients. Along with increased HIF-1α level (cut-off point 0.019 pg/nm), the tumor became more resistant to radiotherapy. Based on this result, assessing HIF-1α level may give benefit to predict the success possibility of treatment before proceeding to radiotherapy. Limitation of this study was a single center of study. It is never attempted before to do multicenter research.

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CONFLICT OF INTEREST

There is no conflict of interest in this study.

REFERENCES

1. Arbyn M, Weiderpass E, Bruni L, de Sanjosé S, Saraiya M, Ferlay J, Bray F. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. Lancet Glob Health. 2020 Feb;8(2):e191-e203. doi: 10.1016/S2214-109X(19)30482-6. Epub 2019 Dec 4. Erratum in: Lancet Glob Health. 2022 Jan;10(1):e41. PMID: 31812369; PMCID: PMC7025157.
2. Nuranna L, Prastasari R, Sutrisna B. Survival of cervical cancer patients and its prognostic factors at Cipto Mangunkusumo Hospital. Jakarta. Med J Indones. 2014;23(3):163-8. https://mji.ui.ac.id/journal/index.php/mji/article/view/739
3. Kusuma F. Kadar survivin, telomerase, dan sitokrom C sebagai prediktor respon terapi pada pasien karsinoma sel skuamosa serviks stadium IIIB. Universitas Indonesia. Universitas Indonesia. 2016.
4. Emami Nejad, A., Najafgholian, S., Rostami, A. *et al.* The role of hypoxia in the tumor microenvironment and development of cancer stem cell: a novel approach to developing treatment. Cancer Cell Int. 2021; **21(1):** 62. https://doi.org/10.1186/s12935-020-01719-5.
5. Wu, Q., You, L., Nepovimova, E. et al. Hypoxia-inducible factors: master regulators of hypoxic tumor immune escape. J Hematol Oncol. 2022; 15(1): 77. https://doi.org/10.1186/s13045-022-01292-6.
6. Moreno Roig E, Groot AJ, Yaromina A, Hendrickx TC, Barbeau LMO, Giuranno L, Dams G, Ient J, Olivo Pimentel V, van Gisbergen MW, Dubois LJ, Vooijs MA. HIF-1α and HIF-2α Differently Regulate the Radiation Sensitivity of NSCLC Cells. Cells. 2019 Jan 12;8(1):45. doi: 10.3390/cells8010045. PMID: 30642030; PMCID: PMC6356534.
7. Nagao A, Kobayashi M, Koyasu S, Chow CCT, Harada H. HIF-1-Dependent Reprogramming of Glucose Metabolic Pathway of Cancer Cells and Its Therapeutic Significance. Int J Mol Sci. 2019 Jan 9;20(2):238. doi: 10.3390/ijms20020238. PMID: 30634433; PMCID: PMC6359724.
8. Weidemann A, Johnson RS. Biology of HIF-1alpha. Cell Death Differ. 2008;15(4):621-7.
9. Burmeister CA, Khan SF, Schäfer G, Mbatani N, Adams T, Moodley J, Prince S. Cervical cancer therapies: Current challenges and future perspectives. Tumour Virus Res. 2022 Jun;13:200238. doi: 10.1016/j.tvr.2022.200238. Epub 2022 Apr 20. PMID: 35460940; PMCID: PMC9062473.
10. Moeller BJ, Dewhirst MW. HIF-1 and tumour radiosensitivity. Br J Cancer. 2006;95(1):1-5.
11. Otrock ZK, Hatoum HA, Awada AH, Ishak RS, Shamseddine AI. Hypoxia-inducible factor in cancer angiogenesis: structure, regulation and clinical perspectives. Crit Rev Oncol Hematol. 2009;70(2):93-102.
12. Zhuang Y, Liu K, He Q, Gu X, Jiang C, Wu J. Hypoxia signaling in cancer: Implications for therapeutic interventions. MedComm (2020). 2023 Jan 23;4(1):e203. doi: 10.1002/mco2.203. PMID: 36703877; PMCID: PMC9870816.
13. Zhong ea. Overexpression of Hypoxia-inducible Factor 1 in Common Human Cancers and Their Metastases. Cancer Research. 1999;59:5830-5.
14. Lee JS, Wang RX, Alexeev EE, Lanis JM, Battista KD, Glover LE, et al. Hypoxanthine is a checkpoint stress metabolite in colonic epithelial energy modulation and barrier function. J Biol Chemist. 2018;293(16):6039-51.
15. Toth RK, Warfel NA. Strange Bedfellows: Nuclear Factor, Erythroid 2-Like 2 (Nrf2) and Hypoxia-Inducible Factor 1 (HIF-1) in Tumor Hypoxia. Antioxidants (Basel). 2017;6(2).

Research Article

Eliminating HPV DNA Positive Result with Large Loop Excision of the Transformation Zone (LLETZ)/Loop Electrosurgical Excision Procedure (LEEP) in Precancerous Cervical Lesions

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Abstract

Objective: To determine the use of LLETZ/LEEP to eliminate HPV DNA positive result in patients with cervical precancerous lesions at General Hospital dr. Mohammad. Hoesin, Palembang.

Methods: A case series with cervical precancerous lesions was undertaken at the Oncology Polyclinic of dr.Mohammad Hoesin Hospital Palembang from January to October 2022. There were 24 samples with positive HPV DNA before LLETZ/LEEP. Samples then checked for HPV DNA after LLETZ/LEEP. The effectiveness of LLETZ/LEEP therapy was analyzed using the Mc Nemar test. Comparison of HPV DNA outcomes (positive or negative) based on procedure, HPV DNA type and histopathological type was analyzed using Fisher Exact and Pearson Chi Square tests. All data were analyzed using SPPS version 22.0.

Results: In this study, it was found that the average age of patients with cervical precancerous lesions was 40.25 ± 7.67 years (28 - 57 years). Based on the diagnosis, 8 samples were found with High-grade Squamous Intraepithelial Lesion (HGSIL) and 16 samples with Low-grade Squamous Intraepithelial Lesion (LGSIL). All samples in this study were housewives and the majority were multiparas (75.0%). History of abortion in the patients in this study was only found in 5 samples (20.8%). The results showed that there were significant differences in the HPV DNA before and after LLETZ/LEEP therapy (p = 0.000). In addition, the results showed that there was no difference in the outcome of HPV DNA based on the procedure (p = 1.000) and the type of HPV DNA (p = 0.643). After LLETZ/LEEP therapy was carried out, it was found that only 1 subject has positive HPV DNA result and the HPV DNA virus found was type 52 and (high risk) and 42 (low risk).

Conclusion: It can be concluded that LLETZ/LEEP therapy is effective in eliminating HPV DNA positive results in cervical precancerous lesions

Keywords: cervical cancer, HPV DNA, LLETZ/LEEP, precancerous lesions, RCT

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INTRODUCTION

Cervical cancer is the fourth common cancer in women worldwide with 341.831 death in 2020. This mortality is mainly related to the delay in diagnosis, where most cervical cancer stages (70% of cases) are diagnosed at invasive, advanced and even terminal stages.[1-4]

Pathogenesis of cervical carcinoma is well known and begins with human papilloma virus infection and progresses to precancerous lesions. Over 50-80% sexually active women was infected by HPV during their life with 20% progressed to cervical cancer. Cervical precancerous lesions will develop into invasive carcinomas over a long period of time, between 10-20 years. Therefore, prevention of this disease is important and can be done by carrying out currently available vaccinations and early detection. Cytology examination with high false negative results

has been screening standard for cervical cancer over 50 years. HPV DNA examination has the advantage of very high negative predictive value is projected to be the new standard.[5,6]

Early detection of cervical precancerous lesions leads to various types of procedures. In developing countries there are three treatment options for cervical precancerous lesions, namely cryotherapy, large loop excision of the transformation zone (LLETZ or LEEP), and cold knife conization (CKC). The recommendations for treatment are for those with confirmed High Grade Squamous Intraepithelial Lesion (HGSIL) or adenocarcinoma in situ (AIS) with cryotherapy, loop electrosurgical excision procedure (LEEP)/ large loop excision of the transformation zone (LLETZ), or cold knife conization (CKC).[5]

The success rate of hot knife conization such as LLETZ/LEEP in cervical precancerous lesions therapy using LLETZ/LEEP is effective if the HPV DNA in patients with precancerous lesions decreases significantly.

This study aims to determine the effectiveness of LLETZ/LEEP in cervical precancerous lesions therapy by examining HPV DNA at dr. Mohammad Hoesin Hospital, Palembang.

METHODS

A case series study to determine the use of LLETZ/LEEP to eliminate HPV DNA positive result in patients with cervical precancerous lesions was undertaken at Dr. Mohammad Hosein Hospital Palembang from January to November 2022. Obtained 30 patient with positive pap smear for cervical precancerous lesions, 6 subjects were excluded due to negative HPV DNA results. The independent variable in this study was LLETZ/ LEEP while the dependent variable was HPV DNA.

The collection of HPV DNA test specimens was carried out by taking cervical mucus with a disposable broom-type cervical brush into the cervical canal before and after LLETZ/LEEP procedure. The direct examination was carried out in Prodia Palembang laboratory. Examination of the HPV genotype using the QIAamp Mini DNA Kit (Qiagen) DN. LLETZ or LEEP were chosen by oncologist based on clinical appearance of the cervix during the procedure.

Data processing used SPSS 22.0. Descriptive analysis was carried out to assess general characteristics and HPV DNA data. The effectiveness of LLETZ/LEEP therapy was analyzed using the Mc Nemar test. Comparison of HPV DNA outcomes (positive or negative) based on procedure, HPV DNA type and histopathological type was analyzed using Fisher Exact and Pearson Chi Square tests.

RESULTS

In this study, it was found that the average age of patients with cervical precancerous lesions was 40.25 ± 7.67 years (28 - 57 years). All samples in this study were housewives and the majority were multiparas (75.0%). History of abortion in the patients in this study was only found in 5 samples (20.8%) (Table 1).

Table 1. General Characteristics of Research Subjects

Variable	Frequency	(%)
Age (years old)		
Mean ± SD	40.25 ± 7.67	
Median (Min- Max)	40 (28 – 57)	
Age at Marriage		
Mean ± SD	23.63 ± 3.93	
Median (Min- Max	23.5 (17 – 30)	
Age, n (%)		
≥ 35	19	79.2
< 35	5	20.8
Occupation, n (%)		
House wife	24	100
Parity, n (%)		
Nulliparous	1	4,2
Primiparas	5	20.8
Multipara	18	75.0
Abortion History, n (%)		
Yes	5	20.8
No	19	79.2
Diagnosis, n (%)		
HGSIL	8	33.3
LGSIL	16	66.7
Anatomical Pathology Results, n (%)		
HGSIL	9	37.5
LGSIL	15	62.5
Procedure, n (%)		
LEEP	12	50.0
LLETZ	12	50.0

Explanation
HGSIL : High-Grade Squamous Intraepithe-Lial Lesion
LGSIL : Low-Grade Squamous Intraepithe-Lial Lesion
LEEP : Loop Electrosurgical Excision Procedure
LLETZ : Large Loop Excision of The Transformation Zone (LLETZ)

Based on the diagnosis, 8 samples were found with High-grade Squamous Intraepithelial Lesion (HGSIL) and 16 samples with Low-grade Squamous Intraepithelial Lesion (LGSIL). Meanwhile, based on the results of anatomical pathology, there were 9 samples with HGSIL histopathological type and 15 samples with LGSIL

histopathological type. There was 1 sample that was initially diagnosed as LGSIL but based on the PA results it was HGSIL (Table 1).

Table 2. Characteristics of HPV DNA

Characteristics	Frequency	%
HPV DNA before, n (%)		0
Positive	24	100.0
HPV DNA Type, n (%)		
High Risk	13	54.2
Low Risk	10	41.7
Other Type	1	4.2
HPV DNA After, n (%)		
Positive	1	4.2
Negative	23	95.8
Alteration, n (%)		
Yes	23	95.8
No	1	4.2

In this study, before LLETZ/LEEP therapy was performed, all 24 subjects (100%) with cervical precancerous lesions had positive HPV DNA, consisting of 13 subjects (54.2 %) with high risk HPV DNA types, 10 subjects (41.7.3%) with low risk HPV DNA types and 1 subject (4.2%) with other types of HPV (Table 2).

After LLETZ/LEEP therapy was carried out, it was found that only 1 subject has positive HPV DNA result. Using the McNemar test, the results showed that there were significant differences in the HPV DNA result before and after LLETZ/LEEP therapy (p = 0.000) (Table 3). Patients who still found positive HPV DNA virus after LLETZ/LEEP therapy had the histopathological type HGSIL, were treated with the LLETZ procedure and the HPV DNA virus found was type 52 and (high risk) and 42 (low risk). This patient was then undergoing hysterectomy.

