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ORIGINAL ARTICLE

Evaluation of Serum Pentraxin 3 and High Sensitive CRP in Female Patients with Gestational Diabetes Mellitus in Second Trimester of Pregnancy

[1]Alyaa A.S. Refae, [1]Hanan G. Abd El-Azeem, [1]Mohamed I. Seddik, [2]Ahmed M. Abbas, [1]Mai M. Hosny*

[1]Department of Clinical Pathology, Faculty of Medicine, Assiut University, Assiut, Egypt
[2]Department of Obstetrics and Gynecological, Faculty of Medicine, Assiut University, Assiut, Egypt

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Corresponding Author:
Mai Mohammed Hosny
Department of obstetrics and Gynecological, Faculty of Medicine, Assiut University, Assiut, Egypt
mai.mo7med266@gmail.com

ABSTRACT

Background: *Gestational Diabetes Mellitus (GDM) is a condition characterized by abnormal glucose metabolism during pregnancy, leading to adverse outcomes for both mother and child. Early intervention can reduce GDM incidence, excessive gestational weight gain, and complications like hypertensive disorders, cesarean delivery, and fetal macrosomia. Both Pentraxin-3(PTX3) and high sensitive C-reactive protein (hs-CRP) may play a role in GDM development, with elevated levels in the second trimester suggesting their potential as biomarkers for early GDM prediction, prevention, and long-term cardiovascular risk assessment.* ***Objective:*** *This study aimed to assess the value of serum Pentraxin-3 and high sensitive CRP in females with gestational diabetes mellitus in second trimester of pregnancy in comparable to females with normal pregnancy and to study correlations between serum Pentraxin-3 and High sensitive CRP in gestational diabetes.* ***Methodology:*** *The study included two groups: 45 pregnant female patients with gestational diabetes in second trimester of pregnancy and 45 pregnant females in second trimester of pregnancy not diagnosed with gestational diabetes. The serum level of PTX-3 was measured by ELISA. The serum level of hs-CRP was assessed by a Dimension Exl 200 system analyzer.* ***Results:*** *PTX3 is significantly elevated in the "Case" group ($p<0.001$). This indicates a pronounced inflammatory state associated with the condition of the "Case" group. Hs-CRP is significantly elevated in the "Case" group($p=0.005$). This indicates a pronounced inflammatory state associated with the condition of the "Case" group.* ***Conclusion:*** *There is significant association between elevated levels of PTX3, hs-CRP in GDM.*

INTRODUCTION

Gestational diabetes mellitus (GDM) refers to abnormal glucose metabolism that occurs or is discovered during pregnancy. GDM can increase the risk of type 2 diabetes mellitus (T2DM) and cardiovascular diseases in pregnant women, and the risk of metabolic disorders in their offspring is high, so It's called a disease that affects two generations[1].

The relationship between maternal hyperglycemia and fetal macrosomia together with other complications has been confirmed [2]. Early intervention during pregnancy can significantly decrease the incidence of GDM, as well as less gestational weight gain and lower adverse outcomes of pregnancy, including hypertensive disorders, cesarean delivery and macrosomia [3].

Pentraxins are a family of multifunctional proteins characterized by the presence of a 200 amino acid. Based on the length and the structure of the subunit, they can be divided into two groups: long pentraxins and short pentraxins [4].

Pentraxin 3 (PTX3) and high sensitive CRP are both the acute phase proteins belonging to the pentraxin family but with different biological characteristics, hs-CRP belongs to the short PTX, which is an acute phase protein synthesized by the hepatocytes when the body is stimulated by microbial invasion or tissue damage [5]. PTX3 is a long-chain PTX protein. A variety of tissue cells can produce PTX3 under the stimulation of pro-inflammatory factors, including the endothelial cells, fibroblasts, monocytes, adipocytes [6].

