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Comparative Analysis of Inflammatory Markers (CRP, TLC, ESR) between Gestational Diabetes and Non-Diabetic Pregnant Females in Rawalpindi, Pakistan

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ABSTRACT

Gestational diabetes mellitus (GDM) is a prevalent metabolic complication in pregnancy, presenting considerable risks to maternal and fetal health. Recent studies indicate that inflammatory processes may contribute to the pathophysiology of GDM, leading to insulin resistance and modified immune responses. Inflammatory markers, including CRP, TLC, and ESR, are frequently utilized to evaluate systemic inflammation. This study aims to evaluate and compare inflammatory markers between GDM and non-diabetic pregnant females to elucidate their potential role in the progression and diagnosis of gestational diabetes mellitus (GDM). This research employed a cross-sectional study design and was conducted from May 2024 to September 2024. 120 participants were included with informed consent and divided into GDM (60) and control groups (60). The clinical features and demographics of patients were recorded using a structured interview questionnaire. Then, a blood sample was collected for laboratory analysis. The predominant clinical symptoms in individuals with gestational diabetes mellitus (GDM) were polydipsia, polyuria, and polyphagia. The unpaired t-test indicated a non-significant correlation of ESR levels between the two groups (p-value 0.06). Nonetheless, TLC and CRP levels exhibited a strong significant correlation between the GDM and control groups, with a p-value of <0.01. This research concluded that CRP and TLC are elevated in GDM patients in comparison to non-diabetic pregnant females.

INTRODUCTION

For many years, gestational diabetes mellitus (GDM) was described as any level of glucose intolerance identified during pregnancy, irrespective of the severity of hyperglycemia. This criterion enabled a standardized approach for the identification and categorization of GDM; nonetheless, it had significant limitations. The most reliable data indicate that several instances of GDM reflect preexisting hyperglycemia identified by regular pregnancy screening, which is not commonly conducted in non-pregnant females of reproductive age.[1] Gestational diabetes (GD) is a metabolic disorder that arises during pregnancy, characterized as diabetes diagnosed in the second or third trimester that was not present before gestation.[2] This disorder profoundly impacts both maternal and fetal health, resulting in both short- and long-term consequences.[3] Hormones such as prolactin and human placental lactogen (HPL) play significant roles in modulating maternal metabolism to support fetal development. During a normal pregnancy, HPL and prolactin cause an increase in insulin levels by inducing pancreatic B-cell hyperplasia. The release of diabetogenic substances from the placenta, including progesterone, corticotropin-releasing hormone, growth hormone, and placental lactogen, results in insulin resistance. Despite beta-cell hyperplasia, gestational diabetes mellitus develops when the body is unable to overcome insulin resistance that occurs during pregnancy.[2, 4, 5] The incidence of GDM has increased globally in recent years, in tandem with the increase in diabetes and obesity rates. It affects 5-25% of pregnancies globally, with 21 million live births impacted as reported by the International Diabetes Federation (IDF).[6] Extensive research suggests an overall GDM prevalence in Pakistan is 16.7%, with 23.9% in the Islamabad and Rawalpindi regions.[7]

The pathophysiology of GDM remains incompletely elucidated; however, new researches indicate it may arise from a confluence of genetic, epigenetic, and environmental influences.[8] Obesity, advanced maternal age, overweight status, excessive prenatal weight gain, genetic polymorphisms, ethnicity, and a family history of GDM and other insulin resistance disorders, such as polycystic ovary syndrome, are significant risk factors for the development of GDM.[9] Women with GDM have a 2-fold heightened chance of getting type 2 diabetes mellitus within ten years postpartum. They face a heightened risk of cardiovascular problems such as pre-eclampsia and gestational hypertension. Gestational diabetes mellitus elevates the likelihood of birth complications, often necessitating a cesarean section. The neonates are subjected to an elevated risk of respiratory distress and hypoglycemia. [9, 10] GDM is primarily diagnosed using the traditional screening method, 75g OGTT (Oral Glucose Tolerance Test). The OGTT entails assessing blood glucose levels after the consumption of a glucose solution and is exceptionally precise, with 99.68 % specificity and 85.74% sensitivity.[11] Conversely, the HbA1c test, which assesses blood glucose levels over a 2-3 month period, is less dependable during pregnancy and may underreport maternal glycaemia. Still, it could be a useful method with OGTT. [12]

