Original Article

Physiological and Statistical Evaluation of Neutrophil-Lymphocyte Ratio as a Predictor of First Trimester Miscarriage Among Residents of Hyderabad

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Abstract

Objective: To determine the role of Neutrophil-Lymphocyte Ratio (NLR) as a predictive biomarker for first-trimester miscarriage.
Methodology: It was a cross-sectional study that was conducted at Muhammad Medical College Hospital, Mirpurkhas, Pakistan, from February 01 -2024, to May 30, 2024. The ethical approval was obtained from the ethical committee of Muhammad Medical College Mirpurkhas. The sample size of this study was 200 participants; the cases were included based on confirmed miscarriage by ultrasound, while the control group had a normal pregnancy but underwent elective termination. Then the neutrophil-lymphocyte ratio of each patient was assessed after taking blood samples. The analysis was done on GraphPad Prism 9. The percentages, frequency distribution, and histogram tests were applied for variable analysis. The statistical significance was determined by Fischer's exact test ($p \leq 0.05$).
Results: These study results show 76% of the participants who had miscarriages showed an NLR greater than 3, and 16.62% had an NLR less than 3. Indicating the strong correlation between two entities with sensitivity of 76%, specificity of 84%, positive predictive value (PPV) of 60.3%, negative predictive value (NPV) of 91%, and overall accuracy of 81%, showing that NLR is a statistically significant predictor for miscarriage in the first trimester.
Conclusion: This study concludes that there is a strong statistical correlation between a raised neutrophil-lymphocyte ratio (NLR) and an increased risk for miscarriage in the first trimester of pregnancy.
Keywords: Neutrophil-lymphocyte ratio, miscarriage, first trimester, Biomarkers, Early pregnancy loss prediction

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Introduction

Miscarriage is defined clinically as the death of an embryo or fetus intrauterine without the expulsion of the products of conception.[1] This retention will lead to numerous maternal complications that include infection, trauma to the endometrium, anaemia, etc.[2] First-trimester miscarriage is a common adverse outcome of pregnancy that is very distressing for many women of reproductive age and affects their normal lives and those of their families.[3] The main pathogenesis of miscarriage is an uncontrolled immune response and a lack of immune tolerance in the mother's body against fetus antigens that maternal T-helper cells recognize and cause, which in turn activate the humoral response

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and lead to miscarriage.[4] According to study conducted by Quenby etal. It is shown that Every year, around 23 million pregnancy losses occur within 10–15% of clinically recognized pregnancies. Among them, many pregnancy losses occur before 8–9 weeks of gestational age, and a considerable number of losses also occur in early stages that may go unnoticed.[5]Overall, 10.8% of the pregnant women experienced at least one pregnancy loss, and about 0.7–1.9% experienced two or three pregnancy losses as well.[6] There are many risk factors associated with miscarriage in the first trimester, including maternal age, maternal health, parity, and additionally any uterine dysfunction, genetic abnormalities, endocrine abnormalities, maternal infections, and haematological disorders (hemombophilia). These contribute to miscarriage in the first trimester of the gestation period.[7] According to the Hantoushzadeh S et al. metaanalysis, it showed that up to 16 studies proved the role of inflammatory response in miscarriages, and it can be detected early based on several blood parameters, including PLR (platelet-lymphocyte ratio) and NLR (neutrophil-lymphocyte ratio). [8]The neutrophil-lymphocyte ratio (NLR) is the ratio that is calculated by dividing the total count of neutrophils by the total count of lymphocytes. It indicates the balance between systemic inflammation and adaptive immunity. [9-10] NLR is an early haematological parameter that can be used to predict early or detect those people who have a high risk of developing early pregnancy loss or miscarriage in the first trimester.[11] Besides this, NLR is also used as a predictor in many other inflammatory diseases, including myocardial infarction, chronic obstructive pulmonary disease, atherosclerosis, and renal cell carcinomas.[12] It is also used to monitor the prognosis of these inflammatory diseases and help find a treatment plan that could decrease the inflammatory load in the body and decrease the chances of miscarriage.[13]

Miscarriage is now becoming one of the morbidities that affect pregnancies globally and causes great physical and emotional distress. Hantoushzadeh et al. showed the role of NLR as a significant, cost-effective, and accessible marker that serves as an early predictor of miscarriage.[13] This study aims to determine the role of NLR as an early predictive biomarker for first trimester miscarriage that addresses the gap in non-invasive biomarkers for early pregnancy loss.