PTX3 and hs-CRP may be related to the pathogenesis of GDM, and they are significantly increased in the second trimester, which provides a new idea for early prevention and treatment of GDM and risk prediction of long-term cardiovascular diseases [7]. The aim of the present work assess the value of serum Pentraxin-3 and high sensitive CRP in females with gestational diabetes mellitus in second trimester of pregnancy in comparable to females with normal pregnancy and to study correlations between serum Pentraxin-3 and High sensitive CRP in gestational diabetes.

METHODOLOGY

This study was carried out between December 2022 and June 2024 in the lab of the Clinical Pathology Department, Assiut University Hospital, Faculty of Medicine, Assiut University, Egypt after ethical committee approval IRB no. 04-2023-200033, and registered as a clinical trial (ClinicalTrials.gov ID: NCT05846191).

Patients:

This study was conducted on 90 pregnant women in the second trimester of pregnancy, were selected from the Women's Health Hospital at Assiut University Hospital . 45 with gestational diabetes after the disease was diagnosed through the oral glucose tolerance test (OGTT) and 45 not diagnosed with gestational diabetes.

Inclusion criteria: Pregnant women in second trimester of pregnancy ,diagnosed with GDM

and Gestational age >20weeks.

Exclusion criteria: Women with pregestational diabetes, women with chronic diseases and Twin pregnancy.

Blood samples:

- Three ml blood for separation of serum which was divided into 2 aliquots; one for routine investigations and the other for serum PTX3 and hs-CRP.
- Specimens for PTX3 and hs-CRP may be stored frozen at (-20°C) for up to one month.

Methods:

Determination of Serum Human pentraxin-3(PTX-3): Cat: ELK3268

The test principle is sandwich enzyme linked immunosorbent assay (ELISA).

Assay Procedure

The assay began by adding 100 µL of standard solutions (ranging from 20 ng/mL to 0.32 ng/mL), blank, or samples to designated wells, followed by an 80-minute incubation at 37°C. After incubation, wells were washed three times with 200 µL of 1× wash buffer. Next, 100 µL of biotinylated antibody was added, incubated for 50 minutes at 37°C, and washed again three times. Streptavidin-HRP (100 µL) was then added, incubated for another 50 minutes, and washed five times. For detection, 90 µL of TMB substrate was added, leading to a blue color development after a 20-minute dark incubation at 37°C. The reaction was stopped with 50 µL of stop solution, turning the mixture yellow. Absorbance was immediately measured at 450 nm.

Determination of Serum High sensitive C-reactive protein (CRP): Cat: DC34

The method is based on a particle enhanced turbidimetric immunoassay (PETIA) technique. Synthetic particles coated with antibody to high sensitive C-Reactive Protein (AbPR) aggregate in the presence of C-Reactive Protein in the sample. The increase in turbidity which accompanies aggregation was proportional to the high sensitive C-Reactive Protein concentration.

Hs-CRP + AbPR → Aggregate (absorbs at 340 nm).

Statistical analysis:

All statistical calculations were done using SPSS (the statistical package for social science; SPSS Inc., Chicago, IL, USA), version 27. Data were statistically described as median, and range when not normally distributed, frequencies (number of cases), and relative frequencies (percentages) when appropriate. Quantitative variables were compared using the Mann-Whitney U test for dichotomous non-normally distributed data. To compare categorical data, the Chi square ($\chi 2$) test was performed. Instead, the exact test was used instead when the expected frequency was less than 5. The correlation between different variables was done using the Spearman rank order correlation test. Receiver Operating Characteristic Curve (ROC) analysis was used to find out the best cut-off values to validate the detection of GDM. The P-value is always two-tailed set significant at the<0.05 level.

RESULTS

Clinical data in patients and the control groups

Table (1) figure (1,2) showed the Comparison between group 1 and patient group 2 regarding clinical data

The baseline clinical characteristics revealed no statistically significant difference in age between the two groups (case vs. control; p=0.142). However, significant differences were observed in body mass index (BMI), prior history of gestational diabetes mellitus (GDM), and previous fetal complications (p <0.001*) , (p=0.006) and (0.029). (Table 1).