Chronic low-grade inflammation throughout gestation results in insulin resistance, with numerous studies indicating elevated levels of inflammatory mediators in individuals with gestational diabetes mellitus (GDM) and their newborns.[8] An inflammatory marker is a biochemical substance in the body that indicates the presence or activity of inflammation, commonly identified by a blood test. GDM is linked to several inflammatory markers that may elucidate its etiology and prognostic value. The inflammatory indicators examined in our study include total leukocyte count (TLC), C-reactive protein (CRP), and Erythrocyte Sedimentation Rate (ESR). CRP is an inflammatory marker produced by the liver that can indicate various conditions, including infections, injuries, autoimmune diseases, and even some cancers. Research suggests that women with GDM may have a slightly elevated CRP level compared to pregnant women without diabetes during the first trimester of pregnancy.[13] This may be due to chronic low-grade inflammation associated with gestational diabetes.[14] CRP levels were seen to be higher in the third trimester of pregnancy compared to the second trimester.[15] TLC might be slightly elevated in GDM, particularly in early pregnancy, although its values vary across laboratory ranges; a rise in TLC can be indicative of a high risk of developing GDM.[16] ESR defines the ability of erythrocytes to sink to the bottom of a test tube within a given time, and it can be elevated during inflammation due to the release of fibrinogen and other inflammatory proteins. ESR is seen to be slightly elevated in patients with GDM than the rise in normal pregnancy. [17]

Although these inflammatory markers are not very specific and accurate, they can assist in the early identification and control of gestational diabetes. This will help to save the mother and fetus from potential complications. However, comparative data on these markers in GDM versus non-GDM pregnancies within the Pakistani population remain limited. This study aims to compare CRP, TLC, and ESR levels in GDM and non-GDM pregnant women in Rawalpindi to understand inflammatory involvement in GDM pathophysiology.

MATERIALS AND METHODS

Ethical Approval

The Institutional Research Board Committee of Zohra Institute of Health Sciences, Rawalpindi (an affiliated college of GCUF), with study location at AFIP Hospital, approved this research.

Data Collection

This comparative cross-sectional study was done at AFIP Rawalpindi from May 2024 to September 2024, with informed consent acquired from the studied participants before sample collection. The current research was conducted on 120 participants (60 were in the GDM group and 60 in the Control group) of the same ethnicity, Pakistani. Randomization was not conducted owing to the study's cross-sectional design. Sixty (60) instances of pregnant women with gestational diabetes mellitus were diagnosed at AFIP according to the criteria established by the International Association of Diabetes and Pregnancy Research Groups (IADPSG), and included in the research. [18] Healthy pregnant females with the same gestational age and normal OGTT were included as controls. The control group individuals' selection was done through a questionnaire, and there was no indication of GDM or other diabetes types or diseases in a single healthy participant. The ethnicity of the patients and controls (Pakistani) was ascertained using an oral questionnaire. Clinical features and demographics of patients were recorded using a structured interview-based questionnaire.

Sample Size

A total of 120 participants—60 with gestational diabetes and 60 healthy controls—were enrolled in the study. **(Fig#1)** While a formal power calculation was not conducted, the sample size was chosen based on practical considerations, including time, budget, and participant availability. Since no previous studies in this region have explored inflammatory markers in gestational diabetes, this research was designed as an initial step to establish local baseline data. The sample size was considered sufficient to observe meaningful trends and differences, and the findings are expected to inform the design of larger, statistically powered studies in the future.

Inclusion Criteria

i. Patients diagnosed with confirmed gestational diabetes mellitus constituted the case group.
ii. Pregnant women exhibiting normal OGTT values constituted the control group.
iii. Pregnant females between 16 and 30 weeks of gestation were chosen for both groups.
iv. Females aged 20 to 40 were recruited for both groups.
v. Neither prior chronic inflammatory nor autoimmune disorders must exist for selection in both groups.

