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Original Article

Association of Inflammatory Hematological Indices (NLR, NMR, PLR, and MLR) with Gestational Diabetes Mellitus: A Case–Control Study

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ABSTRACT

Gestational Diabetes Mellitus is a metabolic disorder affecting approximately 7-14% of pregnant women and is associated with adverse maternal and fetal outcomes. Low-grade chronic inflammation plays a role in the pathogenesis of GDM. Complete blood count parameters related to inflammatory indices, such as the "neutrophil-to-monocyte ratio, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and monocyte-to-lymphocyte ratio," have emerged as accessible and cost-effective biomarkers. **Objective:** To find out the association of Inflammatory Hematological Indices (NLR, NMR, PLR, and MLR) with GDM in pregnant females. **Methods:** This case–control study was conducted at Ziauddin Hospitals, Karachi, from July 2025 to December 2025. A total of 110 pregnant women were enrolled, comprising 51 women diagnosed with GDM and 59 non-GDM controls. GDM was diagnosed on World Health Organization (WHO) criteria, based on oral glucose tolerance test. CBC was obtained, and inflammatory indices were subsequently calculated. **Results:** GDM patients showed significantly higher fasting, 1-hour, and 2-hour plasma glucose levels compared to the control group ($p<0.001$). Highly significant differences were observed in NMR and MLR, and a significant difference in NLR. GDM was significantly associated with NLR and MLR. After adjusting for NMR, PLR, and MLR, the multivariate regression analysis showed that NLR association (OR = 5.32, $p=0.008$) with low discriminative ability. **Conclusion:** Among the inflammatory indices, NLR and MLR were found to be associated with GDM with low discriminative ability. However, it can be used as an adjunctive inflammatory marker.

INTRODUCTION

Gestational Diabetes Mellitus (GDM) is a metabolic disorder of glucose intolerance that is first identified or developed during pregnancy [1]. GDM prevalence is reported to be approximately 7–14% worldwide and possesses serious health risks for both the mother and the fetus if left inadequately controlled [2]. The regional standardized prevalence of GDM was 20.8% in South-East Asia [3]. A study conducted by Inam et al.2022 concluded that the prevalence of GDM in Pakistan is 9.47% [4]. The condition is associated with many maternal, as well as neonatal complications like Caesarean section, Type 2 Diabetes, and Pre-eclampsia in the mother, while respiratory distress, macrosomia, and hypoglycemia in the neonate [5-7]. During normoglycemic pregnancy, mild inflammation is physiologically present to support implantation and fetal growth. The pathophysiology of GDM is multifactorial, involving genetic, hormonal, and environmental factors. However, chronic low-grade inflammation is a major contributing factor in the development of GDM, contributing to pancreatic β-cell malfunction and insulin resistance, which leads to metabolic dysregulation during pregnancy [8, 9]. In recent years, several hematological indices derived from routine complete blood counts (CBC), especially the neutrophil-monocyte ratio (NMR) and neutrophil-lymphocyte ratio (NLR), have gained attention as markers of systemic inflammation [10, 11]. These indices