Table 3. The Effectiveness of LLETZ/LEEP Therapy in Patients with Cervical Precancerous Lesions

Characteristics	before	after	*P-value*
HPV DNA			
Positive	24	1	0.000*
Negative	0	23	
Total	24	24	

DISCUSSION

Cervical precancerous lesions are preceded by HPV infection and are influenced by several factors that increase the risk of precancerous lesions including Early age at sexual debut, multiple sexual partners, history of genital warts and smoking.[7] These risk factors will play a role in the process of carcinogenesis, thereby changing normal cells into abnormal cells that lead to cervical cancer.[8] Most cases of precancerous cervical lesions are diagnosed in women between the ages of the third and fourth decades of life with the peak incidence of cervical cancer being in the age group 40-49 years.[9,10] Women aged 40-49 years have a 2.4 times higher chance of developing precancerous lesions compared to those aged 30-39 years.[5]

In this study, it was found that the average age of patients with cervical precancerous lesions was 40.25 ± 7.67 years and the majority were aged ≥ 35 years (79,2%). These results are in line who reported the majority of patients aged > 35 years.[10-12] Early marriage which lead to early sexual debut is also a risk factor for cervical precancerous lesions. Having sexual intercourse for the first-time during puberty, which is less than 17 years old, is a risk factor for cervical precancerous lesions because the transformation zone and metaplasia tend to mutate easily. In this study, it was found that the average age of patients with cervical precancerous lesions when married ranged between 15-30 years with an average of 23.63 years. These results similar to other studies that found the majority of the age at marriage in patients with precancerous cervical lesions was <30 years (86.3%), aged 16-20 years as many as 41.3%; aged 21-25 years as much as 23.6%; and aged 26-30 years as much as 23.4%.[6,8,9]

The majority of patients with cervical precancerous lesions in this study were multipara (73.47%). This result is in line with the study which reported that the majority of patients with cervical precancerous lesions were multiparas.[6,8] Several studies state that high parity have a significant effect. Women who often give birth (or give birth to many children) automatically experience injuries to their reproductive organs including the cervix, especially in women with short birth intervals, these injuries often include a higher risk of HPV infection.[13,14]

HPV is a member of the Papillomaviridae family which is divided into 2 subfamilies with more than 50 genera, however, only 5 genera (classification based on L1 sequence) are associated with infection in humans namely Alpha-, Beta-, Gamma-, Nu-, and Mu- papillomavirus. In this study, 24 samples (100%) of patients with cervical precancerous lesions had positive HPV DNA, with a high-risk type of 54.2% and a low risk of 41.7% and 4.2% other types of HPV. The most

high-risk HPV DNA types were 18 and 52, while the most low-risk HPV DNA types were 70. These results are in accordance with a study by Kabir et al in 2019 which reported that the five high-risk HPVs that were most often detected in either single or multiple HPV infections were HPV. 16, 18, 45, 51 and 52. At the end of the study after the procedure, it was found that only 1 sample had positive HPV DNA with multiple infections, namely high-risk DNA types 52 and low risk 42.[15]

In this study, both of these procedures had effectiveness in the treatment of cervical precancerous lesions which could be assessed from changes in the HPV DNA. A total of 24 samples that initially had positive HPV DNA after the procedure there was only 1 sample that still had positive HPV DNA.

Research conducted by Petrillo et al in 2020 on 182 (60.7%) women who were vaccinated with the HPV vaccine within 4 weeks after LEEP and 103 (34.3%) women who were not vaccinated. Recurrence of cervical dysplasia following the LEEP procedure occurred in 30 (10.5%) women, of whom 17 were unvaccinated and 13 were vaccinated. Administration of HPV vaccine after LEEP appears to reduce the risk of recurrence, suggesting that HPV vaccination may act as an adjunctive treatment after LEEP.[16]

CONCLUSION

From these results it can be concluded that LLETZ/ LEEP therapy is effective in eliminating HPV DNA positive results in cervical precancerous lesions. After LLETZ/LEEP therapy was carried out, it was found that only 1 sample was still found to have HPV DNA positive.

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REFERENCES

1. GLOBOCAN. The Global Cancer Observatory: All Cancer. 2020. https:// gco.iarc.fr/today/data/factsheets/ cancers/39-all-cancers-fact-sheet.pdf.
2. GLOBOCAN. The Global Cancer Observatory: Cervix Cancer. 2020. https://gco.iarc.fr/today/data/factsheets/ cancers/23-Cervix-uteri-fact-sheet.pdf
3. GLOBOCAN. The Global Cancer Observatory : Cancer Today. Estimated number of new cases in 2020, Indonesia, both sexes, all ages. 2020. https://gco.iarc.fr/today/online-analysis-map?v=2020&mode=population&mode_population.
4. Nurwany R, Sanif R , Novaliani A, Theodorus. The Efficacy of Chemotherapy in Advanced-Stage Cervical Cancer on Vitamin A Serum. Indones J Obstet Gynecol. 2022; 10(2);95–101.
5. Arbyn M, Anttila A, Jordan J, Ronco G, Schenk U, Segnan N, et al. European Guidelines for Quality Assurance in Cervical Cancer Screening. Summary of the supplements on HPV screening and vaccination. Eur J Cancer. 2015; 1: 22-31
6. Chandra R, Rauf S, Malinta U. Human Papilloma Virus Selftesting as an Alternative Method for Cervical Cancer Screening. Indones J Obstet Gynecol. 2017 Apr 5(2);114–19.
7. Wudtisan J, Tantipalakorn C, Charoenkwan K, Sreshthaputra RA, Srisomboon J. Factors Associated with Development of High-Grade Squamous Intraepithelial Lesions of the Uterine Cervix in Women Younger than 30 Years. Asian Pac J Cancer Prev. 2019 Apr 29;20(4):1031-6.
8. Basu P, Taghavi K, Hu SY, Mogri S, Joshi S. Management of cervical premalignant lesions. Curr Probl Cancer 2018;42(2):129–36. from:https://doi.org/10.1016/j. currproblcancer.2018.01.010
9. Teame H, Addissie A, Ayele W, Hirpa S, Gebremariam A, Gebreheat G, et al. Factors associated with cervical precancerous lesions among women screened for cervical cancer in Addis Ababa, Ethiopia: A case control study. PLoS One. 2018;13(1):1–13.
10. Budiana ING, Artha IBRK. Characteristics of Cervical Precancerous Lesions at a Tertiary Hospital in Bali. Indonesia. Indones J Cancer. 2020;14(4):117
11. Getinet M, Taye M, Ayinalem A, Gitie M. Precancerous Lesions of the Cervix and Associated Factors among Women of East Gojjam, Northwest Ethiopia, 2020. Cancer Manag Res. 2021;13(September):9401–10.
12. Wabo B, Nsagha DS, Nana TN, Assob CJN. Prevalence and risk factors associated with precancerous cervical lesions among women in two cities in Cameroon. Pan Afr Med J. 2022;41.
13. Kusuma LF, Yarso KY. Hormonal contraceptive use as risk factor for breast cancer in young Javanese women. J thee Med Sci (Berkala Ilmu Kedokteran). 2016;48(04 (Suplement)):19–19.
14. Ashar H, Kusrini I, Musoddaq A, Asturiningtyas IP. First sexual intercourse and high parity are the most influential factors of precancerous cervical lesion. Maj Obstet Ginekol. 2020;28(3):113
15. Kabir A, Bukar M, Nggada HA, Rann HB, Gidado A, Musa AB. Prevalence of human papillomavirus genotypes in cervical cancer in Maiduguri, Nigeria. Pan Afr Med J. 2019;33:1–11.
16. Petrillo M, Dessole M, Tinacci E, Saderi L, Muresu N, Capobianco G, et al. Efficacy of HPV vaccination in women receiving LEEP for cervical dysplasia: A single institution's experience. Vaccines. 2020;8(1):1 -8.

Research Article

CA-125 Examination as a Predictor the Resectability of Advanced Stage of Ovarian Cancer

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Abstract

Objective: To determine serum CA-125 examination as predictor in predicting the resectability of advanced ovarian cancer at dr. M. Djamil Padang

Methods: The research sample was obtained using a consecutive sampling technique, wherein samples were collected one by one within the specified timeframe until the required sample size was achieved.

Results: The mean CA-125 level among respondents was 589.66 U/mL with a standard deviation of 841.55 U/mL. The study also revealed that a CA-125 cutoff value of 337.5 U/mL demonstrated high sensitivity (92.31%) and specificity (90.90%) in predicting the resectability of ovarian cancer.

Conclusion: Serum CA-125 examination shows a promising result in predicting the resectability of advanced ovarian cancer at dr. M. Djamil Padang

Keywords: CA-125, consecutive sampling technique, ovarian cancer.

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INTRODUCTION

For women everywhere, ovarian cancer is the number one gynecological cancer killer. Ovarian cancer is the fifth leading cause of death among women globally.[1] Globally, 204,000 women are diagnosed with ovarian cancer, and it causes 125,000 deaths each year.[2] In the United States, although ovarian cancer ranks as the eighth most common cancer in women, the number of deaths is far greater than the total number of deaths from gynecological cancer in that country. Every year in the United States, it is estimated that as many as 21,650 new cases are found, and 15,520 women die from ovarian cancer. The 2018 Indonesian Society of Gynecologic Oncology stated that ovarian cancer ranks third in Indonesia after cervical cancer and corpus uterine cancer.[3]

The exact number of cases of ovarian cancer in Indonesia is still unknown, the incidence of ovarian cancer in Indonesia is predicted to be around 537 patients, with a patient mortality rate of 126 cases.[4] At RSUP Dr. M. Djamil, ovarian cancer constitutes the most common malignancy in the Obstetrics and Gynecology department. Ovarian cancer is often asymptomatic, with only a few patients exhibiting specific symptoms. Consequently, about 70% of cases are diagnosed at an advanced stage, with a survival rate below 30%. Conversely, early detection at stage I significantly increases the chances of survival to 90%.[5] However, patients typically present at stages II-IV, resulting in low treatment success rates. Given this context, the author aims to

investigate "the role of CA-125 as a predictor of the resectability of advanced-stage ovarian cancer at Dr. M. Djamil Padang."

METHODS

The is a cross-sectional study, specifically focusing on diagnostic tests. The results were presented in the form of sensitivity output and the area under the curve (AUC) output of CA-125 as a predictor of the resectability of ovarian cancer, utilizing CA-125 levels as a diagnostic test tool. Researchers utilized a consecutive sampling method, collecting samples one by one throughout the study period until an adequate sample size was achieved. Inclusion and exclusion criteria were applied to select the sample for this investigation.

RESULTS

The research aimed to assess the utility of CA-125 examination in predicting the resectability of advanced ovarian cancer at RSUP Dr. M. Djamil Padang. The study involved 35 patients and commenced in August 2021, continuing until the desired sample size was attained at the Obstetrics and Gynecology Department of Dr. M. Djamil Padang Central General Hospital. Among the 35 patients included in the study, the mean CA-125 level was found to be 589.66 U/ml, with a standard deviation of 841.55 U/ml. This result indicates a significantly elevated CA-125 level in advanced-stage ovarian cancer cases.

Table 1. Description of CA-125 Levels at Dr. M. Djamil Hospital Padang

Variable	Advanced ovarian cancer (n=35)
	Mean ± SD
CA-125 Levels	589.66 ± 841.55

The research also found that a CA-125 cutoff value of 337.5 U/mL was highly sensitive (92.31%) and specific (90.90%) for predicting whether or not ovarian cancer could be resected.

Table 2. Description of Mass Resectability of Advance Ovarian Cancer at Dr. M. Djamil Hospital Padang

Variable	Advanced ovarian cancer (n=35)
	f (%)
Resectable	13 (37.1)
Non Resectable	22 (62.9)

Interpretation: Most of advance ovarian cancer are non resectable mass about 22 people (62%).

The results of the analysis show several cut-off points for CA-125 levels. To explain the appropriate cut-off points for this study, researchers used the cut-off point graph in Figure 1 to determine sensitivity and specificity. The results showed that the cut point for CA-125 levels was ≤ 337.50 U/mL (resectable) and > 337.51 U/ml (not resectable) with a specificity of 90.9% and a specificity of 92.3%.