Laboratory data in patients and the control groups

Comparison of serum PTX3 and hs-CRP level in studied groups:

Table (2) figure (3,4) showed the Comparison between group 1 and patient group 2 regarding serum PTX3 and hs-CRP level

The median PTX-3 level was significantly higher in the case group (151.5 ng/mL; range: 63.9– 186.2) compared to the control group (130.6 ng/mL; range: 2.62–168.9; p<0.001), indicating a pronounced inflammatory state in the former. This elevation aligns with the established role of chronic low-grade inflammation in metabolic disorders, including insulin resistance and cardiovascular disease. The identified cut-off value (149.25 ng/mL for PTX-3) may serve as a potential threshold for assessing inflammatory risk in clinical settings.

The case group demonstrated significantly higher median **hs-CRP** levels (11.4 mg/L; range: 0.3– 45.2) compared to the control group (4.7 mg/L; range: 0.3–40.5; p = 0.02), reinforcing a pronounced systemic inflammatory state. This elevation (p = 0.005) aligns with the recognized role of chronic low-grade

inflammation in metabolic dysregulation, including insulin resistance and cardiovascular disease. The derived cut-off value (7.75 mg/L for hs-CRP) may serve as a clinically relevant threshold for stratifying inflammatory risk in affected populations.

Table 1: Comparison between group 1 (control group) and group 2 (Case group) regarding clinical data

Variable	Group 1 (Control) (N=45)	Group 2 (Case) (N=45)	P-value
Age (Years)			
- < 30 years (NO., %)	28 (62.2%)	22 (48.9%)	0.144 (n.s)
- ≥ 30 years (NO., %)	17 (37.8%)	23 (51.1%)	
- Median (Range)	26 (18-37)	30 (20-38)	0.142 (n.s)
Body Mass Index (BMI)			
- Normal (NO., %)	16 (35.6%)	5 (11.1%)	**0.001*** **
- Overweight (NO., %)	25 (55.6%)	24 (53.3%)	
- Obese (NO., %)	4 (8.9%)	16 (35.6%)	
- Median (Range)	25.8 (21.5-34.3)	28.1 (20.4-39.5)	**<0.001*** **
Past History of GDM			
- No Past History (NO., %)	45 (100%)	38 (84.4%)	**0.006** **
- History of GDM (NO., %)	0 (0%)	7 (15.6%)	
Past History of Fetal Complication			
- NO (NO., %)	44 (97.8%)	38 (84.4%)	**0.029***
- YES (NO., %)	1 (2.2%)	7 (15.6%)	

n.s: Not statistically significant.
*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

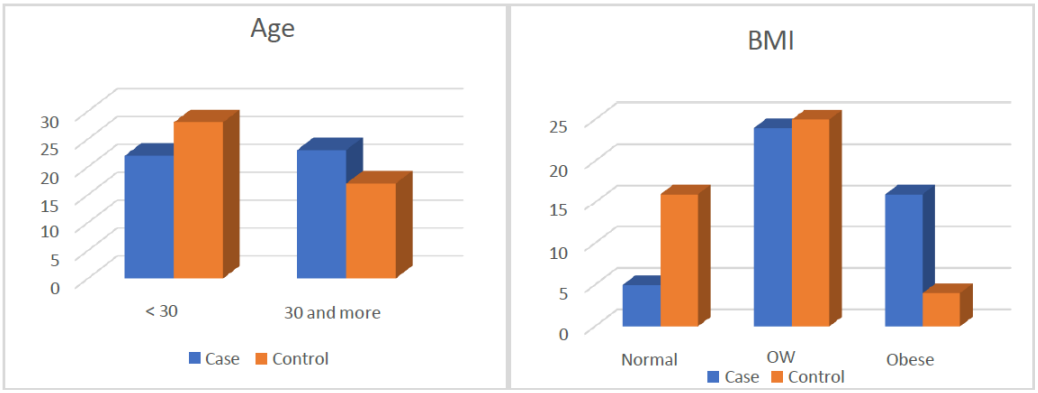

Fig. 1: Comparison between group 1 (control group) and group 2 (Case group) regarding clinical data (Age and BMI)