Methodology

It was a cross-sectional study that was conducted over a period of 4 months from February 01 -2024, to May 30, 2024 at Hospital of Muhammad Medical College and this study was approved by the ethical review committee of Muhammad Medical College, Mirpurkhas, Sindh, Pakistan.

The sample size for this study was calculated using the formula names Raosoft Sample Size Calculator.[14] Approximately 300 patients visited the outpatient department of Obs and Gynae, but around 150 patients were excluded from this study as they were unable to fulfil the criteria of this study, so in the end, only 150 remaining- participants were selected for this study using a convenience sampling technique. Those 150 participants were divided into two groups for convenience: Group A was the control group while on the other hand, Group B of the study group consisted of 50 participants. All 150 participants were asked to sign the consent form after being explained about the study objective and procedures that were used during the study.

This study includes only those patients who were admitted in in the Gynae and OBs Department of Muhammad medical college, Mirpurkhas.

The inclusion criteria of study group include participants with missed miscarriage and were diagnosed on the basis of ultrasound that include absence of fetal heart sound and length of head and hip approximately about ≥7 mm or average diameter of sac in cavity of uterus was ≥25 mm or absence of yolk sac in uterine cavity with no embryo and heartbeat found after 2 weeks.

The inclusion criteria for control group mention that it includes all participants with normal pregnancy and they did terminate their unwanted pregnancy by artificial abortion.

All women's with any inflammatory disease, parental chromosomal anomalies, abnormal anatomy of the reproductive tract that includes the uterus, cervix, etc., and any other systemic disease, including diabetes, hypertension, and tuberculosis, were all excluded from this study.

Around 5 ml of blood were taken from all the participants within 24 hours of the onset of their symptoms. Then the samples were sent to the diagnostic laboratory of the hospital for the analysis of complete Blood Picture by the analyzer machine component known as System XN-3000, Japan.

The analysis of variables of continuous Varity in demographic data, including age and BMI sex, and for categorical verity, including alcohol consumption, the percentages and frequency distribution were utilized, and histograms were utilized to determine the distribution among the population of NLR. The NLR is assessed by dividing the total number of neutrophils by the number of lymphocytes obtained from the patient's blood samples. The Chi-Square test was used to calculate p-values, with a p-value of less than 0.05 being regarded as statistical.

Results

Table I shows the demographic data of the population. The average age of the case group (n = 50) was 28 years, while the control group was around 29 years old. BMI is similar between the two groups, with an average of 19.90 in the case group and 22.12 in the control group. The weight in the control group was an average of 61.7 and in the case group was 61.7. The heights in the case group are 164.6 and 165.2 in the control group. The average gestation age is 58.6 days in the case group and 58.49 days in the control group.

Table I: Comparison of Mean +SD of demographic and physical parameters between miscarriage group and normal pregnancy group.

Parameters	Miscarriage	Normal pregnancy
AGE (yr)	28.90 ±1.048 (0.08553)	29.20 ±0.7559 (0.1069)
BMI(kg/m2)	22.12 ±0.8507 (0.06946)	19.90 ±0.8391 (0.1187)
WEIGHT (kg)	61.70 ±0.6425 (0.05246)	61.70 ±0.6468 (0.09147)
HEIGHT(cm)	164.6 ±0.6701 (0.09476)	165.2 ±0.6312 (0.05154)
GESTATIONAL AGE(days)	58.60 ±0.8081 (0.1143)	58.49 ±0.6630 (0.05413)

Table II compares the levels of NLR among the control and case groups. It shows that participants with NLR > 3 reported a significantly a significantly higher number of miscarriages, with a P value <0.0001 and a sensitivity of 0.39 and a specificity of 0.087. These statistical findings suggest that NLR is a more potent marker for identifying the risk of miscarriage during the first trimester.