Exclusion Criteria

i. Individuals with any preexisting type of diabetes were excluded from the research.
ii. Females with chronic inflammatory illnesses and those utilizing NSAIDs are excluded from the research.
iii. Females having any acute infection occurring within four weeks before recruitment were excluded from the study.

Sample Collection and Lab Analysis

After taking a clinical history through a questionnaire, a blood sample was collected in EDTA tubes (TLC & ESR) and a Gel Vacutainer (Standard CRP) under antiseptic conditions. Then, inflammatory marker analysis was done. For C-reactive protein (CRP), an automated clinical analyzer (Cobas c-311) was used, for white blood cells (WBCs), a hematology analyzer (BC-Mindray 6000) was used, and for erythrocyte sedimentation rate (ESR), the Westergren method was used. **(Fig#1)**

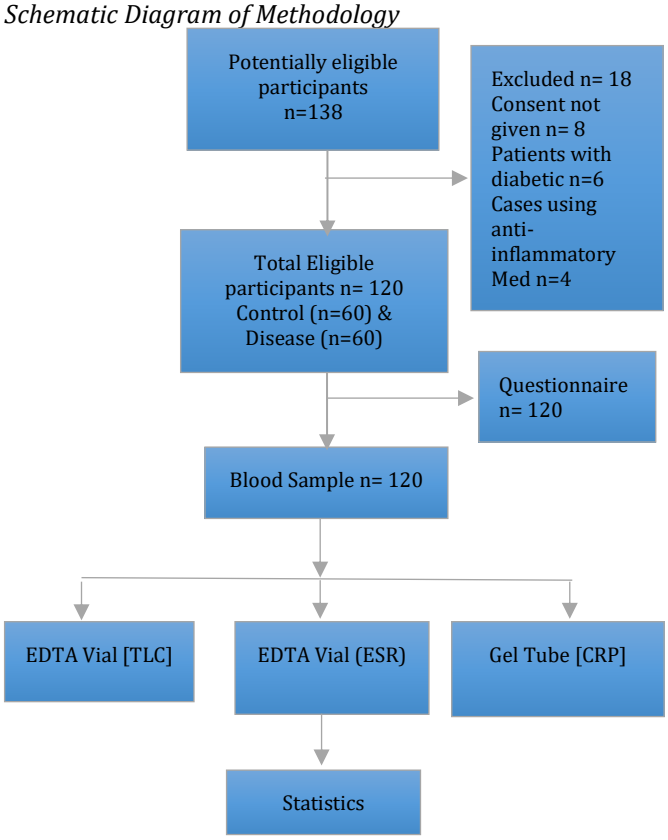
Statistical Analysis

The collected data were analyzed using both GraphPad Prism and SPSS software. GraphPad Prism was used for visual representation of results (e.g., bar graphs, scatter plots), while SPSS was used for statistical comparisons and descriptive statistics. GraphPad Prism

Qualitative variables data (gestational age group, clinical features) were represented in frequencies and percentages, and the results of quantitative variables (Age, inflammatory markers) were presented as means with standard deviation (±). Both groups' means were compared using an unpaired t-test to see significant differences among inflammatory parameters.

Figure 1

Schematic Diagram of Methodology

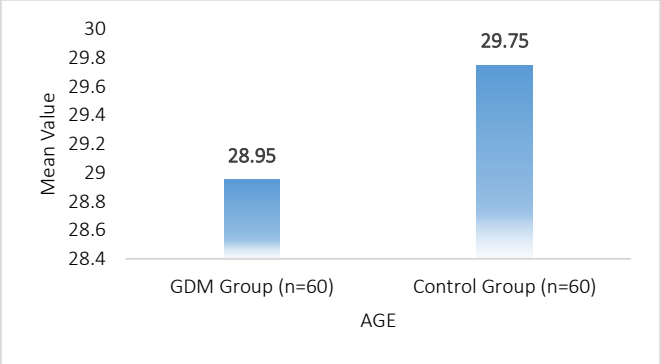


RESULTS

A total of 60 patients with gestational diabetes mellitus (disease group) and 60 healthy pregnant females (control subjects) were included in the study. The mean age of the studied groups was 28.95 ± 5.15 years in GDM patients and 29.75 ± 5.65 in the control group (Figure#2), with 29 females (48.3%) in the disease group having diabetes family history (Figure#3), but in the control group, not a single individual had a positive family history of diabetes. Both groups were classified into four categories based on gestational age, with the maximum gestational age period being 26-30 weeks. The control group had 32 females (53.3%), while the GDM group included 36 females (60.0%). (Figure#4) The GDM patients were classified into five categories according to their clinical outcomes. The largest proportion of patients experienced increased thirst and urination, totaling 22 (36.7%), followed by 14 (23.3%) who reported excessive hunger. (Figure#5)