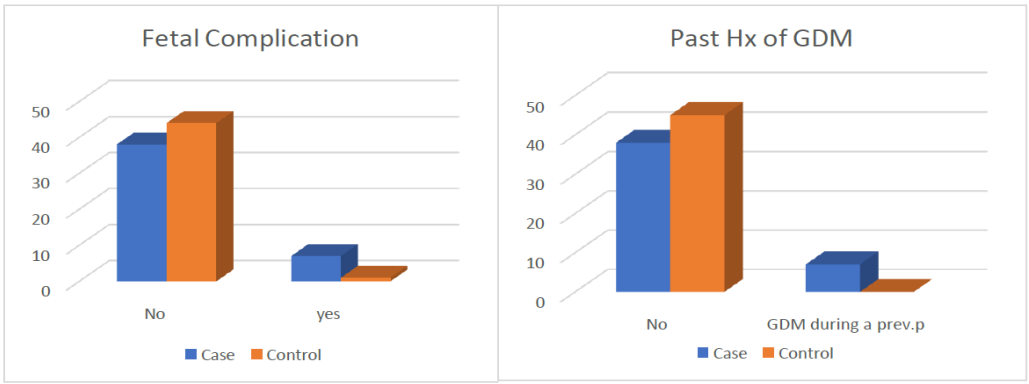

Fig. 2: Comparison between group 1 (control group) and group 2 (Case group) regarding clinical data (past history of GDM and fetal complication)

Table 2: Comparison of serum PTX3 and hs-CRP level in studied groups

Variable	Group 1 (Control) (N=45)	Group 2 (Case) (N=45)	P-value
PTX-3			
PTX-3 (Median, Range)	130.6 (2.62–168.9)	151.5 (63.9–186.2)	**<0.001****
PTX-3 Cutoff (149.25)			
- Normal (<149.25) (NO., %)	36 (80.0%)	19 (42.2%)	**<0.001****
- High (>149.25) (NO., %)	9 (20.0%)	26 (57.8%)	
hs-CRP			
hs-CRP (Median, Range)	4.7 (0.3–40.5)	11.4 (0.3–45.2)	**0.02***
hs-CRP Cutoff (7.75)			
- Normal (<7.75) (NO., %)	32 (71.1%)	19 (42.2%)	**0.005**
- High (>7.75) (NO., %)	13 (28.9%)	26 (57.8%)	

* Mild Statistical significant difference.
** Highly Statistical significant difference.

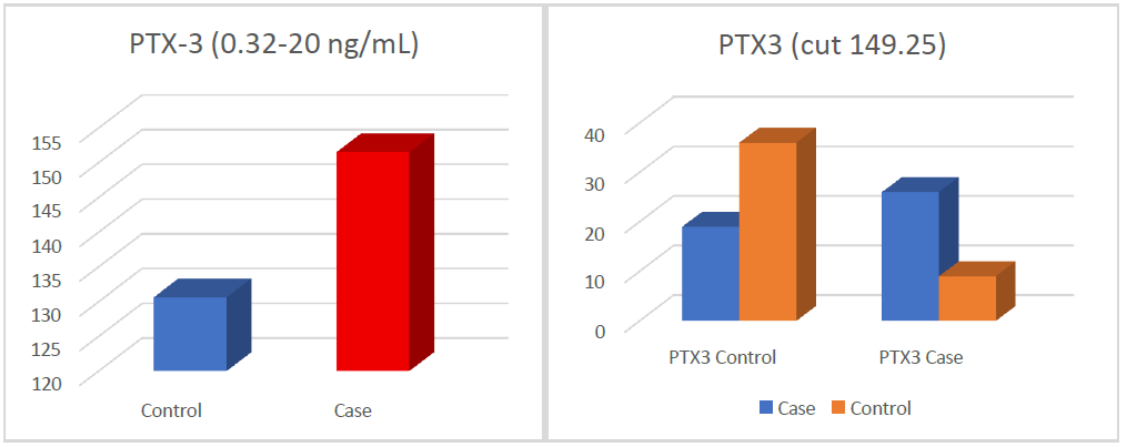

Fig. 3: Comparison between group 1 (Control) and group 2 (Case) regarding PTX-3 level