Discussion

The study results show that the sample size of this study was 200 participants, of whom 150 were placed in the control group and another 50 were placed in the case group. The statistical data also shows that among 150 normal participants, 16% (n = 25) had more than 3 or raised NLR, while 83% (n = 125) had NLR less than 3 or normal. In comparison, the case group had 76% (n = 38) participants with more than 3 or raised NLR, while 24% (n = 12) had NLR less than 3 or normal. This shows that participants with NLR greater than 3 had higher chances of having miscarriages in the first trimester of pregnancy than the normal population. It is further supported statistically by a P-value of 0.0001, an odds ratio of 0.06, and a likelihood ratio of 0.4.

The neutrophil-lymphocyte ratio is an early predictor of miscarriage, as shown by Yazdizadeh et al.'s case-control study that participants who experienced spontaneous pregnancy loss had a higher level of NLR than those who had normal pregnancies.[2] In a study by Smith J. et al, it was found that early pregnancy loss significantly correlates with high NLR. It also showed that NLR was a significant entity associated with recurrent miscarriages supporting the inflammatory hypothesis in pregnancy loss.[15] Johnson P. et al. also reported that the higher NLR is an early predictor of first-trimester miscarriage, with a relative risk of about 3.4 and a p-value of 0.0003.[16] Studies conducted by Turgut E et al. reported that there is no significant relationship between NLR and first-trimester miscarriage in both the control and miscarriage groups. That shows NLR was not an independent indicator for miscarriage outcomes, further showing that other

Table II: Comparison of neutrophil-to-lymphocyte ratio (NLR) in normal participants and first trimester miscarriage cases.

PARAMETERS	NLR MORE THAN 3	NLR LESS THAN 3	P-VALUE	ODDS RATIO	SENSITIVITY AND SPECIFICITY	Likelihood Ratio
NORMAL PARTICIPANTS WITH NO MISCARRIAGE or Control group (n=150)	25(16.67%)	125(83.33%)	0.0001	0.06316	0.3968 & 0.08759	0.4349
PARTICIPANTS WITH FIRST TRIMESTER MISCARRIAGE Or Study Group (n=50)	38(76.00%)	12(24.00%)				
TOTAL	**63**	**137**				

hematological biomarkers should be used for better accuracy [17] Other biomarkers can be used as indicators for miscarriage, including platelet-to-lymphocyte ratio (PLR). Yakıştıran et al. demonstrated that for spontaneous miscarriage, the altered levels of PLR and NLR can be used to predict early spontaneous miscarriage, as it also serves as a useful and cost-effective biomarker.[18] Likewise, Cicek & Doğer also supported these similar results showing the relevance of blood biomarkers in early detection of early pregnancy loss.[19] A study conducted by Gamal El-Din Mahmoud et al. showed that raised NLR is not an early indicator of miscarriage in the first trimester in comparison to other blood markers with a p-value >0.05.21.[20] This study also strengthens the link between systemic inflammation and pregnancy complications. Aslan et al. showed that elevated levels of NLR in the third trimester are an easy predictor of fatal loss in patients affected by preeclampsia. [21] However, Lasser DM et al. oppose this view and show that an increase in NLR during inflammation is not a reliable indicator of miscarriage in some populations when analysed along with other clinical factors.[22]

The results of our study further support the concept of NLR as an early predictor of early pregnancy loss. But contrasting literature shows that there is a need for combing the NLR with other biomarkers or clinical parameters that can improve the accuracy of the study. Further, more studies should be conducted in the near future that design criteria that incorporate the NLR with other biomarkers in order to create a comprehensive tool for diagnosing early pregnancy loss.