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offer valuable insights into the balance between innate and adaptive immune responses and have been studied as indicators in various metabolic, cardiovascular (CVS), and inflammatory disorders, including diabetes. The NLR represents the ratio of neutrophils to lymphocytes, key components of innate immunity and mediators of acute inflammation, which are essential for adaptive immune regulation [12]. An elevated NLR reflects a pro-inflammatory state and has been shown to correlate with insulin resistance and oxidative stress [13]. Recent studies have demonstrated that women exhibit significantly higher NLR values in inflammatory conditions during pregnancy. This elevation suggests the presence of systemic inflammation even before clinical manifestations of glucose intolerance. For instance, Zhao et al. reported that NLR levels were markedly elevated in pregnancy-induced hyperglycemia, supporting its potential role as a diagnostic and predictive biomarker [14]. Furthermore, NLR correlates with hyperglycemia and insulin resistance, highlighting the link between systemic inflammation and metabolic dysregulation during pregnancy [15]. The NMR is another inflammatory biomarker that reflects the interaction between two innate immune cells, neutrophils and monocytes [16]. Neutrophils are rapidly recruited to inflammatory sites, while monocytes contribute to chronic inflammation and tissue remodeling [17]. However, it is important to note that these markers are influenced by several physiological and pathological factors, including infections, stress, and other inflammatory conditions. Thus, their interpretation should be contextualized within comprehensive clinical assessment and laboratory findings [12]. In studies suggesting pregnancy-induced hyperglycemia, exploring NMR is relatively limited compared to NLR [18]. However, the monocyte-to-lymphocyte ratio (MLR) and platelet-to-lymphocyte ratio (PLR) are also widely used indicators to assess the inflammatory state of the body. PLR and MLR are calculated as the ratios of platelet-to-lymphocyte counts and monocyte-to-lymphocyte counts, respectively. Previous studies have demonstrated that platelets secrete numerous inflammatory mediators that contribute to immune and inflammatory responses via both autocrine and paracrine mechanisms. Simultaneously, they facilitate the chemotaxis, aggregation, and infiltration of immune cells into sites of tissue injury. PLR has consequently emerged as a valuable biomarker, reflecting alterations in platelet and lymphocyte counts associated with acute inflammatory conditions and prothrombotic states [19]. Recent studies have indicated that MLR also has the potential to serve as an innovative indicator of the systemic inflammatory response in a range of medical conditions, including pregnancy complications, cerebral hemorrhage, ischemic stroke, cardiac events, sepsis and infectious pathologies, cancers, and other diseases. These cost-effective and easily accessible inflammatory markers can assist in assessing patient risk and modifying treatment strategies in routine clinical practice [20]. The oral glucose tolerance test (OGTT) is currently regarded as the 'gold-standard diagnostic method' for Gestational Diabetes Mellitus [21]. The measurement of NLR, NMR, PLR, and MLR can offer several clinical advantages. These indices are derived from routine blood tests, making them easily obtainable and reproducible [10, 16, 20].

Data on the local population, however, about the role of NMR, NLR, PLR, and MLR are limited, and there is a lack of specific local data on the correlation of these inflammatory indices with GDM. There is limited evidence that can be found, which results in a knowledge gap about the potential use in detecting inflammatory changes in the studied population that are associated with GDM. Therefore, the study aimed to find out the association of NLR, NMR, PLR, and MLR hematological inflammatory indices with GDM.

METHODS

This case-control study was carried out at Ziauddin Hospitals (Clifton and Kemari). The study was conducted from July 2025 to December 2025, after receiving approval from the committees (GEC, ERC, and BASR) with ERC reference number: 10520625AABC dated 9th July, 2025. Convenience sampling was done. Written informed consent was obtained from all participants before their enrollment in the study.The sample size was calculated using OpenEpi version 3.01 (OpenEpi: Open Source Epidemiologic Statistics for Public Health), specifically the "Sample Size for Unmatched Case-Control Studies" module. The calculation assumed a two-sided confidence level of 95%, statistical power of 80%, and a control-to-case ratio of 1:1. Based on previous local and regional data, the proportion of exposure (inflammatory hematological index elevation) was assumed to be 3.07% in controls and 24.5% in cases, corresponding to an odds ratio of approximately 10.25 and an absolute difference of 21.43 percentage points. With these assumptions, OpenEpi returned a minimum required sample of 49 cases and 49 controls [22]. To account for potential incomplete records, missing data, and exclusions after file review, the sample was increased to 51 cases and 59 controls, yielding a total of 110 pregnant women who visited the outpatient department (OPD) of Dr. Ziauddin Hospital. During the second trimester of pregnancy, patients diagnosed with GDM were taken as cases, and normoglycemic pregnant women were taken as controls. Patients with infections, autoimmune disorders, medication, or other chronic conditions were excluded from the study. Inflammatory hematological indices were calculated from absolute cell counts obtained from the complete blood count. The