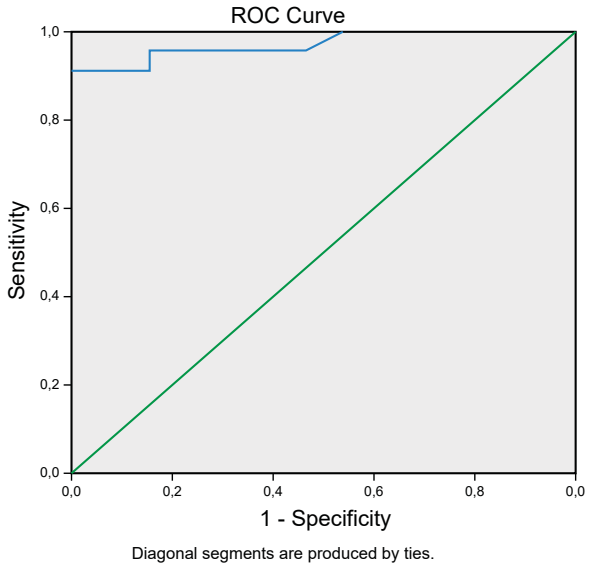

Figure 1. Receiver Opertaing Curve (ROC) for CA 125 and Resectability

Table 3. Sensitivity and Specificity Data of CA-125 *cut-off point* as Resectability Predictor of Advanced Ovarian Cancer

Variable		Resectability	
CA – 125	(≤ 337.5 U/mL)	**(Resectable)**	**(Unresectablele)**
	(> 337.5 U/mL)	12 (85.7)	2 (14.3)
		1 (4.8)	20 (95.2)
Total		13	22

From 35 patients, 12 patients had COP < 337.5 U/ml (resectable), 2 patients had COP < 337.5 U/ml (not resectable), 1 patient had COP > 337.5 U/ml (resectable), and 20 patients COP > 337.5 U/ml (not resectable). The results of the analysis showed that the sensitivity (92.31%), specificity (90.90%), positive predictive value (85.71%), negative predictive value (95.24%) were very good. All these values are considered good if ≥ 70%.

DISCUSSION

CA-125 Levels in Ovarian Cancer Patients

CA-125, a glycoprotein with a high molecular mass, is produced by both ovarian cancer cells

and normal cells in coelomic epithelial tissue. It serves as a crucial marker for monitoring tumor activity in patients with established ovarian cancer. CA-125 levels are routinely monitored to assess how well a patient is responding to therapy and to detect any signs of cancer recurrence after treatment. In approximately 90% of patients with epithelial ovarian cancer, changes in blood CA-125 levels are closely associated with disease remission and progression. Over the past two decades, CA-125 has been regarded as the gold standard tumor marker in medicine, playing a pivotal role in tracking treatment effectiveness and identifying potential cancer recurrence.[6]

Serum CA-125 levels before surgery may help rule out patients as candidates for primary cytoreduction and laparotomy. In order to identify patients who might benefit from decreased suboptimal cytorelation, they employed a preoperative blood CA-125 level of 500 U/ml or above as a cutoff criterion.[7] Based on the research results it is known that the mean CA-125 level of respondents is equal to 589.66 U/ml with a standard deviation of 841.55U/ml. Patients who had resectability of a non-resectable tumor mass (>1 cm), which was 62.9%, were higher than those who had resectable tumors (<1 cm), which was 37.1%. In women with histologically confirmed ovarian cancer, serum CA-125 levels exceed 35 U/ml in over 80% of cases. CA-125 testing can detect 85% of clinically severe ovarian cancer cases. However, it's important to note that elevated CA-125 levels can also be observed in various other malignancies besides ovarian cancer, including lung, bladder, gastric, hepatic, and pancreatic cancers, as well as leukemia, non-Hodgkin's lymphoma, and mediastinal teratomas. The CA-125 mucin is considered the most reliable marker for epithelial ovarian cancer, with a normal range typically falling between 0-35 kU/L. However, in premenopausal women, normal CA-125 levels may reach up to 100 kU/L or higher, particularly during menstruation. Over the past two decades, CA-125 has consistently demonstrated its efficacy as the primary tumor marker for clinical applications in medicine.[6]

Sensitivity, Specificity, Positive and Negative Predictive Value, and Cut-Off Point Rate CA-125 as a Resectability Predictor of Ovarian Cancer

Malignancy prediction in ovarian tumor patients necessitates a comprehensive assessment that incorporates not only the patient's medical history and physical examination but also the utilization of tumor markers, such as CA-125. Preoperatively, CA-125 is widely employed to evaluate the severity of epithelial ovarian cancers. In particular, serum CA-125 has shown the strongest association with a borderline diagnosis or malignancy among ovarian mucinous tumors. The diagnostic efficacy of serum CA-125 is thus maximized in these cases.[8]

Ovarian cancer is frequently diagnosed at an advanced stage because patients often do not experience symptoms until the disease has progressed significantly. Only about a quarter of ovarian cancer cases are detected early. In ovarian cancer management, CA-125 is utilized as a predictor of recurrence and assesses resectability. Approximately 80% of ovarian cancer patients will exhibit elevated CA-125 levels before any recurrence is evident on clinical examination or imaging studies.[9] The CA-125 test may be used to analyze, monitor, and assess the response to treatment in ovarian cancer, despite the fact that it is not specific for the diagnosis of ovarian cancer itself. A decrease in CA-125 levels over time indicates that the treatment is effective. Moreover, CA-125 expression is typically much higher in serous type epithelial ovarian cancer compared to other forms of epithelial ovarian cancer.[10]

Some epidemiologists have established a minimum positive predictive value of 10% for early detection tests of ovarian cancer, requiring a sensitivity of at least 75% and a specificity of better than 99.6%. In the study, the Area Under the Curve (AUC) value was found to be 97%, with a p-value of 0.0001. This indicates that CA-125 can serve as a reliable predictor in forecasting the resectability of advanced ovarian cancer, as assessed by the Receiver Operating Characteristic (ROC) curve. The ROC curve evaluates the performance of tumor markers by correlating the sensitivity and specificity of the diagnostic test. The AUC obtained from this study's findings surpassed the AUC value reported in a study, which utilized ultrasonography to predict hip carcinoma with an AUC value of 89%. The CA-125 predictor exhibited a 78% positive predictive value and a 73% negative predictive value.[11]

The research also identified a CA-125 cutoff value of 337.5 U/mL, which exhibited high sensitivity (92.31%) and specificity (90.90%) in predicting the resectability of ovarian cancer. These findings align with previous studies, which

demonstrated that preoperative IL-6 and CA-125 levels were associated with surgical outcomes (suboptimal and optimal cytoreduction), with a cutoff point of 418.5 U/mL, yielding a sensitivity value of 88.9% and a specificity value of 77.2%. Furthermore, another study revealed that a CA-125 cutoff value of 248.55 U/mL had a sensitivity of 73.2%, specificity of 73.6%, and accuracy of 73.3% (p=0.0001) in predicting cytoreduction for epithelial ovarian cancer. Additionally, a CA-125 cutoff of 100 U/mL was shown to have a sensitivity of 72% and a specificity of 73% for predicting inadequate cytoreduction surgery in stage III ovarian cancer patients.[12]

Determining a cutoff point for assessing a predictor involves establishing normal and abnormal limit values, also known as the threshold values for positive and negative test results. A test result is considered positive if it surpasses the threshold value, while it is deemed negative if it falls below this threshold. The findings of this investigation, with a sensitivity of 78% and a specificity of 73%, are consistent with previous reports suggesting that optimal cytoreduction success decreases beyond certain CA-125 levels.[11]

CA-125 expression in ovarian cancer masses is attributed to its lubrication and hydration properties, which create a protective and anti-adhesive barrier, facilitating mass development. In a study by Mani et al. (2007), high CA-125 levels were observed in 90% of ovarian cancer cases, with an initial rise detected in 34% of cases and a median CA-125 level of 1,733 U/ml. Given its excellent sensitivity and specificity, CA-125 testing continues to be advocated for in the diagnosis of ovarian cancer.[13]

CONCLUSIONS

The CA-125 examination yielded highly promising results as a predictor for forecasting the resectability of advanced ovarian cancer at RSUP Dr. M. Djamil Padang, particularly with a Cutoff Point (COP) 337.5 U/mL for the resectability of advanced ovarian cancer at RSUP dr. M. Djamil Padang.

REFERENCES

1. Daniel W. Cramer and Kathryn L. Terry, Epidemiology and Biostatistics. Berek & Hacker's gynecologic Oncology 2022.
2. Aleksandra GM, Ian Jacobs, Usha Menon, Ovarian Cancer-Tumor Markers and Screening. Berek & Hacker's gynecologic Oncology 2022
3. Patricia GL, Lourdes A M, Kassandra MP, Epidemiology of Ovarian Cancer. Chin Clin Oncol J. 2020;9(4):47.doi: 10.21037/cco-20-34. Epub 2020 Jun 30.
4. Li-yuan Feng, Sheng-bin Liao,Li li. Preoperative serum levels of HE4 and CA125 predict primary optimal cytoreduction in advanced epithelial ovarian cancer: a preliminary model study. J Ovarian Res. 2020;13(1):17. doi: 10.1186/s13048-020-0614-1.
5. Harry J. Whitwell, Jenny Worthington, Oleg Blyuss, at al,. Improved early detection of ovarian cancer using longitudinal multimarker models. Br J Cancer. 2020;122(6):847-56.doi: 10.1038/s41416-019-0718-9. Epub 2020 Jan 15.
6. Doufekas K, Olaitan A. Clinical epidemiology of epithelial ovarian cancer in the UK.Int J Women's Health. 2014; 6:537.
7. Gatot NAW, Yudi MH, Setiawan S, Sofie RK, Maringan DLT, Syahrul Rauf. The Ability Pre-operative Serum (Cancer Antigen-125, Fatty Acid Synthase, and Glucose Transporter) to Predict Primary Suboptimal Cytoreduction in Epithelial Ovarian Cancer. Macedonian J Med Sci. 2020;8(A):858-65.
8. Lertkhachonsuk A, Buranawongtrakoon S, Lekskul N, Rermluk N, Wee-Stekly W, Charakorn C. Serum CA19-9, CA-125 and CEA as tumor markers for mucinous ovarian tumors' Obstet Gynecol Res. 2020;46(11):2287-91.
9. Wiwi Irawan, Syahrul Rauf, Nasrudin A, Maisuri TC. HE4 Levels in Ovarian Cancer-Resistant Menopausal Women. Indones J Obstet Gynecol. 2019;(3):224-7.doi. https://doi.org/10.32771/inajog.v7i3.991.
10. Pradjatmo H, Siswishanto R, Prawitasari S. Relationship of Preoperative CA-125 Levels to the Survival Prognosis of Epithelial Ovarian Cancer Patients at Dr. Sardjito.J Reprod Health. 2014;5(1):15-23.
11. Kurniadi A, Hidayat YM, Suardi D, Susanto H, Gatot NAW, Prayitno H. Role of Serum IL-6 and CA-125 Prabedah as Predictors of Tumor Resectability in Epithelial Type Ovarian Cancer. Indones J Cancer. 2018;11(4):151-8.
12. Luis AH, Angel EM. How far should we go in optimal cytoreductive surgery for ovarian cancer? . Chin Clin Oncol J. 2020;9(5):70. doi: 10.21037/cco-20-40. Epub 2020 Sep 11
13. Dongyong Shan, Shulin Cheng, Yechen Ma, Honghua Peng. Serum Level of Tumor Markers and the Clinical Significance in Epithelial Ovarian Cancer. J Central South University (Medical Science). 2023;48(7):1039-49.

Research Article

Concordance and Acceptability of HPV DNA Genotyping Test by Patient's Self-Sampling Against Clinician Sampling

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Abstract

Objectives: To determine the effectiveness of self-sampling method, especially during the COVID-19 pandemic and considering Indonesia's cultural context.

Methods: This study utilized a cross-sectional design, and involved patients at the Gynecology and Colposcopy Clinic of Dr. Cipto Mangunkusumo General Hospital. The estimated sample size was 48, determined using a diagnostic test formula. The sample population consisted of female patients with positive VIA or abnormal Pap smear results. Each patient underwent HPV DNA self-sampling and clinician sampling tests using the GenoFlow HPV Array technique and continued with colposcopy. All patients were also administered a questionnaire consisting of eight questions about their perspective on the self-sampling HPV DNA test. The data analysis employed a 2 × 2 table using SPSS version 20, and Cohen's kappa coefficient was calculated to measure the agreement between the sampling results of patients' and clinicians'.

Results: Among the examinations conducted by clinicians, there were 33 patients with positive HPV results, whereas through self-sampling, there were 28 patients with positive HPV (p=0.00). High risk HPV was the most commonly observed, with HPV type 16 appearing the most (15%). Based on these data, the self-sampling sensitivity, specificity, positive predictive value, and negative predictive value were 85%, 100%, 100%, and 75%, respectively, with a concordance rate of 89.6%. The Cohen's Kappa coefficient between samples taken by the clinician and self-sampling resulted in K=0.778, which is considered a good agreement (K=0.61-0.80). All patients concluded that the procedure was easy (100%), and the majority (60.5%) expressed a preference for the self-sampling method.

Conclusion: There is a good agreement between the results of self-sampling and clinician sampling for detecting HPV DNA, with patients positively accepting the self-sampling method, indicating its potential as an effective cervical cancer screening method.

Keywords: Cervical Cancer Screening, Clinician Sampling, Human Papillomavirus, Self-Sampling.