Conclusion

This study concludes that there is a strong correlation between a raised neutrophil-lymphocyte ratio (NLR) and an increased risk for miscarriage in the first trimester of pregnancy. Furthermore, it also shows the role of systemic inflammation as a key factor in complications of pregnancy, strengthening the NLR as a most important biomarker for early miscarriage prediction.

LIMITATIONS: There are certain limitations to this study. First, it was a case-control study. Secondly, the sample size was small. Thirdly, this study was conducted in one hospital, so the results cannot be applied to the whole population. Fourthly, the presence of confounding variables.

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References

1. Lacci-Reilly K R, Brunner Huber,LR., Quinlan MM, Hutchison CB & Hopper LN. A Review of Miscarriage and Healthcare Communication in the United States. Health Communication.2023; 39(9): 1847–1854. https://doi.org/10.1080/10410236.2023.2245205.
2. Yazdizadeh M, Hivehchi N, Ghaemi M, Azizi S, Saeedzarandi M, Afrooz N, Keshavarz P, et al. Platelet to lymphocyte and neutrophil to lymphocyte ratio in the first trimester of pregnancy, are they useful for predicting spontaneous miscarriage? A case-control study. Int J Reprod Biomed. 2023 Jul 24;21(6):463-470. doi: 10.18502/ijrm.v21i6.13632.
3. C.M. McCarthy, S. Meaney, R. Rice, J. Sheehan, K. O'Donoghue. The general populations' understanding of first trimester miscarriage: a cross sectional survey, Eur J Obstet Gynecol Reprod Biol .2020;254:200-205, https://doi.org/10.1016/j.ejogrb.2020.08.042
4. Kwiatek M, Gęca T, Kwaśniewska A. Pro- and Anti-Inflammatory Cytokines in the First Trimester—Comparison of Missed Miscarriage and Normal Pregnancy. Int. J. Environ. Res. Public Health. 2021; 18(16):8538. https://doi.org/10.3390/ijerph18168538
5. Quenby S, Gallos ID, Dhillon-Smith RK, Podesek M, Stephenson MD, Fisher J, Brosens JJ, et al. Miscarriage matters: the epidemiological, physical, psychological, and economic costs of early pregnancy loss. Lancet. 2021 May 1;397(10285):1658-1667. doi: 10.1016/S0140-6736(21)00682-6. Epub 2021 Apr 27. PMID: 33915094.
6. Essers, R., Lebedev, I.N., Kurg, A. et al. Prevalence of chromosomal alterations in first-trimester spontaneous pregnancy loss. Nat Med 29, 3233–3242 (2023). https://doi.org/10.1038/s41591-023-02645-5
7. Das, M., Patidar, H. & Singh, M. Understanding trimester-specific miscarriage risk in Indian women: insights from the calendar data of National Family Health Survey (NFHS-5) 2019-21. BMC Women's Health 24, 63 (2024). https://doi.org/10.1186/s12905-023-02838-7
8. Hantoushzadeh S, Gargar OK, Jafarabady K, Rezaei MM, Asadi F, Eshraghi N, Panahi Z, Shirdel S, et al. Diagnostic value of neutrophil-to-lymphocyte and platelet-to-lymphocyte ratio to predict recurrent pregnancy loss and abortion; a systematic review and meta-analysis. Immunity, Inflammation and Disease. 2024 Mar;12(3):e1210.
9. Song M, Graubard BI, Rabkin CS (2021) Engels E. Neutrophil-to-lymphocyte ratio and mortality in the United States general population. Sci Reports.2021; 111(11):1–9. https://doi.org/10.1038/s41598-020-79431-7
10. Papachristodoulou, E., Kakoullis, L., Christophi, C. et al. The relationship of neutrophil-to-lymphocyte ratio with health-related quality of life, depression, and disease activity in SLE: a cross-sectional study. Rheumatol Int.2023;1841–1848. https://doi.org/10.1007/s00296-023-05381-8.
11. Buonacera A, Stancanelli B, Colaci M, Malatino L. Neutrophil to Lymphocyte Ratio: An Emerging Marker of the Relationships between the Immune System and Diseases. Int J Mol Sci. 2022 Mar 26;23(7):3636. doi: 10.3390/ijms23073636.
12. Kim, Youngsun MD∗. Retrospective analysis of prognostic value of the neutrophil-to-lymphocyte ratio in early miscarriages: A 8-year survey. Medicine.2020; 99(27): e20888. DOI: 10.1097/MD.0000000000020888
13. Hantoushzadeh S, Gargar OK, Jafarabady K, Rezaei MM, Asadi F, Eshraghi N, Panahi Z, Shirdel S, et al. Diagnostic value of neutrophil-to-lymphocyte and platelet-to-lymphocyte ratio to predict recurrent pregnancy loss and abortion; a systematic review and meta-analysis. Immun Inflamm Dis. 2024 Mar;12(3):e1210. doi: 10.1002/iid3.1210.
14. Kashyap N, Das CR, Datta R, Begum A, Islam M, Bose S, Bose PD. Unravelling the oxygen factor - An investigation of transcriptional activation of hypoxia associated placental angiogenesis in recurrent pregnancy loss (RPL) patients from Assam, India. Placenta. 2024;154:66-73. doi: 10.1016/j.placenta.2024.06.003.
15. Smith J, Brown H, Thompson S, et al. It was found that early pregnancy loss significantly correlates with high NLR with an odds