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neutrophil-to-lymphocyte ratio (NLR) was calculated as the absolute neutrophil count divided by the absolute lymphocyte count; the neutrophil-to-monocyte ratio (NMR) as the absolute neutrophil count divided by the absolute monocyte count; the platelet-to-lymphocyte ratio (PLR) as the platelet count divided by the absolute lymphocyte count; and the monocyte-to-lymphocyte ratio (MLR) as the absolute monocyte count divided by the absolute lymphocyte count. All ratios were computed using absolute counts rather than percentage values, because percentage-based counts would not yield valid NLR, NMR, PLR, or MLR results. The formulas were applied uniformly to all participants, and the derived indices were calculated in SPSS version 27.0 after data entry and verification by two independent investigators. In addition, anthropometric data, including height, weight, blood pressure (B.P.), and laboratory data comprising OGTT, as well as hematological parameters for all participants, were recorded. In 2013, the World Health Organization (WHO) adopted the diagnostic criteria for GDM proposed by the International Association of Diabetes and Pregnancy Study Groups (IADPSG). The participants were told to fast for 8–14 hours the night before the test. The OGTT was performed between 24 and 28 weeks of gestation. After an overnight fast of 8–14 hours, a fasting venous blood sample was collected at baseline (time 0). Participants then ingested 75 g of anhydrous glucose dissolved in 250–300 mL of water over 5 minutes. Additional venous blood samples were collected at 1 hour and 2 hours after the glucose load, in accordance with WHO recommendations. GDM was diagnosed using the 2013 WHO criteria based on the 75-g OGTT performed at 24–28 weeks of gestation [23]. Diagnosis was made if any one threshold was met: fasting plasma glucose ≥5.1 mmol/L (92 mg/dL), 1-hour ≥10.0 mmol/L (180 mg/dL), or 2-hour ≥8.5 mmol/L (153 mg/dL) [23]. Active infections, hematological disorders, malignant hypertension, malignancies, immunodeficiency, liver disorders, and inflammatory conditions were exclusion criteria, not diagnostic thresholds [9,12].

SPSS version 27.0 was utilized for the analysis of data. Quantitative variables were summarized as median and interquartile range (IQR), whereas qualitative variables were presented as frequencies and percentages. The Shapiro-Wilk test was done to check the normality of the data. General characteristics were compared between the GDM and non-GDM groups by using the Chi-square. Similarly, differences in quantitative variables between the two groups were evaluated using the Mann–Whitney U test. Binary logistic regression was used for the predictors of GDM. All the indices with (p=<0.2) in univariate analysis were included in the multivariate model. In the multivariate logistic regression model, only inflammatory hematological indices (NLR, NMR, PLR, and MLR) were included. Potential confounders such as BMI, socioeconomic status, dietary factors, and comorbidities were not adjusted for and should be considered in future studies. Subsequently, The prediction power of the hematological parameters for GDM was analyzed by receiver operating characteristic (ROC) curve analysis. The graph was made using GraphPad Prism version 9.

RESULTS

A total of 110 pregnant females were included in this study, with a median age of 28 years [7]. Among them, there were 51 pregnant females with GDM patients with a median age of 30 [5] years, while 59 normoglycemic pregnant females were recruited as controls with a median age of 28 [6] years, with significant differences between the two groups (p=0.002). No significant difference in parity was observed between the GDM and non-GDM groups (p=0.558) (Table 1). No significant difference in parity was observed between the GDM and non-GDM groups (p=0.558) (Table 1).