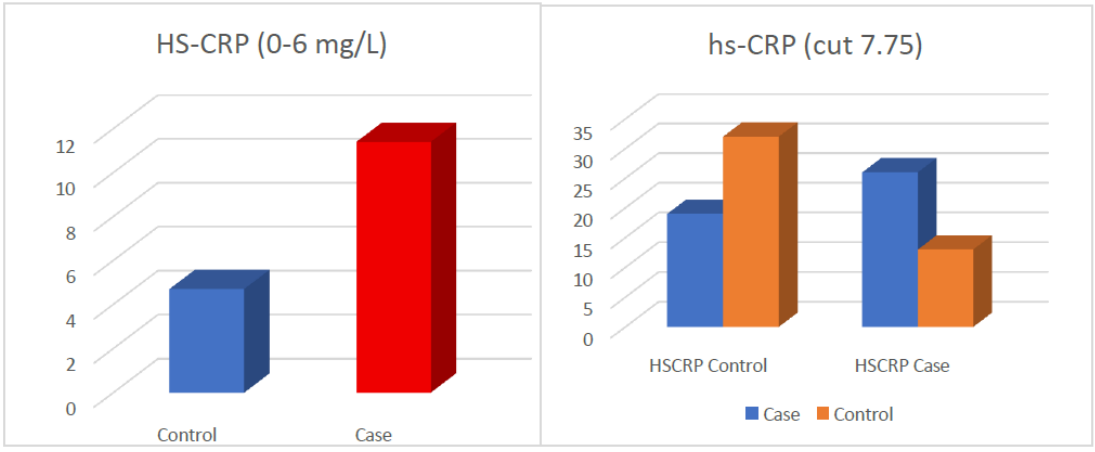

Fig. 4: Comparison between group 1 (Control) and group 2 (Case) regarding hs-CRP level

Receiver Operating Characteristic Curve (ROC) analysis of PTX3 and hs-CRP.

Pentraxin-3 (PTX3), (Cut-off: 149.25 ng/mL):

The PTX3 marker displayed lower sensitivity (34.5%) and specificity (25.7%), with an AUC of 74.2%. Although statistically significant ($p < 0.001$), its performance indicates that PTX3 may not be a strong standalone diagnostic marker. Nevertheless, the moderate AUC suggests potential utility in a complementary role alongside other biomarkers.

High-Sensitivity C-Reactive Protein (hs-CRP) (Cut-off: 7.75 mg/L):

The hs-CRP marker demonstrated moderate sensitivity (66.7%) and specificity (62.7%), with an AUC of 64.2%. Its role in inflammation-related conditions suggests that while it may be useful, it should ideally be used in conjunction with other diagnostic criteria for a more comprehensive assessment.

Table 3: (ROC) analysis of PTX3 and hs-CRP.

Biomarker (Cutoff)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC (%)	P-value	95% CI
PTX-3 (149.25)	34.5	25.7	42.2	20.0	74.2	**<0.001****	0.642 – 0.842
hs-CRP (7.75)	66.7	62.7	57.8	71.1	64.2	**0.02***	0.526 – 0.758

* Mild Statistical significant difference.
** Highly Statistical significant difference.