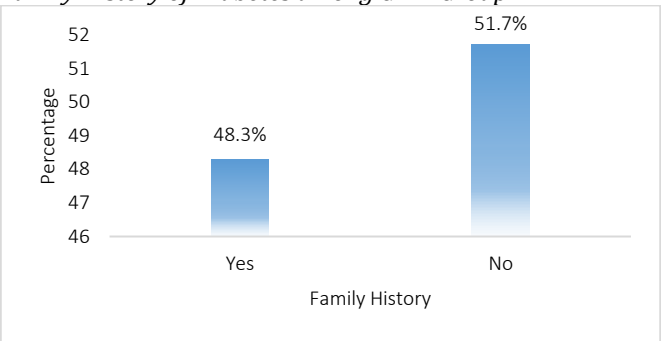
Figure# 2

Age of Studied GDM Patients and Normal Pregnant Females (Control)



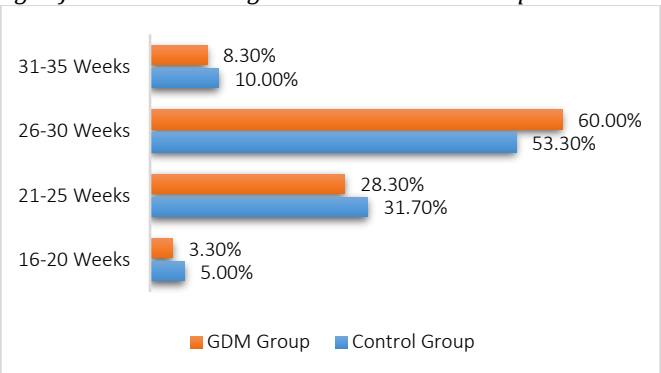
Figure# 3

Family History of Diabetes among GDM Group



Figure# 4

Age of Gestation among GDM and Control Group



Figure# 5

Clinical Findings of Studied GDM Patients

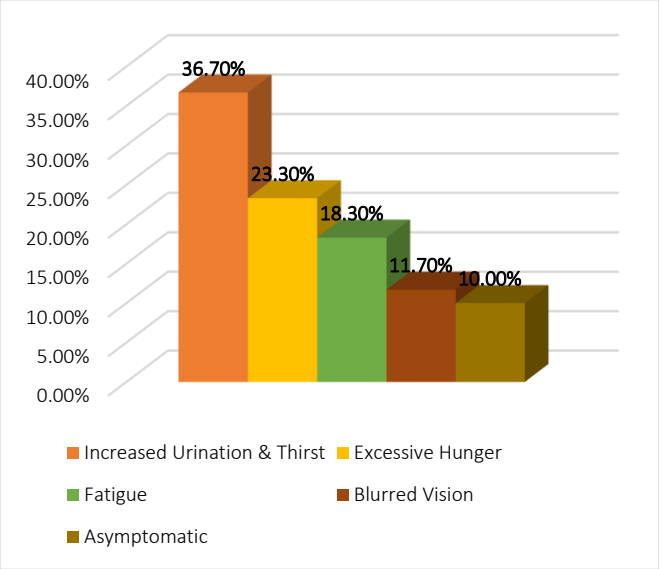


Table 1

Descriptive and Comparative Analysis of Inflammatory Markers (Unpaired-t-test)