Table 1: General Characteristics of the GDM and Non-GDM Participants

Characteristics	GDM (n=51)	Non-GDM (n=59)	p-value
Ethnicity, n (%)			
Urdu speaking	2 (3.9%)	6 (10.2%)	0.088
Pathan	39 (76.5%)	31 (52.5%)	
Sindhi	1 (2%)	1 (1.7%)	
Kachi	3 (5.9%)	2 (3.4%)	
Balochi	0	1 (1.7%)	
Others	6 (11.8%)	18 (30.5%)	
Occupation, n (%)			
Housewife	49 (96.1%)	56 (94.9%)	1.000
Working	2 (3.6%)	3 (5.1%)	
Parity, n (%)			
Nulliparous	14 (27.5%)	19 (32.2%)	0.588
Parous	37 (72.5%)	40 (67.8%)	
Educational level, n (%)			
Illiterate	20 (39.2%)	29 (49.2%)	0.138
Primary	14 (27.5%)	12 (20.3%)	
Secondary	11 (21.6%)	5 (8.5%)	
Intermediate	4 (7.8%)	4 (6.8%)	
Graduate	1 (2%)	7 (11.9%)	
Masters	1 (2%)	2 (3.4%)	
Family history of Diabetes, n (%)			
Yes	24 (47.1%)	17 (28.8%)	0.048
No	27 (52.9%)	42 (71.2%)	

The anthropometric variables and glycaemic parameters of the subjects of the study. It provides a comparison of parameters related to body composition and glucose metabolism between pregnant females with GDM and normoglycemic controls to see if there are any metabolic differences between the two groups (Table 2).

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Table 2: Maternal Anthropometric and Glycemic Profile of Study Participants

Variables		GDM Median (IQR)	Non-GDM Median (IQR)	p-value
Maternal Age (Years)		30 (5)	28 (6)	0.002*
Height (cm)		158 (8)	157 (6)	0.817
Weight (kg)		77 (12)	75 (17.5)	0.084
Gestational Age at Diagnosis (Weeks)		26 (3)	–	–
OGTT	FBS (mg/dl)	96 (12)	82 (9)	<0.001**
	1- h (mg/dl)	181 (29)	149 (22)	<0.001**
	2- h (mg/dl)	138 (29)	120 (21)	<0.001**

Significant differences were observed in OGTT at fasting, 1-hour, and 2-hour blood sugar levels between the GDM and non-GDM groups. The GDM group showed lower Hb and RBC with reduced lymphocytes and higher monocytes (Table 3).

Table 3: Comparison of CBC Parameters between the GDM and Non-GDM Patients

Characteristics	GDM Median (IQR)	Non-GDM Median (IQR)	p-value
Hb (gm/dl)	10.6 (1.6)	11 (1.2)	0.022*
Red cell count (x1012/L)	3.99 (0.43)	4.33 (0.80)	0.001**
H.C.T (%)	34 (3%)	34 (3%)	0.782
MCV (fL)	82 (5)	83 (5.5)	0.107
MCH (Pg)	27 (2)	28 (3)	0.281
MCHC (g/dL)	32 (2)	32 (2)	0.820
RDW (%)	14.4 (2.1%)	14.2 (2.8%)	0.773
Platelet (x109/L)	261 (110)	272 (89)	0.797
Total WBC count (×109/L)	8.9 (13)	9.6 (9)	0.203
Neutrophils (%)	67 (20%)	66 (14%)	0.952
Lymphocytes (%)	19 (8%)	23 (8.5%)	0.004*
Monocytes (%)	6 (3%)	4 (4%)	<0.001**
Eosinophils (%)	2 (2%)	2 (2%)	0.870

*Significant
**highly significant

The inflammatory hematological indices in pregnant females with GDM and non-GDM were compared. The figure shows the differences in NLR, NMR, PLR, and MLR between the two groups. The statistical significance between groups is represented by *, and the highly significant by ** (Figure 1).