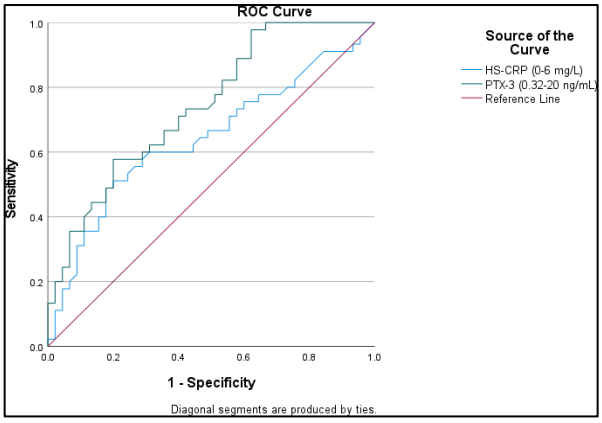

Fig. 5: ROC curve output of hs-CRP and PTX3

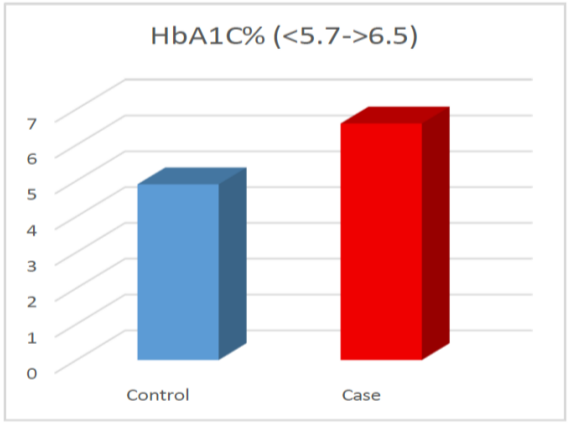

Fig. 6: Comparison between group 1 (control group) and group (Case group) regarding Median and Range of HBA1c level.

Hemoglobin A1C (HbA1C) level in the studied groups

Tables (4) figures (6) show the results of HbA1C in group 1 and patient group2

The median HbA1C for cases was 6.6% (range: 5.9-7.3), while for controls it was 4.9% (range: 4.2- 5.5). The $p < 0.001$, indicating a significant difference, suggesting poor glycemic control in the case group.

Table 4: Comparison between group 1 (control group) and group (Case group) regarding Median and Range of HBA1C level.

HBA1C	Median	Range
Group 1(Control) (N=45)	4.9	(4.2-5.5)
Group 2(Case) N=45)	6.6	(5.9-7.3)
P-value	<0.001***	

** Highly Statistical significant difference.

Correlation coefficients between hs-CRP & PTX3 levels:

There is **no statistically significant** correlation between hs-CRP and PTX3 (r=0.066, p=0.535)

Table 5: Correlation between hs-CRP & PTX3.

Test	r-value	P-value
Hs-CRP (mg/L) vs PTX3 (ng/ml)	0.066	0.535

Spearman rank order correlation test.
n.s.: no Statistical significant difference.

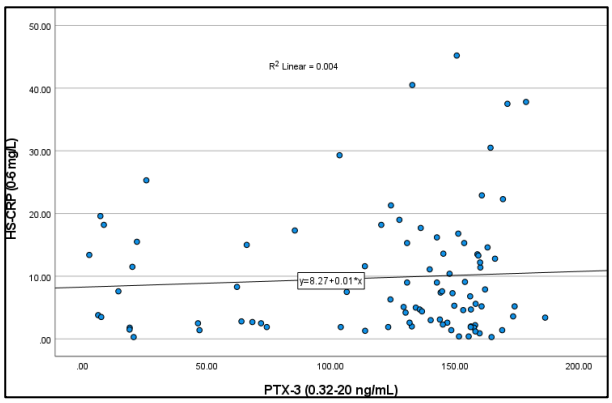

Fig. 7: Correlation coefficients between hs-CRP & PTX3 levels

DISCUSSION

In this study, we assessed the value of serum Pentraxin-3 (PTX3) and high-sensitive C- reactive protein (hs-CRP) in females with gestational diabetes mellitus (GDM) compared to healthy pregnant females during the second trimester. This was a case control study that was conducted at Antenatal Care Clinic of Woman Health Hospital and Clinical Pathology Department in Assiut University Hospitals on 90 females divided into 2 groups. Group 1 (control group): included 45 females not diagnosed and Group 2 (Case group): included 45 patients with gestational diabetes in the second trimester of pregnancy.