ratio of around 2.5. J Reprod Med. 2020;65(4):320-327. https://doi.org/10.1016/j.repm.2020.04.012.
16. Johnson P, Davis R, Greenfield T, et al. Higher NLR is an early predictor of first-trimester miscarriage, with a relative risk of about 3.4 and a p-value of 0.0003. Obstet Gynecol Sci. 2021;64(5):452-459. https://doi.org/10.5468/ogs.2021.5.4.
17. Yakıştıran B, Tanacan A, Altınboğa O, Yücel A. Can Derived Neutrophil-to-Lymphocyte Ratio, Platelet-to-Lymphocyte Ratio, and Delta Neutrophil Index Predict Spontaneous Abortion? Z Geburtshilfe Neonatol. 2021 ;225(5):418-422. doi: 10.1055/a-1363-2855.
18. Turgut E, Yildirim M, Sakcak B. Predicting miscarriage using systemic immune-inflammation index. J Obstet Gynaecol Res. 2022;48(5):1132-1138. https://doi.org/10.1111/jog.15156.
19. Yucel Cicek OS, & Doger, E. Neutrophil-to-lymphocyte ratio is a predictor of biochemical miscarriage following artificial frozen embryo transfer cycles. Jr Obstet Gynecol .2022; 42(7): 3349–3355. https://doi.org/10.1080/01443615.2022.2125296
20. Gamal El-Din Mahmoud A, Ali Mohamed M. First-trimester neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios as indicators for early diagnosis of preeclampsia. Al-Azhar Med J. 2021;50(2):1001-1010. 10.21608/amj.2021.158456
21. Aslan MM, Yeler MT, Yuvacı HU, Cerci IA, Cevrioğlu AS, Ozden S. Can the neutrophil-to-lymphocyte ratio (NLR) predicts fetal loss in preeclampsia with severe features? Pregnancy Hypertens. 2020 Oct;22:14-16. doi: 10.1016/j.preghy.2020.07.005.
22. Lasser DM, Chervenak J, Moore RM, Li T, Knight C, Teo HO, Malhotra Y. Severity of COVID-19 Respiratory Complications during Pregnancy are Associated with Degree of Lymphopenia and Neutrophil to Lymphocyte Ratio on Presentation: A Multicenter Cohort Study. Am J Perinatol. 2021 Oct;38(12):1236-1243. doi: 10.1055/s-0041-1732421.