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INTRODUCTION

Cervical cancer is a malignancy that is commonly experienced by women. Globally, cervical cancer ranks fourth after breast, lung, and colorectum cancers.[1] According to the International Agency for Research on Cancer, there were 660,000 new cases of cervical cancer worldwide in 2022, with 94% occurring in developing countries. Approximately 350,000 deaths were attributed to cervical cancer, accounting for 14.1% of cancer-related deaths among women.[2] In Indonesia, Globocan reported that there was an increase in the incidence and mortality rates due to cervical cancer with 36,964 cases (23.3 per 100,000 women) and 20,708 deaths (13.2 per 100,000 women).[2]

High-risk Human Papillomavirus (HPV) infections, particularly types 16 and 18, are the primary causes of cervical cancer. The disease often shows no clear signs or symptoms until it reaches an advanced stage, leading to delayed diagnosis and treatment. Therefore, the World Health Organization (WHO) recommends HPV

vaccination before individuals become sexually active as a means of cervical cancer prevention. Early detection methods such as Visual Inspection with Acetic Acid (VIA), Pap smears, and testing for high-risk HPV strains are also suggested.[3]

HPV DNA examination is an early detection method for cervical cancer that uses amplification techniques. The GenoFlow HPV Array (DiagCor) is a recognized tool for HPV DNA genotyping, capable of detecting high-risk HPV strains, which can significantly impact patient management. Patients with negative test results have a very low likelihood of developing cervical cancer, with the test showing a sensitivity of up to 90% and a specificity of 84.61%.[4] The combined use of HPV DNA examination and Pap smear achieves a sensitivity of 93.7% for detecting cervical intraepithelial neoplasia grades 2 and 3 (CIN 2/3). In contrast, a Pap smear alone has a sensitivity of 60%, while HPV DNA examination alone has a sensitivity of 85% for detecting high-grade lesions.[3]

Sample collection for the GenoFlow HPV Array (DiagCor) examination can be performed by clinicians or independently by the patient. Self-sampling for HPV DNA testing involves using vaginal specimens and is considered more acceptable for women who are reluctant to undergo VIA or a Pap smear due to cultural reasons or discomfort. This method allows women to perform the test at home, offering greater convenience. It also reduces logistical and financial burdens while enhancing privacy and comfort.[6]

The Directorate of Diseases Prevention and Control of the Indonesian Ministry of Health reported that the screening coverage in 2021 was still around 6.8%.[7] Indonesia, with its diverse educational levels and habits, adds another layer of complexity. Cultural and normative differences also influence the acceptance rates of this examination. Self-sampling of the HPV DNA test presents as an alternative to cervical cancer screening. This is expected to enhance the cervical cancer screening coverage in Indonesia, thus reducing the incidence of cervical cancer. Therefore, this study aimed to determine the accuracy and patient perspective of a self-sampling HPV DNA genotyping test for cervical cancer detection.

METHODS

This study was a diagnostic test utilizing a cross-sectional design to determine the accuracy of self-sampling HPV DNA examination in women with positive VIA or Pap smear results at Dr. Cipto Mangunkusumo General Hospital (RSCM). The approach of this research was qualitative, as the information or data to be presented consisted of statements of both positive and negative outcomes, both from the self-sampling results of patients and direct examinations conducted by clinicians. This study was conducted in accordance with the Declaration of Helsinki. Ethical approval was granted by the Ethics Committee of the Faculty of Medicine, University of Indonesia (KET-280/UN2.F1/ETIK/PPM.00.02/2022).

The sample population consisted of female patients who visited the Gynecology and Colposcopy Clinic at the RSCM with positive VIA or abnormal Pap smear results. Each patient underwent HPV DNA self-sampling using cervical sampling brush and clinician sampling, and continued with colposcopy. The population consideration was based on the population with positive VIA results, as the likelihood of obtaining a positive HPV DNA result was higher than that of normal patients, and adding a normal population results in higher costs. The sample size in this study was estimated using two diagnostic test formulas. We reviewed both these calculations and obtained an estimated sample size of 48.

The data collection technique used in this study employed a limited consecutive sampling from March 1st to August 31, 2022. All patients fulfilled the inclusion criteria, and no exclusion criteria were requested for their willingness to participate. The data underwent analysis using SPSS version 20. In order to ascertain sensitivity, specificity, positive predictive value, and negative predictive value, a diagnostic test employing a 2 × 2 table was employed. Cohen's Kappa coefficient was also calculated to measure the agreement between the patients' and Clinicians' sampling results. The interpretation of the Cohen's kappa coefficient as following <0.20 = poor, 0.21-0.40 = fair, 0.41-0.60 = moderate, 0.61-0.80 = good, >0.80 = very good.[8]

Patient perspectives were assessed using a questionnaire to evaluate concerns when taking samples independently, perceived concerns, ease of use of self-sampling, preferred sampling method, problems encountered in screening, affordable and reasonable price for self-examination of HPV DNA, interest in routine HPV DNA testing every 1-3 years, and the desire to convey the subject of independent HPV DNA

testing to friends and relatives. The questionnaire will be presented in the form of a descriptive table, using percentages.

RESULTS

The research subjects were selected based on inclusion criteria and were conducted at Gynecology and Colposcopy Clinic in RSCM from March 1st to August 31, 2022, resulting in a total of 48 subjects. As shown in **Table 1**, among the examinations conducted by clinicians, 33 patients (68.75%) had positive HPV results, whereas 28 patients (58.33%) had positive HPV results through self-sampling. The p-values comparing the two examination methods were p= 0.00 (p<0.05).

Table 1. Comparison between Examinations Conducted by Clinician and Self-sampling

Variable	Positive HPV		Negative HPV		*P-value*
	N	%	N	%	
Clinician Sampling	33	68.75	15	31.25	0.00
Self-Sampling	28	58.33	20	41.67	

Self-sampling sensitivity, specificity, positive predictive value, and negative predictive value were 85%, 100%, 100%, and 75%, respectively. We also calculated Cohen's Kappa coefficient between samples taken by clinicians and samples taken by patients, resulting in a kappa value of 0.778. From this kappa value, it can be concluded that the level of agreement between patients and clinician's sampling results is good agreement (*K*=0.61-0.80).

Table 2. HPV Type in Clinician and self-sampling results

HPV Type	Clinician Sampling (N)	Self-Sampling (N)
HR 16	5	4
HR 16, 18	1	1
HR 16, 59	1	1
HR 18	2	2
HR 18, 58	1	1
HR 33	2	2
HR 39	1	-
HR 39, 56	1	-
HR 45	2	2
HR 51	3	3
HR 52	1	-
HR 52, 53	1	1
HR 52, LR 44	1	1
HR 53	1	1
HR 56	1	1
HR 56, 58	1	1
HR 58	3	3
HR 66	1	-
HR 66, LR 43	1	1
HR 68, LR 43	1	1
LR 42, 81	1	1
LR 81	1	1
Negative	15	20

The types of HPV detected by either method of sampling are presented on **Table 2**. High-risk HPV was the most commonly observed, with HPV type 16 appearing the most frequently (15%). Identification of two low-risk HPV types were also found in both samples. Concordance was observed in 43 pairs (28 positive for the same HPV types and 15 negative). In general, there was an 89.6% agreement rate (43 out of 48 pairs) between the outcomes acquired through HPV DNA testing via self-sampling and clinician sampling.

Table 3. Patient Perspective on HPV DNA Self-Sampling Test

Question	Number of Patients (N)	(%)
Concerns when taking samples independently		
Yes	20	41.7
No	28	58.3
Perceived concerns		
Painfull	10	21
The sample taken is not good	7	15
The sampling tool falls	7	7
Others	1	2.1
Ease of use of self-sampling		
Easy	48	100
Difficult	0	0
Do not know	0	0
Preferred sampling method		
By clinician	19	39.5
Self-sampling	29	60.5
Problems encountered in screening		
Fear of precancerous or cancerous results	42	87.5
Facilities (Hospital) away from home or office	4	8.4
Feeling unnecessary because there are no symptoms/pain	5	10.5
Transport issues	2	4.2
The cost of the examination is quite expensive	18	37.5
If the result is positive, it is feared that the cost will be expensive	25	52.1
Not approved by the husband/spouse	5	10.5
Affordable and reasonable price for self-examination of HPV DNA		
<500,000 rupiah	37	77
500,000-1,000,000 rupiah	9	18.7
>1,000,000 rupiah	2	4.3
Interest in routine HPV DNA testing every 1-3 years		
Yes	46	95.8
No	2	4.2
Desire to convey the subject of independent HPV DNA testing to friends and relatives		
Yes	48	100
No	0	0

In **Table 3**, where questionnaire responses were collected from patients after undergoing the examination, 28 patients (58.3%) expressed concerns about conducting self-sampling. The greatest concern was related to pain during the examination, with 10 patients (21%) mentioning it. After self-sampling, all patients concluded that the procedure was easy (100%), and the majority of patients (29 patients, 60.5%) expressed a preference for the self-sampling method. A significant proportion of patients (42 patients, 87.5%) were admitted to being fearful of undergoing screening, as a positive result might lead to worries. According to the questionnaire, the anticipated cost of the self-sampling method was <500,000 rupiah (37 patients, 77%).

DISCUSSION

In developing countries, cervical cancer is often detected at advanced stages, leading to high mortality rates. According to Globocan data from 2022, there were 36,964 new cases of cervical cancer in Indonesia, out of a total of 69,886 cases in Southeast Asia. The estimated number of deaths due to cervical cancer was 20,708 out of 38,703 cases in the same region. This makes Indonesia the highest-ranking country in Southeast Asia in terms of new case detection and cervical cancer-related deaths. Cervical cancer is prioritized in Indonesia due to the low coverage of screening and early detection efforts.

Our study found that high-risk HPV type 16 was the most frequent (15%). This was consistent with the study which reported HPV 16 (18.4%) as the most prevalent type among the 61 HPV DNA genotypes.[9] Chan et al. also demonstrated HPV type 16 to be the most detected virus worldwide, with it being responsible for around 32% of all infections in South Asia alone.[10]

The self-sampling tests in this study generated a sensitivity, specificity, positive predictive value, and negative predictive value of 85%, 100%, 100%, and 75%, respectively. Several studies have examined the accuracy of self-sampling HPV DNA tests compared to those obtained by clinicians. A study conducted in India found that the diagnostic values between self-sampling and clinician sampling did not significantly differ. In self-sampling, the sensitivity, specificity, positive predictive value, and negative predictive value were 66.7%, 98.1%, 83.3%, and 95.3%, respectively.[11] In a study which investigated the effectiveness of self-sampling for HPV DNA testing in Ghana, the results were highly promising. Self-sampling exhibited a sensitivity and specificity of 92.6% and 95.9%, respectively. The concordance with samples collected by clinicians was also high, at 94.2% with a kappa value of 0.88.[12]

Our study also observed a high concordance rate between the self-sampling and clinician sampling results. Overall, a 89.6% concordance rate was observed between the tests for combined high- and low-risk types. This percentage is consistent with other studies, such as those conducted in Netherlands showed concordance rates of 96.8%.[13] A study conducted in Brazil also revealed that 88% of self-collected HPV DNA test results matched samples collected by clinicians.[14] A study in Singapore showed sensitivity 83.3%, specificity 94.6%, positive predictive value 79.4%, negative predictive value 95.8%, accuracy 93.3% and kappa value of 0.77.[15]

Cohen's kappa coefficient was also calculated to assess the agreement between samples collected by clinicians and those taken by patients. The resulting kappa value of 0.778 falls within the range indicative of good agreement (K=0.61-0.80). A study conducted in India also found that HPV DNA testing with self-sampling yielded diagnostic values equivalent to those obtained with clinician-collected samples at 94.1% with a kappa of 0.73.[11] This value would further increase when combined with other screenings, such as VIA or Pap smear. A study in Hongkong showed a kappa value of 0.652.[16] This signifies a robust level of consistency between the two sampling methods employed by patients and clinicians. Our findings suggest reliable and good agreement in the sampled data, highlighting the strong concordance between the samples obtained from both clinicians and patients during the study. These values led to the conclusion that self-sampling can be performed when there is a limited availability of human resources in particular area.

Based on questionnaires administered to patient samples, there is a reluctance to undergo early detection of cervical cancer due to the fear of receiving a positive diagnosis and discomfort with examinations. However, they preferred screening by self-sampling in future tests because of their ease of use. This finding is consistent with other research conducted in Argentina, a middle-income country, which 85.8% accepted self-sampling as a screening method.[17] Another study in Malaysia, 84.5% also found that self-sampling was easy and 81.7% good experience about it.[18] A study in Thailand showed 91.5% women felt comfort and 80.8% rated very good to excellent for overall experience compared to clinician collected method.[19] Other study in USA showed that 59.1% preferred self HPV testing with 82.7% said the reason more convenient, easier, and time saving.[20] In a study conducted in Norway, the majority of respondents concluded that self-sampling was easy to perform (94.5%), painless (90.7%), and devoid of embarrassment (89.7%), as it was performed independently.[21] These findings align with the results of the study

CONCLUSION

There is a good agreement between the results of self-sampling and clinician sampling for detecting HPV DNA, with patients positively accepting the self-sampling method, indicating its potential as an effective cervical cancer screening method.

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CONFLICT of INTEREST

There is no conflict of interest associated with this article.

DATA AVAILABILITY STATEMENT

The authors affirm that the data supporting the findings of this research are accessible within the article and its supplementary resources.