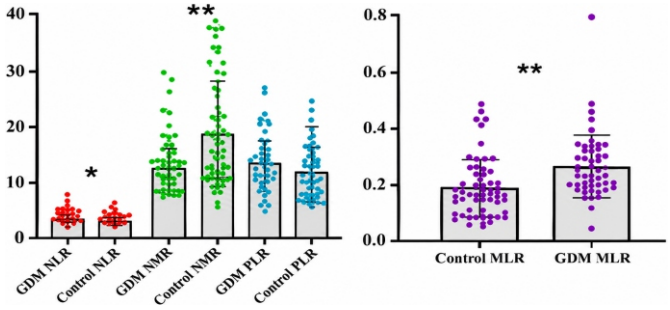


Figure 1: (a) Comparison of inflammatory hematological indices (NLR, NMR, and PLR) between the GDM and non-GDM (b) Comparison of MLR between the GDM and non-GDM groups

The NLR showed statistically significant association with GDM in multivariate logistic regression analysis (OR=5.32, 95%CI 1.55–18.16, p=0.008), but the AUC showed low discriminative ability, AUC=0.620 (95% CI: 0.513–0.726), and the diagnostic cutoff value was 2.265 (Youden index=0.268). The sensitivity and specificity of NLR were 88.2% and 28.8%, respectively. The cut-off value was determined using the Youden index (Table 4).

Table 4: Multivariate Logistic Regression Analysis associated with GDM

Variables	Multivariate Analysis		
	OR	CI	p-value
NMR	0.702	0.546 – 0.904	0.006
NLR	5.320	1.558 – 18.165	0.008
PLR	0.966	0.852 – 1.094	0.583

In this model, the monocyte to lymphocyte ratio (MLR) showed a statistically significant association with GDM (95% CI: 0.000–0.749; p=0.045), but showed an extremely low odds ratio and wide confidence interval, suggesting model instability

DISCUSSION

This case-control study was done to find out the association of hematological inflammatory indices as a biomarker of inflammation in GDM during pregnancy. Our study showed significant associations of GDM with NLR and MLR, whereas NMR is inversely associated. PLR, however, remained non- significant between the GDM group and the non-GDM group. Our NLR results are aligned with earlier studies demonstrating that women with GDM exhibit a heightened inflammatory state reflected by elevated NLR. A Turkish case-control study involving 228 pregnant women reported significantly higher NLR values among women with GDM, emphasizing that systemic inflammation is evident early in pregnancy and persists throughout gestation. The consistently significant p-values across trimesters reinforce the robustness of NLR as a marker associated with GDM across different stages of pregnancy [15]. Similarly, a large prospective cohort study conducted in China, including 1,200 women in their first trimester, reported that those who subsequently developed GDM exhibited significantly elevated baseline NLR values compared with normoglycemic pregnancies. These findings highlight the potential role of inflammatory markers not only in the pathophysiology of GDM but also in its early prediction [14]. These findings are consistent with growing evidence indicating that maternal systemic inflammation can influence placental function. Inflammatory processes may modify placental gene expression, impair vascular development, and disrupt nutrient transport mechanisms, which can ultimately contribute to abnormal fetal growth patterns. In particular, neutrophil-mediated inflammation appears to exert more targeted effects on the maternal-fetal interface, potentially affecting the efficiency of maternal-fetal