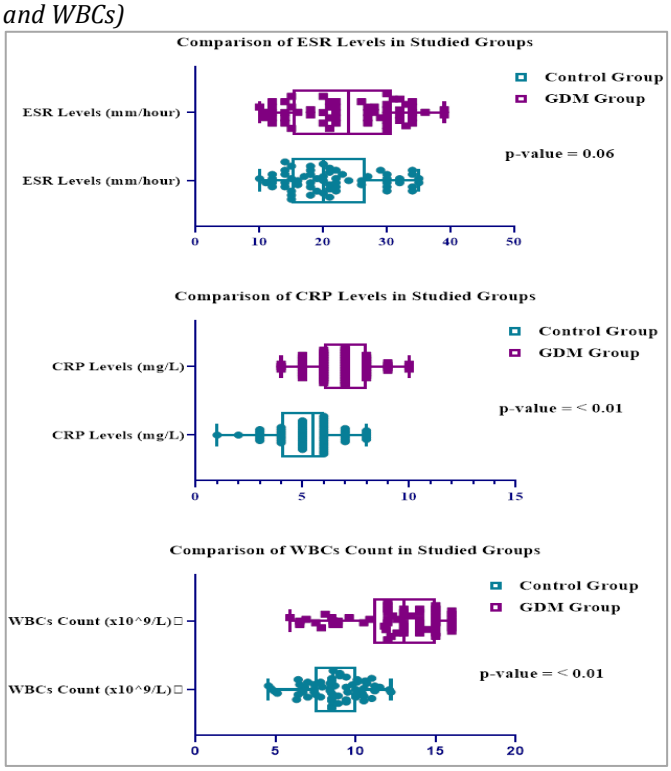
Variable Name	GDM Patients	Control Group	t-value	p-value
ESR (mm/hour)	23.95 ± 8.28	21.32 ± 7.11	0.0288	0.064
WBCs Count (x10^9/L)	12.42 ± 2.81	8.68 ± 1.31	0.3876	< 0.01
CRP (mg/L)	6.73 ± 1.48	5.33 ± 1.55	0.1783	< 0.01

±= Means & Standard Deviation, ESR (Erythrocyte Sedimentation Rate), WBCs (White Blood Cells), CRP (C-Reactive Proteins)

Table#1 and **Figure#6** showed the differences in inflammatory markers (CRP, WBCs, and ESR) between gestational diabetes mellitus (GDM) and normal pregnant females (control group). The values are given as mean ± standard deviation (descriptive statistics), and the p-values (unpaired t-test) indicate the statistical significance of the differences between the groups. ESR serves as an indicator of chronic inflammation. Although the GDM group exhibits a marginally elevated ESR, the disparity is not statistically significant with a p-value of 0.064, showing that ESR may not serve as a robust indicator of inflammation in GDM during gestation. The WBCs count is a key indicator of immune system activation and inflammation. The GDM group exhibits a significantly higher WBC count than the control group (p-value < 0.01). This indicates that GDM is linked to heightened immunological activation and systemic inflammation, potentially leading to pregnancy complications. Acute-phase proteins like CRP rise in response to inflammation. The significantly higher CRP levels (p-value = <0.01) in the GDM group indicate increased systemic inflammation. Elevated CRP levels in GDM may be linked to insulin resistance, oxidative stress, and a pro-inflammatory state, which can contribute to pregnancy complications such as preeclampsia or preterm birth.

Figure#6

Comparative Analysis of Inflammatory Markers (ESR, CRP, and WBCs)



DISCUSSION

Gestational Diabetes Mellitus is a disorder that arises solely during pregnancy and is defined by any level of glucose intolerance, endangering both fetal and maternal health. Its prevalence varies globally, with a rate of 16.7% in Pakistan, 23.9% in Islamabad and Rawalpindi regions specifically. As observed in the studied population, 48.33% of patients have had a positive family history of GDM, suggesting it to be a key risk factor. However, the rest 51.67% showed no family history of GDM, and hence no clear relationship can be established between GDM and a family history of gestational diabetes mellitus. Numerous studies, including those by Nakshine & Jogdand (2023), [19] Monod et al. (2023). [20] and Gao et al. (2022), [21] suggest that a family history of any diabetes is associated with an elevated risk of Gestational Diabetes Mellitus.