REFERENCES

1. World Health Organization: WHO. Cervical cancer. https://www.who.int/news-room/fact-sheets/detail/cervical-cancer.
2. International Agency for Research on Cancer. Cancer today [Internet]. Available from: https://gco.iarc.fr/today/en/dataviz/bars?sexes=2&mode=cancer&group_populations=1.
3. WHO. Comprehensive Cervical Cancer Control, a guide to essential practice. 2nd ed. Geneva; 2014: 81-98.
4. Indarti J, Fernando D, Fernando F, Putri RA, Mahardika A, Ikhsan M. Incidence of Positive Human Papillomavirus High Risk in Negative Cytology Result: Indones J Obstet Gynecol. 2019;7(4) 30;244–50.
5. Indarti J. The Role of HPV-DNA Test Combined with Cervical Cytology to Improve the Accuracy of Cervical Precancerous Lesion Screening. Indones J Obstet Gynecol. 2013;37(2):62–62.
6. Lozar T, Nagvekar R, Rohrer C, Dube Mandishora RS, Ivanus U, Fitzpatrick MB. Cervical Cancer Screening Postpandemic: Self-Sampling Opportunities to Accelerate the Elimination of Cervical Cancer. Int J Womens Health. 2021 Sep 18;13:841–59.
7. Kementerian Kesehatan Republik Indonesia. Profil Kesehatan Indonesia 2021. https://www.kemkes.go.id/id/profil-kesehatan-indonesia-2021.
8. Tang W, Hu J, Zhang H, Wu P, He H. Kappa coefficient: a popular measure of rater agreement. Shanghai Archives Psych. 2015 Feb 2;27(1):62.
9. Chan CK, Aimagambetova G, Ukybassova T, Kongrtay K, Azizan A. Human Papillomavirus Infection and Cervical Cancer: Epidemiology, Screening, and Vaccination—Review of Current Perspectives. J Oncol. 2019 Oct 10;2019:3257939.
10. Niyazmetova L, Aimagambetova G, Stambekova N, Abugalieva Z, Seksembayeva K, Ali S, et al. Application of molecular genotyping to determine prevalence of HPV strains in Pap smears of Kazakhstan women. Int J Infectious Dis. 2017 Jan 1;54:85–8.
11. Madhivanan P, Nishimura H, Ravi K, Pope B, Coudray M, Arun A, et al. Acceptability and Concordance of Self- Versus Clinician-Sampling for HPV Testing among Rural South Indian Women. Asian Pac J Cancer Prev. 2021 Mar;22(3):971–6.
12. Obiri-Yeboah D, Adu-Sarkodie Y, Djigma F, Hayfron-Benjamin A, Abdul L, Simpore J, et al. Self-collected vaginal sampling for the detection of genital human papillomavirus (HPV) using careHPV among Ghanaian women. BMC Women's Health. 2017 Sep 26;17(1):86.
13. Ketelaars PJW, Bosgraaf RP, Siebers AG, Massuger LFAG, van der Linden JC, Wauters CAP, et al. High-risk human papillomavirus detection in self-sampling compared to physician-taken smear in a responder population of the Dutch cervical screening: Results of the VERA study. Prev Med. 2017 Aug 1;101:96–101.
14. Rodrigues LLS, Morgado MG, Sahasrabuddhe VV, Paula VSD, Oliveira NS, Chavez-Juan E, et al. Cervico-vaginal self-collection in HIV-infected and uninfected women from Tapajós region, Amazon, Brazil: High acceptability, hrHPV diversity and risk factors. Gynecol Oncol. 2018 Oct 1;151(1):102–10.
15. Lim LM, Chan MFG, Win PPT, Shen L, Arunachalam I, Ng SYJ, et al. Self-sampling HPV DNA test for cervical cancer screening in Singapore: A prospective study. Ann Acad Med Sing. 2022 Nov;51(11):733–5.
16. Wong ELY, Chan PKS, Chor JSY, Cheung AWL, Huang F, Wong SYS. Evaluation of the Impact of Human Papillomavirus DNA Self-sampling on the Uptake of Cervical Cancer Screening. Cancer Nurs. 2016 Jan;39(1):E1–11.
17. Arrossi S, Ramos S, Straw C, Thouyaret L, Orellana L. HPV testing: a mixed-method approach to understand why women prefer self-collection in a middle-income country. BMC Public Health. 2016 Aug 19;16(1):832.
18. Ma'som M, Bhoo-Pathy N, Nasir NH, Bellinson J, Subramaniam S, Ma Y, et al. Attitudes and factors affecting acceptability of self-administered cervicovaginal sampling for human papillomavirus (HPV) genotyping as an alternative to Pap testing among multi-ethnic Malaysian women. BMJ Open. 2016 Aug;6(8):e011022.
19. Phoolcharoen N, Kantathavorn N, Krisorakun W, Taepisitpong C, Krongthong W, Saeloo S. Acceptability of Self-Sample Human Papillomavirus Testing Among Thai Women Visiting a Colposcopy Clinic. J Community Health. 2018 Jun 1;43(3):611–5.
20. Mao C, Kulasingam SL, Whitham HK, Hawes SE, Lin J, Kiviat NB. Clinician and Patient Acceptability of Self-Collected Human Papillomavirus Testing for Cervical Cancer Screening. J Womens Health (Larchmt). 2017 Jun 1;26(6):609–15.
21. Leinonen MK, Schee K, Jonassen CM, Lie AK, Nystrand CF, Rangberg A, et al. Safety and acceptability of human papillomavirus testing of self-collected specimens: A methodologic study of the impact of collection devices and HPV assays on sensitivity for cervical cancer and high-grade lesions. J Clin Virol. 2018 Feb 1;99–100:22–30.

Case Report

Successful Management of an Unruptured Extrauterine Pregnancy in a Woman with a History of Prior Miscarriage at Tertiary Hospital in Indonesia

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Abstract

Objective: To describe the prompt and successful treatment of an extrauterine pregnancy case in a tertiary-level hospital in Indonesia.

Methods: We report a case of prompt and successful management of unruptured tubal pregnancy in the dr. Soetomo General Hospital Surabaya.

Results: A 32-years-old woman presented with lower abdominal pain and vaginal bleeding one days before admission. She was sexually active, used no contraceptives, and had a history of one miscarriage. On examination, she was hemodynamically stable. A bimanual exam revealed cervical motion tenderness and pain. A high human chorionic gonadotropin (hCG) level (1,725 IU/L) and a left-sided mass, highly suspected as an extrauterine gestational sac confirming a 6 week, 1 days age of pregnancy without sign of free fluid in the abdomen nor fetal heart rate on ultrasound, prompted diagnostic laparoscopy. We found a tubal pregnancy located on the ampullae of the left fallopian tube with minimal hemoperitoneum (50 ml). A chromopertubation test was done to ensure a patent right fallopian tube, so we did a salpingectomy. The patient recovered well and was discharged home on day 2 post-procedure.

Conclusion: Early diagnosis is vital and feasible to prevent morbidity and mortality in women with ectopic pregnancy. All sexually active women complaining painful abdomen or vaginal bleeding must be examined for an ectopic pregnancy to enable early diagnosis and prompt treatment. A laparoscopic surgery done by a trained individual provided a safe and minimally invasive intervention to this case.

Keywords: case report, ectopic pregnancy, laparoscopy, salpingectomy, tubal pregnancy.

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INTRODUCTION

Occurring in about 1-2% of all pregnancies worldwide, ectopic pregnancy is a common etiology of pregnancy loss. It also jeopardizes maternal health and life, especially when it is not diagnosed and promptly managed. Almost all (95%) percent of ectopic pregnancies took place in fallopian tubes.[1] Ampullae is the most common site where ectopic tubal pregnancies happen, accounting for 70% of all cases of ectopic pregnancies.[2] Women with ectopic pregnancy may complain about blatant symptoms, such as overt pain in the abdomen and vaginal bleeding, or more vague symptoms, such as nausea and vomiting.[3] Diagnosis and exact location of ectopic pregnancy can be made during the 1st trimester of pregnancy using ultrasonography.

Early diagnosis plays a crucial role in preventing life-threatening complications like hemorrhagic shock resulting from ruptured ectopic pregnancy. The prevalence of ruptured extrauterine pregnancy varies between developed and developing countries, often due to factors such as access to early diagnostics. In Ghana, for instance, only 5.43% of tubal pregnancies remain unruptured, primarily due to the low detection rate stemming from factors such as limited early pregnancy awareness, delayed reporting, and suboptimal utilization of diagnostic tests in

healthcare facilities.[4] Similar figure was found in a study from Bangladesh and India,[5, 6] where the proportion of unruptured ectopic pregnancy was only 5.7%5 and 18.75%, respectively. Inversely, the rupture rate in a population-based study conducted in France was only 18%.[7] Similarly, a European review approximated that only 20% of ectopic pregnancies failed to be diagnosed while still intact.[8]

Indonesia is a developing country with around 5 million live births annually, with ectopic pregnancy happening in approximately 60.000 pregnancies annually.[9] Our center, dr. Soetomo General Hospital is a tertiary-level referral hospital managing about 600 births in 2021. A previous study in our center found 98 patients with ectopic pregnancy in 2 years (2013-2014). It is quite rare to find a case with unruptured sacs. Mostly came with ruptured ectopic pregnancy and life-threatening hemorrhagic shock.[9] Currently, no previous estimate exists on the rate of unruptured ectopic pregnancy and ectopic pregnancy-related complications in Indonesia. A systematic search in significant databases also confirmed that there is no case report of unruptured tubal pregnancy from Indonesia which from the technical aspects perfomed minimally invase approach with laparoscopy and performed chromopertubation. Through this report, we would like to describe a case of unruptured tubal pregnancy and demonstrate the feasibility of its management to prevent maternal complications in an Indonesian hospital.

CASE PRESENTATION

A 32-year-old woman gravida 2, on her second pregnancy at 6-7 weeks gestational age [GA] came to our hospital's emergency room with a chief complaint of abdominal pain one days before admission. Along with the abdominal pain, she also experienced vaginal bleeding (Spotting) twice 12 hours before admission. She had a previous history of miscarriage and had undergone a curettage for the treatment. She used no contraceptives. The patient was hemodynamically stable with positive elevated β human chorionic gonadotropin (β-hCG) levels (1,725 IU/L) and normal serial hemoglobin levels (12–13 g/dl). From the physical examination, we found cervical motion tenderness and pain found on the bimanual examination. Ultrasound by obstetrician revealed a left-sided mass, highly suspected as an extrauterine gestational sac confirming a 6 week, 1 days age of pregnancy. It also showed no sign of free abdominal fluid and no fetal heart rate (Figure 1). It

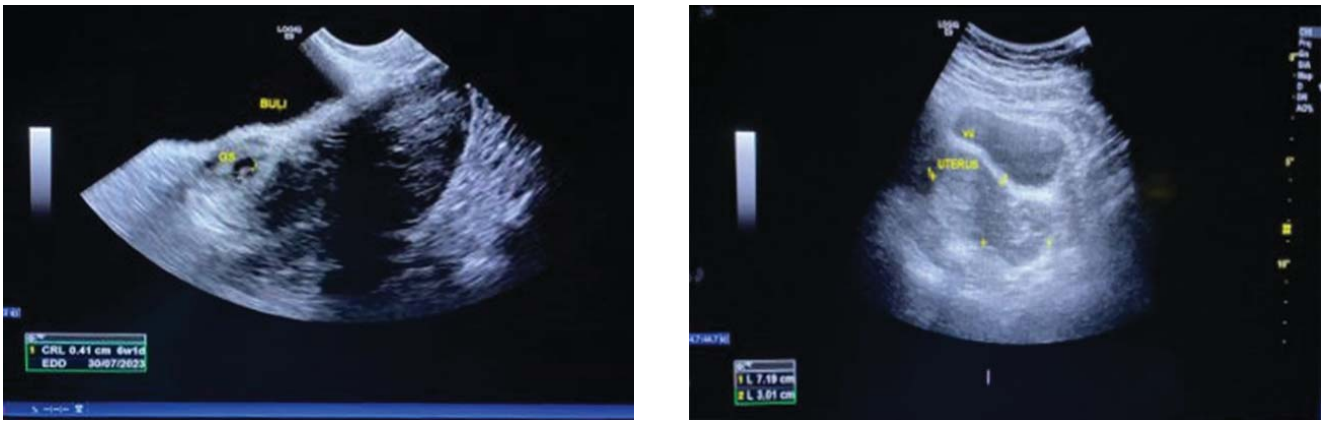

Figure 1. Transvaginal ultrasound revealed a left-sided mass suspicious of an extrauterine gestational sac with biometry similar to 6 weeks, no sign of free abdominal fluid, and no fetal heart rate.

Due to the unclear specific location of the gestational sac from the ultrasound, we then commenced diagnostic laparoscopy. We found a tubal pregnancy located on the ampullae of the left fallopian tube with minimal hemoperitoneum (50 ml) (Figure 2). A chromopertubation test revealed a patent right fallopian tube. We then performed a left total salpingectomy (Figure 3). The patient then recovered well without any event. She was discharged home on day 2 post-procedure.