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exchange. However, not all studies reported consistent associations. There were no significant differences in NLR between the GDM and non-GDM groups in a Turkish study including 310 pregnant women [24]. These discrepancies in the findings may be attributed to differences in ethnic background or timing of hematological assessments, underscoring the need for standardized methodologies and population-specific reference values. We have explored other potential inflammatory markers, such as NMR, that were inversely correlated with GDM. This suggests higher monocyte activity, indicating enhanced chronic inflammatory status, as monocytes play a key role in sustained inflammation and insulin resistance. However, a Turkish study reported no difference in NMR between the GDM and non-GDM groups [23]. We also observed highly significant values of MLR between the GDM and non-GDM, but no association of PLR with GDM. Our study was in agreement with Zhao *et al.* who showed highly significant MLR in the GDM group in contrast to the non-GDM group [14]. Another analysis from Turkey, which included 310 pregnant women, showed similar results, highlighting the positive association of GDM with MLR [25]. The elevation of MLR reflects monocyte-driven inflammation in insulin resistance, suggesting a state of immune activation. Both the above-mentioned studies also observed the association of PLR with GDM; however, no differences were found in PLR between the GDM and non- GDM groups [14, 23]. As data are scarce on MLR and PLR in GDM, a study done on 348 diabetic foot ulcer (DFU) patients demonstrated significantly higher all-cause mortality among Chinese individuals with elevated PLR, indicating their association with the severity of diabetes mellitus (DM) and adverse outcomes like tissue damage, microangiopathy, and macrovascular complications, often leading to end-organ damage. It can also cause cardiovascular events, infectious complications such as sepsis, pneumonia, tissue necrosis, systemic infection, and inflammation [13]. Further supporting the role of inflammatory markers, a study conducted on type 2 diabetics, HBA1C showed a strong positive correlation with NLR and PLR, highlighting their role in glycemic control [24]. Additionally, a Pakistani study on inflammatory markers (CRP, TLC, and ESR) revealed significantly higher WBCs and CRP in the GDM group compared to controls [26]. Taken together, these findings affirm that different inflammatory hematological indices robustly reflect underlying immune dysregulation and metabolic stress in GDM. In particular, the significance of NLR and MLR underscores their heightened immunological activation and ties to systemic inflammation, insulin resistance, oxidative stress, and a pro-inflammatory state. Although these inflammatory cells are routinely measured in clinical practice, our results, supported by evidence from international studies, suggest that NLR and MLR may be used as an adjunctive inflammatory indicator in future studies of GDM. In GDM, elevated NLR (AUC=0.620) demonstrated low discriminatory ability for risk identification, while MLR showed no significant predictive value. Increased monocyte levels and disrupted lymphocyte profiles observed in women with GDM likely reflect a state of chronic low-grade inflammation, which is known to contribute to metabolic stress. Despite their low predictive performance, these accessible, cost-effective markers derived from routine complete blood counts offer valuable insights into the inflammatory mechanisms and understanding immune dysregulation mechanisms driving GDM pathogenesis.

This single-center study had a relatively small sample size, particularly in participants with gestational diabetes mellitus (GDM). Additionally, important confounders such as BMI, socioeconomic status, and dietary factors were not adjusted in the analysis. This was a single-centre, retrospective case-control study. Although consecutive sampling was used to reduce selection bias, residual selection bias may remain because participants were drawn from one hospital setting and data were obtained from existing medical records. The retrospective design and single-centre nature may limit the generalizability of the findings. Future multicentre prospective studies with larger sample sizes and standardized sampling protocols are recommended to validate these results and to confirm the clinical utility of NLR, NMR, PLR, and MLR as adjunctive inflammatory markers in GDM. The retrospective nature of data collection and potential variability in the timing of laboratory measurements may also affect interpretation. Future multicenter studies are recommended to be validated and generalized with studies that have larger sample sizes in diverse populations and to ascertain their potential utility as adjunctive inflammatory markers in GDM.

CONCLUSIONS

Among the hematological inflammatory indices, NLR and MLR showed significant associations with GDM. These hematological indices may serve as potential adjunctive inflammatory markers, but further large-scale prospective studies are required to establish their clinical utility.

Authors' Contribution

Conceptualization: AB, AIS
Methodology: AA, HSA
Formal analysis: AA, AB, AIS
Writing and Drafting: AA, AB, HSA
Review and Editing: AA, AB, HAS, AIS

All authors approved the final manuscript and take responsibility for the integrity of the work

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Conflict of Interest

All the authors declare no conflict of interest.

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