Clinical findings among disease group patients, as perceived from the results, show increased thirst and urination (36.7%), excessive hunger (23.3%), blurred vision (11.7%), and fatigue (18.3%), though 10% of the patients were asymptomatic. These symptoms align with various other studies that have also reported that Polyuria (Frequent urination) and Polydipsia (Excessive thirst) are quite common symptoms and key factors in identifying GDM among pregnant women. However, both these symptoms are not universally present in all cases, as some studies, like Al-Azemi et al., (2014)[[22] reported cases that did not exhibit the symptoms of frequent urination and thirst.

Similarly, irregular eating habits, mainly excessive eating, are also associated with GDM. This may be due to mood dysregulation and stress that is recorded in patients with GDM. This can influence eating patterns and lead to

excessive eating and poor dietary choices, as reported by several studies.

During the study, ESR, CRP, and WBC (inflammatory markers) were assessed, revealing significantly higher levels in GDM patients as compared to the control group. The present study findings indicate that the CRP levels for GDM patients are slightly elevated compared to the control group individuals, with a statistically significant p-value < 0.01. This signifies a 40% increase in CRP levels in pregnant women diagnosed with GDM. Our results align with previous studies showing elevated CRP levels in GDM patients. Torun (2022)[23] carried out research with analogous findings, indicating that CRP levels in women with gestational diabetes mellitus were elevated compared to those in healthy pregnant women, with a statistically significant p-value of 0.027. Although their p-value was slightly higher than our results, both studies contribute to the growing evidence that CRP is a key inflammatory marker associated with GDM. Similar relations were found by Naureen et al. (2023) [24] who observed a positive correlation between CRP and GDM among pregnant females. A statistically significant difference with p=0.005 was seen in the mean serum CRP levels among the diseased and control groups. This aligns with our inference that CRP is a significant inflammatory marker while identifying GDM. In contrast, a study conducted by Fazeleh Hezareh et al. (2021) [25] in Iran showed a non-significant correlation of CRP with GDM.

Our study found that the WBC levels in GDM patients were also slightly higher than those of the standard women's group, which is statistically significant with a p-value <0.01. This corresponds to the research conducted by Ghasemi et al. (2025) [26] who determined that women with gestational diabetes mellitus exhibited a greater white blood cell count (p=0.009) in comparison to healthy pregnant women. The findings support the higher level of WBC parameters among women with GDM during their second trimester. Similarly, Kağıtçı (2024)[27] also inferred that high WBC concentration is associated with GDM, as the white blood cell count of women diagnosed with GDM was seen to be higher than that of normal pregnant females. The p-value of 0.015 corresponds to the p-value of our findings. Similarly, Duo et al. (2024)[16] noted that the levels of WBC were substantially elevated in pregnant women with GDM, yielding a p-value of 0.002, in contrast to healthy pregnant women.

Furthermore, the findings of our study revealed that the Erythrocyte Sedimentation Rate (ESR) appears to be higher in GDM patients compared to control group females, although this result was not statistically significant with a p-value of 0.064. Current findings were consistent with Bahar et al. (2020) [28], who reported a non-significant difference between case and control groups with a p-value of 0.821. In contrast, Habib (2009) [17] showed a significant elevation in ESR levels with GDM. (p=0.001) Currently, there is no clear evidence to support or refute the theory that ESR levels are significantly elevated among women with GDM, as the p-value is not statistically significant. To fully elucidate this relationship, further research is needed to evaluate the association between ESR levels and GDM more comprehensively.

CONCLUSION

The current study concludes that a significant increase in CRP and WBCs is associated with gestational diabetes mellitus (GDM), suggesting a condition of heightened systemic inflammation. Although the GDM group exhibits a marginally elevated ESR, the disparity is not statistically significant, indicating that ESR may not serve as a robust indicator of inflammation in GDM during gestation. Elevated CRP, TLC, and ESR levels in GDM patients suggest a potential involvement of inflammation in the disease's pathophysiology. These findings highlight the need to keep an eye on inflammatory markers throughout pregnancy since prompt intervention and management of complications associated with GDM may be possible if excessive levels are detected early. However, the current study has several limitations, including a relatively small sample size and its cross-sectional design, which limits the ability to make definitive conclusions. It is recommended that future cohort explores larger and more diverse populations to enhance the generalizability of results. Additionally, integrating more specific inflammatory markers, such as cytokines and adipokines, may offer a clearer understanding of the underlying inflammatory pathways involved in GDM.

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