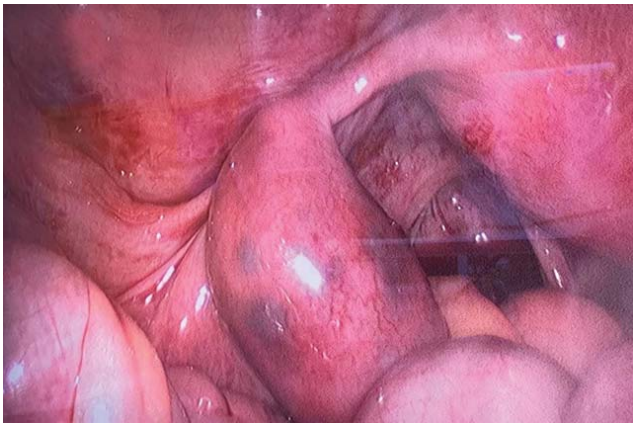

Figure 2. Intraoperative documentation showed the engorged left fallopian tube filled with an ectopic gestational sac (diameter size 3.5 cm) with minimal hemoperitoneum

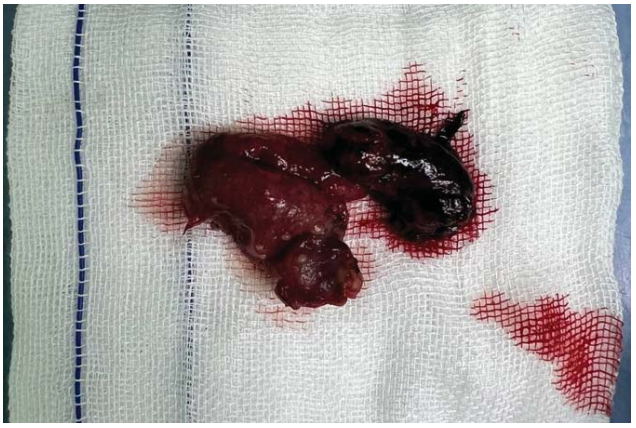

Figure 3. The pathology specimen was taken from the salpingectomy procedure.

DISCUSSION

Ectopic pregnancy contributes significantly to pregnancy-related morbidity and mortality, especially in the earliest three months of pregnancy. A diagnosis of ectopic pregnancy had a critical clinical implication; however, it could also be easily misdiagnosed. Early diagnosis of ectopic pregnancy may prevent women from complications, such as ruptured ectopic pregnancy and life-threatening hemorrhagic shock.

A suspicion of ectopic pregnancy should arise in all reproductive-aged women who are sexually active, especially those who presented with classic symptoms like the patient in our case report. Vaginal bleeding and pain in the abdomen are classic symptoms of extrauterine pregnancy. A study found that those symptoms appeared in 76% and 66% of women with ectopic pregnancy who presented to an emergency room.[10] Those symptoms may result from ectopic implantation, which subsequently causes inflammation and damage to the fallopian tubes.[6]

The suspicion may be strengthened with further history taking to explore factors or conditions that can heighten the risk of extrauterine pregnancy. Some known risk factors were mainly those which interfere with the normal tubal anatomy or the ciliary function, such as infertility, prior tubal surgery, infection in the reproductive tract or pelvic inflammatory disease, endometriosis, diethylstilbestrol (DES) exposure, vaginal douching, smoking, contraceptive (oral contraceptive pills, intrauterine device, or surgical sterilization) failure, and finally, a prior history of ectopic pregnancy.[1,11,12] In this case, the patient underwent a curettage procedure for her miscarriage history, which, although rare, had previously been recorded as a risk factor of ectopic pregnancy.[9]

The diagnosis can then be confirmed with positive serum β-hCG levels and ultrasound. Transvaginal ultrasound may be used to diagnose ectopic pregnancy during 5–9 weeks GA, with positive signs such as a sac of gestation that contains a fetal pole and heartbeat in the adnexal structure.[13] Free fluid may be found if the gestational sac has ruptured.[12] In our patient, we found a left-sided mass suspicious of an extrauterine gestational sac without any sign of abdominal free fluid or fetal heart rate. Her β-hCG level was also much lower than the level that was usually correlated with an increased risk of ruptured extrauterine pregnancy (>5,000 IU/ml).[12] She also did not have any other risk factor of rupture, such as a history of infertility, ovulation induction, or tubal damage, except that she had no previous history of contraceptive use.[7]

Due to her clinical condition, diagnostic laparoscopy was then chosen over laparotomy.[1,14] During the procedure, the gestational sac was found in the ampullae with a diameter of 3.5 cm, below the size associated with a higher risk of rupture (>4 cm).[12] Chromopertubation was performed to demonstrate the right fallopian tube's patency to ensure possible future natural pregnancy.[15] Due to the presence of a patent contralateral tube, salpingectomy was then decided because salpingostomy did not increase the chance of obtaining intrauterine pregnancy while putting the patient at risk of bleeding and persistent trophoblast tissue.[16] Finally, the patient was counseled for the subsequent pregnancy, as

the history of tubal surgery for prior extrauterine pregnancy heightened the odds of future extrauterine pregnancy by approximately three times (95% confidence interval [CI] 1.21-36.51).[11]

The successful management of unruptured extrauterine pregnancy, particularly in this case, relies heavily on early detection and prompt treatment. Generally, when diagnosed early and managed appropriately, the success rate of treating an unruptured ectopic pregnancy is relatively high. However, the success rate may vary based on the experience and proficiency of the medical team handling the patient. Timely diagnosis and suitable treatment typically lead to higher success rates in managing unruptured ectopic pregnancies.

In conclusion, in developing countries with limited access to ultrasound during early pregnancy, many women with ectopic pregnancies present with ruptured sacs, with or without hemodynamic deterioration. Nevertheless, this case highlights that early diagnosis and timely minimally invasive surgery are achievable, thus helping to prevent ectopic pregnancy-related morbidity and mortality. Therefore, ectopic pregnancy should be considered an important differential diagnosis among sexually-active reproductive-aged women experiencing lower abdominal pain and subsequent vaginal bleeding.

CONCLUSIONS

Early diagnosis is crucial and feasible for preventing morbidity and mortality in women with ectopic pregnancy. It carries significant clinical implications and can help prevent serious complications, such as ruptured ectopic pregnancy and life-threatening hemorrhagic shock. All sexually active women who present with abdominal pain and vaginal bleeding should be evaluated for possible extrauterine pregnancy to enable early diagnosis and prompt treatment. Ultrasonography and β-hCG level test must be done to exclude this differential diagnosis. Then, a laparoscopic surgery done by a trained individual provides a safe and minimally invasive intervention, while laparotomy can be done if the patient's condition deteriorates.

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REFERENCES

1. Tulandi T. Ectopic pregnancy: Epidemiology, risk factors, and anatomic sites. Wolters Kluwer; 2023 [updated Mar 29, 2023July 17, 2023]. https://www.uptodate.com/contents/ectopic-pregnancy-epidemiology-risk-factors-and-anatomic-sites.
2. Bouyer J, Coste J, Fernandez H, Pouly JL, Job-Spira N. Sites of ectopic pregnancy: A 10 year population-based study of 1800 cases. Hum Reprod. 2002;17(12):3224-30.
3. Mummert T, Gnugnoli DM. Ectopic pregnancy. Statpearls. Treasure Island (FL): StatPearls Publishing LLC. 2023.
4. Obed S. Diagnosis of unruptured ectopic pregnancy is still uncommon in ghana. Ghana Med J. 2006;40(1):3-7.
5. Banu SA, Pervin M, Akther R, Kabir R, Tasnim S. Pattern of presentation and management of ectopic pregnancy in tertiary care hospital. Bangladesh J Obstet Gynecol. 2023;36(1):28-32.
6. Singh T, Mohan S, Aggarwal S, Maji D. A study on presentation and management of ectopic pregnancy at tertiary care hospital. Int J Reprod Contracept Obstet Gynecol. 2021;10(5):1997-2000.
7. Job-Spira N, Fernandez H, Bouyer J, Pouly JL, Germain E, Coste J. Ruptured tubal ectopic pregnancy: Risk factors and reproductive outcome: Results of a population-based study in france. Am J Obstet Gynecol. 1999;180(4):938-44.
8. Alkatout I, Honemeyer U, Strauss A, Tinelli A, Malvasi A, Jonat W, et al. Clinical diagnosis and treatment of ectopic pregnancy. Obstet Gynecol Surv. 2013;68(8):571-81.
9. Nugraha AR, Sa'adi A, Tirthaningsih NW. Profile study of ectopic pregnancy at department of obstetrics and gynecology, dr. Soetomo hospital, Surabaya. Indonesia. Maj Obstet Ginekol. 2020;28(2):75-8.
10. Casanova BC, Sammel MD, Chittams J, Timbers K, Kulp JL, Barnhart KT. Prediction of outcome in women with symptomatic first-trimester pregnancy: Focus on intrauterine rather than ectopic gestation. J Womens Health (Larchmt). 2009;18(2):195-200.
11. Zhang D, Shi W, Li C, Yuan JJ, Xia W, Xue RH, et al. Risk factors for recurrent ectopic pregnancy: A case-control study. BJOG. 2016;123 Suppl 3:82-9.
12. Anaswara T, Sheelamoni A, Sheela T. A comparison between ruptured and unruptured ectopic pregnancy and its association with the risk factors: A single center study. Int J Clin Obstet Gynecol. 2021;5(4):79-84.
13. Biswas JK, Tewari V. A rare case of unruptured tubal ectopic pregnancy at 14 weeks gestation with live foetus in situ and review of literature. Int J Reprod Contracept Obstet Gynecol. 2021;10(1):422-4.
14. Acog practice bulletin no. 94: Medical management of ectopic pregnancy. Obstet Gynecol. 2008;111(6):1479-85.

15. Sunkara S, Bardawil E, Nijjar JB. Chromopertubation of an ectopic pregnancy. Obstet Gynecol. 2021;137(1):123-5.
16. Mol F, van Mello NM, Strandell A, Strandell K, Jurkovic D, Ross J, et al. Salpingotomy versus salpingectomy in women with tubal pregnancy (esep study): An open-label, multicentre, randomised controlled trial. Lancet. 2014;383(9927):1483-9.

Literature Review

Management of Vulvovaginal Candidiasis in Pregnancy

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Abstract

Objective: This study aimed at describing VVC therapy that has been proven to be safe in pregnancy.

Background: Pregnancy is a risk factor for vulvovaginal candidiasis (VVC). The most common cause of VVC in pregnancy is *Candida albicans.* When symptoms and signs of vulvar pruritus, pain, swelling, redness, burning sensation, dyspareunia, dysuria, vulvar edema, fissures, excoriation and vaginal discharge are found which suggest VVC, it is necessary to perform microscopic examination and/or fungal culture to establish the diagnosis of VVC. In pregnancy, VVC must be treated as soon as possible because it can cause adverse perinatal outcomes such as premature labor, premature rupture of membranes, low birth weight babies and fetal brain problems. Unfortunately, prescription oral antifungal therapy in pregnancy is still found. Treatment with oral antifungal is not recommended because of the risk of causing congenital abnormalities in the fetus.

Methods: Literatures in English and Indonesian were searched with topic restrictions on the type of publication for the last thirty years.

Summary: Topical intravaginal antifungal therapy such as clotrimazole and nystatin, are the recommended treatment for VVC in pregnancy that has been shown its safety. In addition, giving prophylaxis in the last trimester of pregnancy in asymptomatic VVC cases provides good pregnancy and neonatal outcomes but is still debated. In severe, prolonged or recurrent cases of VVC, other co-infections may be sought which may also need to be managed. Administration of probiotics for VVC therapy still requires further research.

Keywords: Candidiasis, Clotrimazole, Nystatin, Pregnancy, Topical

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INTRODUCTION

Vulvovaginal candidiasis (VVC) is defined as *Candida* infection of the vagina and vestibule which can extend to the labia minora, labia majora, intercrural and perianal.[1,2] VVC is one of the most common causes of vulvovaginal itching and vaginal discharge.[3] After bacterial vaginosis, VVC is the second most common cause of vaginitis.[2] At least 70-75% of women of childbearing age have experienced symptomatic VVC once during their life and 40-50% have one reinfection.[3,4] As many as 5-8% have recurrent infections (VVC ≥3 episodes per year).[3,5]

Healthy women may experience VVC sporadically.6 However, the infection is often associated with host-related factors and changes in the vaginal environment including pregnancy.[6,7] The risk of developing VVC in healthy women is 20% and higher in pregnancy (30-50%).[4,8] This is related to physiological changes in pregnancy.[3,5] Because there is an increased risk of VVC in pregnancy, early detection and management must be performed immediately so that the poor outcomes in pregnancy and the fetus can be prevented.

Several antifungal therapy options for VVC are available in Indonesia. It is important to know

that the use of antifungals in pregnancy must be administered with caution and the appropriate form chosen. The recommended antifungal treatment in pregnancy is topical antifungal, while oral antifungal is not recommended because of the risk of causing congenital abnormalities in the fetus. However, it cannot be denied that oral antifungal therapy in pregnancy is still found. In Indonesia, there is no literature review that specifically discusses about VVC therapy in pregnancy.

This literature review aims to describe VVC therapy that has been proven to be safe in pregnancy based on the correct diagnosis of VVC. Literature searching in English and Indonesian with topic restrictions on the type of publication for the last thirty years was performed, sourced from PubMed-MEDLINE, Google Scholar, university databases, electronic journals, electronic books, and official health organization websites. Hopefully, this literature review can be a guidance for providing safe VVC therapy in pregnancy.

LITERATURE REVIEW

Prevalence of Vulvovaginal Candidiasis in Pregnancy

In a meta-analysis study in Africa, the prevalence of VVC in pregnancy was 29.2% of 4,185 pregnant women subjects.[9] In other studies, the prevalence of VVC in pregnancy was reported from Lebanon, Jamaica, Argentina and China respectively 44.8%, 30,7%, 25% and 21,8%.[10-13] The species of *Candida* that causes most VVC in pregnancy is *Candida albicans.* However, other species have also been reported, such as *Candida glabrata, C.krusei, C.tropicalis,* and *C.parapsilosis* (table 1).

Table 1. Candida species cause Vulvovaginal Candidiasis in Pregnancy

Author, Country, year	Percentage of Candida Species					
	C. albicans	***C. glabrata***	***C. krusei***	***C. tropicalis***	***C. parapsilosis***	**Others**
Farr A *et al,* Austria, 2021[1]	92.4	3.8	1.9			1.9
Ghaddar *et al,* Lebanon, 2020[14]	42	41	17			
Tsega *et al,* Etiopia, 2019[15]	56.25.	17.7	21.9	1		3.1
Mushi MF *et al,* Tanzania, 2019[16]	63.4	16.8		17.8		
Zhai *et al,* Cina, 2018[13]	79.8	13.5	2.2	1.1	3.7	

Pathophysiology of Vulvovaginal Candidiasis in Pregnancy

One-third of pregnant women have at least once experienced VVC during pregnancy.[17] Change of *Candida* yeast cell into hyphae (pathogen) can occur during changes in the physiology of pregnancy.[18] The increased risk of VVC and asymptomatic colonization in pregnancy may be due to pregnancy-related factors, namely increased levels of estrogen, lower vaginal pH, increased production of vaginal mucosal glycogen and immunological changes.[3,5]

Increased estrogen facilitates adhesion and penetration of yeast to vaginal mucosal epithelial cells.[5,17] Estrogen also promotes the hyphal formation and elaboration of enzymes (such as Secreted Aspartyl Proteinase) that enhance *Candida* colonization.[5] The lower vaginal pH during pregnancy also increases yeast adhesion in the vaginal mucosa.[4] Increased glycogen creates an optimal environment for the development of *Candida*.[17] Glycogen metabolism in *C. albicans* contributes to the restoration of its survival and virulence in the host.[19] Immunological changes during pregnancy may contribute to the severity and susceptibility to infection during pregnancy.[5] The anti-inflammatory state from the second trimester of pregnancy until delivery results in a weaker local genital immune response to *Candida* so that although colonization frequency increases, symptoms are less pronounced.[5]

Vulvovaginal Candidiasis and Perinatal Outcomes

VVC in pregnancy can cause extensive inflammation, which affects perinatal outcomes.[20] Fungal infections of the female genital tract cause inflammation and increase proinflammatory mediators in vaginal fluids, such as interleukin-8, resulting in cervical ripening and contractions which are associated with preterm birth.20 These outcomes are associated with inflammation in pregnancy in symptomatic VVC.[20]

In one study, it was reported that 35% of patients with preterm labor were infected with *C. albicans*. Out of 12 pregnant women with premature rupture of membranes, 4 mothers (33.3%) showed positive results for *C. albicans* infection. Among 29 mothers who had low birth weight babies, 5 (17.2%) were positively infected with *C. albicans.* Of the 40 pregnant women who gave birth prematurely, 14 (35%) were positive for *C. albicans.*[21] However, the results of this study when associated with VVC in pregnancy were not statistically significant.[21] Likewise in a meta-analysis study on various perinatal outcomes related to VVC in pregnancy was not significant.[21] Factors that influence the results are different specimen collection times (if it was taken from pregnant women in the late trimester, it is unlikely to affect the occurrence of premature labor), the diagnostic were performed only on the lower genital tract (otherwise, upper genital tract infection is an early sign of preterm labor) and the different methods of diagnosis were used in the studies included in the studies (there could be misclassification resulting in no association in this meta-analysis).[21]

It has been reported in other studies that VVC in pregnancy and infected fetus is directly associated with an increased risk of childhood epilepsy.[22] Although the mechanism is not fully understood, it is possible that cytokines produced by the immune system during infection in pregnancy may harm the developing brain of fetus.[22,23] High concentrations of certain cytokines in amniotic fluid and neonatal blood have been associated with cerebral palsy.[23] The association between VVC in pregnancy and the risk of epilepsy for children born in the first 3 years of life from mothers who experience VVC during pregnancy (especially in the second trimester), the born children 2.79 times at risk of experiencing epilepsy (only in children born prematurely).[23] These various outcomes remind us of the importance of screening for infection during antenatal examinations to prevent VVC from expanding or ascending so that unwanted perinatal outcomes can be prevented.[24]

Clinical Manifestations and Diagnosis of Vulvovaginal Candidiasis

From a clinical perspective, it is necessary to distinguish between complicated and uncomplicated VVC cases related to management.[1,4] VVC in pregnancy is included in the complicated classification.[4] VVC clinical manifestations are vulvar pruritus, pain, swelling, erythema, burning, dyspareunia and dysuria.[1,25] The signs include vulvar edema, fissures, excoriation and vaginal discharge (thick or watery like curd, odorless).[1,25,26] The diagnosis is based on clinical and mycological laboratory test (microscopic examination and/or fungal culture).[25]

On a direct microscopic examination with saline or 10% potassium hydroxide from vaginal secretion specimens, fungal elements (yeast, budding yeast, hyphae or pseudohyphae) can be found using 400x magnification (10x ocular lens plus 40x objective lens).[1,25] In fungal cultures, yeast colonies can grow.[25] The media used for culturing *Candida* spp. are Sabouraud Dextrose Agar and parallel with Chromogenic Agar with its pigmentation ability to differentiate *Candida*.[1] In vitro antifungal sensitivity test is needed, especially in cases of chronic VVC due to *Candida* non-albicans.[1] To get accurate results, it is important to pay attention to how to obtain vaginal secretion specimens, namely: the correct way to take specimens, the amount is adequate, the sterility of the tools (sterile swab and 0.9% NaCl in a sterile closed tube) and safe-fast transport of specimens to the laboratory.[27]

The direct microscopic examination should be performed for all women with symptoms or signs of VVC. If the results are positive, treatment should be performed.[25] Fungal elements can be found by microscopy in 50-80% of VVC.[1] If the test results are negative but there are signs and symptoms of VVC, it is recommended to have fungal cultures from vaginal secretion specimens.[25] Fungal cultures are needed for unclear cases.[1] No fungal elements were found on microscopic examination possibly because the number of microorganisms is very small, thereby reducing the sensitivity of the examination.[1] Even

though the fungal burden is low, this condition still can trigger inflammation and therefore fungal cultures should be performed for species identification in some cases, for example in patients with recurrent chronic VVC.[1]

In 90% of VVC cases, itching is the dominant symptom.[1] But not all itching complaints must be VVC, only 35-40% of itching complaints are actually caused by VVC.[1] In one study, clinical findings of maceration of the vulva and white, curd-like vaginal discharge had a good diagnostic accuracy with PPV values 58% and 100% respectively.[26] These clinical signs can be a characteristic to direct us to VVC.[26] There is no evidence that VVC symptoms in pregnancy are more severe than in non-pregnant condition, but the VVC adds discomfort to pregnancy, especially recurrent VVC.[5]

In reality, microscopic examination or culture is not always possible to perform.[1] In these conditions, empirical treatment may be considered.[25] However, identifying *Candida* spp. without any signs and symptoms of VVC, is not an indication for treatment because around 10% -20% of women experience *Candida* colonization in the vagina.[25]

Serological assay is not used in diagnosing VVC because antibody levels can be detected in women with and without VVC (e.g. intestinal colonization).[1] Most PCR tests for yeast are not FDA-approved.[25] Fungal culture tests remain the gold standard for diagnosing VVC.[25]

Vulvovaginal Candidiasis Therapy in Pregnancy

Given the risk of teratogenesis during pregnancy, prescribing antifungal drugs during this period must be cautious.[28] A systematic review of congenital malformations and use of fluconazole during the first trimester of pregnancy found that women who have fluconazole during pregnancy was 1.29 times the risk of developing cardiac abnormalities.[3] The association between fluconazole treatment during pregnancy and the risk of malformations in the fetus in 40,000 mothers and babies in case-control study was found that the use of fluconazole during pregnancy has a risk of causing not only labioschisis and/ or palatoschisis but also dextro-transposition of the great arteries 5.53 times and 7.56 times respectively.[3] A cumulative dose of fluconazole 150-6,000 mg given during the first trimester of pregnancy, has been associated with a 3.16 times increased risk of significant fetal tetralogy of fallot, according to study in Denmark.[1]

Fluconazole use during pregnancy was also associated with spontaneous abortion, although there was no increased risk if consuming fluconazole before pregnancy or using a topical azole class.[3] Studies of other oral antifungal therapies such as itraconazole, ketoconazole and voriconazole have been associated with various outcomes, such as abortion, fetal musculoskeletal malformations (in experimental animals) and fetal skeletal-visceral abnormalities (in experimental animals).[28] Current guidelines state that oral antifungal therapy should be avoided and only topical antifungal therapy can be used to treat VVC in pregnancy.[1,2,3]

Several studies have reported a significant reduction in preterm birth following intravaginal clotrimazole treatment in cases of VVC during the first trimester of pregnancy.[1] Treatment of VVC in pregnancy with topical clotrimazole during the first trimester, can prevent fetal malformations and miscarriage.[1] In a study in Australia, a trend toward decreased preterm birth was shown after clotrimazole treatment in the first trimester.[1] Another study reported an increase in preterm birth rates after recurrent asymptomatic colonization with *Candida* in early pregnancy.[1]

According to the British Association of Sexual Health and HIV, treatment of asymptomatic CCV is not given due to the colonization of *Candida* spp. not associated with preterm labor and low birth weight.[2] In contrast, prophylaxis is recommended during the third trimester of pregnancy to reduce the rate of neonatal candidiasis, particularly oral candidiasis and diaper dermatitis.[1,2] Term neonates are more likely to develop oral candidiasis or diaper dermatitis during the first year of life in pregnant women who experience *Candida* colonization through mother-to-neonatal transmission during vaginal delivery.[1] Therefore, prophylactic antifungal treatment may be recommended in cases with asymptomatic colonization during the last weeks of pregnancy to prevent transmission to newborns in vaginal delivery.[1] Prophylaxis administration significantly reduced the risk of oral candidiasis and diaper dermatitis from 10% to 2% at 4 weeks of life.[1] The association of asymptomatic *Candida* spp. colonization and perinatal outcome remains unclear.[2] The fact that treatment with topical clotrimazole during the first trimester of pregnancy has been shown to reduce preterm birth rates warrants further

prospective study.[2,4]

Topical azole therapy can be used at all stages of pregnancy because of minimal systemic exposure to treatment by intravaginal administration.[4,28] The Food and Drug Administration (FDA) has assigned topical clotrimazole as pregnancy category B agents.[28] Imidazoles and other topical triazoles have been approved by the FDA as pregnancy category C.[4,28] Several clinical trials confirmed the safety of clotrimazole in pregnancy.[4] There is no association between intravaginal clotrimazole treatment with congenital abnormalities.[4] 55,64 55, 55 67

Treatment of VVC in pregnancy with clotrimazole 100 mg (vaginal tablet), administered for approximately 1 week, has a high cure rate (78-88%).[4] Similar results were obtained with application of clotrimazole 1% vaginal cream for 1 week.[4] Intravaginal clotrimazole was significantly more effective than placebo treatment.[4] Treatment of VVC with clotrimazole 500 mg vaginal tablets or vaginal cream was as effective as administration of fluconazole 150 mg orally in a single dose.[1] Comparison of the use of a single dose of clotrimazole 500 mg decreased the effectiveness of treatment compared to clotrimazole 200 mg for 3 days.[4]

Topical nystatin is an alternative treatment that has been extensively studied for administration in the first trimester of pregnancy.[29] No risk associated with major malformations has been observed in multiple studies.[29] According to the FDA, nystatin include in the A category for pregnancy used.[28] The dose of nystatin recommended during pregnancy is 100,000 IU intravaginally once daily for 14 days.[1,29] Nystatin is recommended as an alternative therapy for *Candida* non-albicans.[30] In European guidelines, nystatin is used as a first-line treatment of chronic VVC due to *C. glabrata.*[30] However, for all *Candida* species, nystatin's antifungal activity is also effective.[30]

Tabel 2. List of Intravaginal Topical Antifungals

Topical (Intravaginal) Antifungals	Dosage	Medicinal Preparations	Duration of drug administration (days)	Availability in Indonesia	FDA Category for Pregnant Women
Nistatin	100.000 IU[1]	100.000 IU vaginal tablet	14	Available *	A[28]
	200.000 IU[1]		6		
Klotrimazol	1 x 100mg[4]	100mg vaginal tablet	7	Available **	B[28]
	1 x 200mg[1]		3		
	1 x 500mg[1]	500mg vaginal tablet	1	Available	
	1x5 g[25]	1% vaginal cream	7-14	Not available	
	1x5 g[25]	2% vaginal cream	3	Not available	
Ekonazol	2x150 mg[1]	150 mg vaginal suppository	1	Not available	C[28]
	1x150 mg1		3		
Mikonazol	1x5 g[25]	2% vaginal cream	7	Not available	C[28]
	1x5 g[25]	4% vaginal cream	3	Not available	
	1x100 mg[25]	100 mg, 200 mg, 1.200 mg	7	Not available	
	1x200 mg[25]	vaginal suppository	3	Not available	
	1x1.200mg[25]		1	Not available	

Note: FDA: *Food and Drug Administration;* IU: *International Unit;* g: gram; mg: milligram
* Health care facility level 1,2, and 3 (Indonesian National Formulary 2021)[31]
** Health care facility level 2 and 3 (Indonesian National Formulary 2021)[31]

During pregnancy, treatment with topical imidazole has been shown to be more effective than treatment with topical nystatin.[1] The cure rate with clotrimazole therapy is higher than with nystatin.[4] Although clotrimazole and nystatin have been used to treat VVC for more than 40 years, both drugs are still effective.[4,30] The goal of VVC treatment is not to eradicate all the fungi in the lower genital tract, but to reduce their number so that the patient is free from clinical symptoms.[1]

In pregnancy, the susceptibility to infectious diseases increases so that it can cause not only mixed vaginitis infections, especially in the last trimester of pregnancy but also cause complicated vaginal dysbiosis so that clinical manifestations become more severe.[32] The prevalence of mixed vaginal infections is 6.5–61.0% during pregnancy and 2.4–10.0% for non-pregnant women.[32] Mixed vaginal infections in late pregnancy can lead to an increased incidence and risk of peripartum infections.[32] In one Indonesian research, the most common cause of vaginal discharge was infection with Candida spp. along with bacterial vaginosis.[26]

In addition, other co-infections in small amounts, namely *Candida* spp. along with *Trichomonas vaginalis, Chlamydia trachomatis,* and *Neisseria gonorrhoeae* were also found.[26] It is necessary to consider the presence of infections other than *Candida* spp. if clinical manifestations of vaginitis are more severe, prolonged or repeated for more comprehensive management.

Probiotic Therapy for Vulvovaginal Candidiasis

Some lactobacilli have antagonistic effects against *Candida,* for example *Lactobacillus rhamnosus.*[1] Administering *L. rhamnosus* twice daily vaginally for one week after the administration of intravaginal miconazole, decreased the recurrence rate of VVC within 6 months after treatment.[1] Decreased *Lactobacillus* is found in vaginitis due to mixed infections.[32] Several investigators demonstrated that vaginal *Lactobacillus* has a major inhibitory effect on *C. albicans* and *C. glabrata.*[18]

This protective effect is mainly due to its ability to attach to vaginal epithelial cells, compete for nutrients, inhibit adhesion of pathogens to receptors on epithelial cells, and inhibit the growth of pathogens.[1] Lactobacilli have been identified as having antifungal or immunostimulatory effects in vitro.[1] It is also known to significantly reduce in vivo fungal colonization after VVC therapy.[1] Another study reported that *L. plantarum* increased the effectiveness of a single dose of 500 mg clotrimazole in preventing recurrence of VVC.[1]

Although probiotic marketing for VVC treatment is increased, further research is needed to prove its benefits.[33] In addition, there is considerable heterogeneity across studies (e.g. route of administration, type of probiotic and duration of use of probiotic).[33] Before recommending the use of probiotics for the treatment of VVC, further quality studies are needed.

CONCLUSION

Pregnancy is a risk factor for increasing Candida spp. colonization in the vulvovagina. Colonization of Candida spp. during pregnancy can be symptomatic or asymptomatic. The diagnosis of the VVC is made based on clinical manifestation and mycological laboratory tests (microscopic examination and/or fungal culture). Intravaginal clotrimazole or nystatin are options in the management of VVC in pregnancy. Do not give any oral antifungal as the management of VVC in pregnancy because of the risk causing congenital abnormalities in the fetus. Therapy for asymptomatic Candida spp. colonization in pregnancy is still debated. The use of probiotics in the management of VVC still requires further research.

REFERENCES

1. Farr A, Effendy I, Frey Tirri B, Hof H, Mayser P, Petricevic L, et al. Guideline: Vulvovaginal candidosis (AWMF 015/072, level S2k). Mycoses. 2021;64(6):583–602.
2. Chatzivasileiou P, Vyzantiadis TA. Vaginal yeast colonisation: From a potential harmless condition to clinical implications and management approaches—A literature review. Mycoses. 2019;62(8):638–50.
3. Nyirjesy P, Brookhart C, Lazenby G, Schwebke J, SOBEL JD. Vulvovaginal Candidiasis: A Review of the Evidence for the 2021 Centers for Disease Control and Prevention of Sexually Transmitted Infections Treatment Guidelines. Clin Infect Dis. 2022;74(Suppl 2):S134–43.
4. Mendling W, Shazly MA El, Zhang L. Clotrimazole for vulvovaginal candidosis: More than 45 years of clinical experience. Pharmaceuticals. 2020;13(10):1–26.
5. Aguin TJ, Sobel JD. Vulvovaginal Candidiasis in Pregnancy. Curr Infect Dis Rep. 2015;17(6):15–20.
6. de Cássia Orlandi Sardi J, Silva DR, Anibal PC, de Campos Baldin JJCM, Ramalho SR, Rosalen PL, et al. Vulvovaginal Candidiasis: Epidemiology and Risk Factors, Pathogenesis, Resistance, and New Therapeutic Options. Curr Fungal Infect Rep. 2021;15(1):32–40.
7. Vaginal Candidiasis | Fungal Diseases | CDC https://www.cdc.gov/fungal/diseases/candidiasis/genital/index.html
8. Bagga R, Arora P. Genital Micro-Organisms in Pregnancy. Front Public Health. 2020;8(June):1–10.
9. Mohamed AO, Mohamed MS, Mallhi TH, Hussain MA, Jalloh MA, Omar KA, et al. Prevalence of vulvovaginal candidiasis among pregnant women in Africa: A systematic review and meta-Analysis. J Infect Dev Ctries. 2022;16(8):1243–51.
10. Ghaddar N, El Roz A, Ghssein G, Ibrahim JN. Emergence of Vulvovaginal Candidiasis among Lebanese Pregnant Women: Prevalence, Risk Factors, and Species Distribution. Infect Dis Obstet Gynecol. 2019;2019.
11. Kamara P, Hylton-Kong T, Brathwaite A, Del Rosario GR, Kristensen S, Patrick N, et al. Vaginal infections in pregnant women in Jamaica: Prevalence and risk factors. Int J STD AIDS. 2000;11(8):516–20.
12. Mucci MJ, Cuestas ML, Cervetto MM, Landaburu MF, Mujica MT. A prospective observational study of vulvovagintis in pregnant women in Argentina, with special reference to candidiasis. Mycoses. 2016;59(7):429–35.
13. Zhai Y, Liu J, Zhou L, Ji T, Meng L, Gao Y, et al. Detection of candida species in pregnant chinese women with a molecular beacon method. J Med Microbiol. 2018;67(6):783–9.

14. Ghaddar N, Anastasiadis E, Halimeh R, Ghaddar A, Dhar R, Alfouzan W, et al. Prevalence and antifungal susceptibility of Candida albicans causing vaginal discharge among pregnant women in Lebanon. BMC Infect Dis. 2020;20(1):1–9.
15. Tsega A, Mekonnen F. Prevalence, risk factors and antifungal susceptibility pattern of Candida species among pregnant women at Debre Markos Referral Hospital, Northwest Ethiopia. BMC Pregnancy Childbirth. 2019;19(1):1–8.
16. Mushi MF, Mmole A, Mshana SE. Candida vaginitis among symptomatic pregnant women attending antenatal clinics in Mwanza, Tanzania. BMC Res Notes 2019;12(1):1–5. https://doi.org/10.1186/s13104-019-4793-z
17. Zisova LG, Chokoeva AA, Amaliev GI, Petleshkova P V., Miteva-Katrandzhieva T, Krasteva MB, et al. Vulvovaginal Candidiasis in Pregnant Women and its Importance for Candida Colonization of Newborns. Folia Med (Plovdiv). 2016;58(2):108–14.
18. Liu XL, Xiao Y, Zhang H, Wu LJ. Comparison of vaginal flora in patients with spontaneous abortion and women with normal first-trimester. Reprod Dev Med. 2018;2(3):150–6.
19. Miao J, Regan J, Cai C, Palmer GE, Williams DL, Kruppa MD, et al. Glycogen Metabolism in Candida albicans Impacts Fitness and Virulence during Vulvovaginal and Invasive Candidiasis. MBio. 2023;
20. Gigi RMS, Buitrago-Garcia D, Taghavi K, Dunaiski CM, van de Wijgert JHHM, Peters RPH, et al. Vulvovaginal yeast infections during pregnancy and perinatal outcomes: systematic review and meta-analysis. BMC Womens Health. 2023;23(1):1–12. https://doi.org/10.1186/s12905-023-02258-7
21. Rasti S, Asadi MA, Taghriri A, Behrashi M, Mousavie G. Vaginal candidiasis complications on pregnant women. Jundishapur J Microbiol. 2014;7(2):1–2.
22. Andersen ML, Tufik S, Colombo AL, Cavalheiro EA, Cysneiros RM, Scorza FA. Sudden unexpected death in children with epilepsy: The many faces of fungal pathogenicity. Med Hypotheses. 2012;79(2):127–8. http://dx.doi.org/10.1016/j.mehy.2012.03.015
23. Sun Y, Vestergaard M, Christensen J, Nahmias AJ, Olsen J. Prenatal exposure to maternal infections and epilepsy in childhood: A population-based cohort study. Pediatrics. 2008;121(5).
24. Farr A, Kiss H, Hagmann M, Marschalek J, Husslein P, Petricevic L. Routine use of an antenatal infection screen-and-treat program to prevent preterm birth: Long-term experience at a tertiary referral center. Birth. 2015;42(2):173–80.
25. Hazra A, Collison MW, Davis AM. CDC Sexually Transmitted Infections Treatment Guidelines, 2021. JAMA. 2022;327: 870–1 .
26. Hasan FD, Ocviyanti D. Identifying Causes of Vaginal Discharge: The Role of Gynecologic Symptoms and Signs. Indones J Obstet Gynecol. 2015;3(1):3-10. DOI: https://doi.org/10.32771/inajog.v3i1.17
27. Wahyuningsih R, Bramono K, Kurniawan A, Karuniawati A. Pemeriksaan Laboratorium Infeksi Oportunistik pada Pasien Terinfeksi HIV & AIDS. Perhimpunan Mikologi Kedokteran Indonesia dan CHAI. 2015.
28. Patel MA, Aliporewala VM, Patel DA. Common Antifungal Drugs in Pregnancy: Risks and Precautions. J Obstet Gynecol India. 2021;71(6):577–82. https://doi.org/10.1007/s13224-021-01586-8
29. Soong D, Einarson A. Vaginal yeast infections during pregnancy. Can Fam Physician. 2009;55(3):255–6.
30. Choukri F, Benderdouche M, Sednaoui P. In vitro susceptibility profile of 200 recent clinical isolates of Candida spp. to topical antifungal treatments of vulvovaginal candidiasis, the imidazoles and nystatin agents. J Med Mycol. 2014;24(4):303–7. http://dx.doi.org/10.1016/j.mycmed.2014.05.001
31. Kemenkes. Kemenkes. Keputusan Menteri Kesehatan Republik Indonesia Nomor Hk.01.07/MENKES/6485/2021 tentang Formularium Nasional. 2021;1–167.
32. Li H, Dong M, Xie W, Qi W, Teng F, Li H, et al. Mixed Vaginitis in the Third Trimester of Pregnancy Is Associated With Adverse Pregnancy Outcomes: A Cross-Sectional Study. Front Cell Infect Microbiol. 2022;12(March):1–15.
33. Buggio L, Somigliana E, Borghi A, Vercellini P. Probiotics and vaginal microecology: Fact or fancy? BMC Womens Health. 2019;19(1):1–6.