In our study, regarding age distribution, there was no statistically significant difference between the GDM group and the control group ($p = 0.144$). This suggests that age was well-matched between the groups and is unlikely to have confounded the inflammatory markers being studied. Our findings are consistent with those of Shatha and Sameer[8] who reported that there was no significant association between neither GDM and age of the patients nor the parity in the study groups as the P value was> 0.208, 0.183 respectively. In our study, in terms of body mass index (BMI), the GDM group exhibited a significantly higher BMI than the control group ($p < 0.001$). This fact supported by many studies such as a study in California by Dai et al.[9] identified that BMI > 25 Kg /m2 a risk for developing GDM of (P value = 0.001). The present study revealed a highly significant association between previous history of GDM and current gestational diabetes, with 15.6% of cases having a prior GDM history compared to 0% in the control group ($p=0.006$). This finding strongly supports the established understanding that women with a history of GDM are at substantially increased risk for recurrent gestational diabetes in subsequent pregnancies. Ehrlich et al.[10] found that women with previous GDM had 17-fold higher risk of developing the condition again. The lower recurrence rate observed in our study (15.6%) may be attributed to differences in study population characteristics, diagnostic criteria employed, or the interval between pregnancies. Our findings reinforce the importance of preconception counseling and early screening in women with previous GDM history, as recommended by current clinical guidelines. Our study demonstrated a statistically significant increase in past history of fetal complications among the GDM group compared to controls (15.6% vs. 2.2%, $p=0.029$). This finding is consistent with the well-established association between maternal hyperglycemia and adverse perinatal outcomes. The rate of past history of fetal complications observed in our control group (2.2%) aligns with baseline rates reported in normoglycemic pregnancies, while the 15.6% rate in the GDM group falls within the range reported in previous studies. Ramezani et al.[11] demonstrated a continuous relationship between maternal glucose levels and adverse outcomes, even below the diagnostic threshold for GDM. The past history of fetal complication rate observed in our study emphasizes the clinical significance of early diagnosis and appropriate management of GDM to minimize maternal-fetal morbidity.

The current study revealed a statistically significant elevation in median serum Pentraxin-3 (PTX3) levels among pregnant women with gestational diabetes mellitus (GDM) compared to normoglycemic controls. Specifically, the median PTX3 level in the GDM group was 151.5 ng/mL (range: 63.9– 186.2), while it was 130.6 ng/mL (range: 2.62–168.9) in the control group ($p < 0.001$). This significant elevation reflects a pronounced inflammatory state associated with GDM, consistent with the notion that chronic low-grade inflammation plays a critical role in the pathophysiology of metabolic disorders, including insulin resistance and endothelial dysfunction. The current study identified a PTX3 cut-off value of 149.25 ng/mL, above which GDM cases were more frequent. Although PTX3 levels were significantly higher in the GDM group, the diagnostic performance of PTX3 was limited. Receiver Operating Characteristic (ROC) analysis revealed a sensitivity of 34.5% and specificity of 25.7%, with a moderate area under the curve (AUC) of 74.2% (95% CI: 0.642 to 0.842; $p < 0.001$). These values suggest that while PTX3 is statistically associated with GDM, it may not be a reliable standalone diagnostic marker due to its low discriminative power. This finding aligns with previous literature suggesting that PTX3, an acute-phase reactant produced by vascular endothelial cells and macrophages in response to pro-inflammatory cytokines, is elevated in metabolic and vascular conditions. For instance, Khamis et al.[12] reported that maternal serum level of pentraxin 3 was found to be significantly higher in pregnant women with gestational diabetes than

normoglycemic patients when measured at 24-28 weeks of gestation. Interestingly, no significant correlation was observed between PTX3 and hs-CRP (r = 0.066, p = 0.535), which suggests that these markers may reflect distinct inflammatory pathways—a hypothesis consistent with the findings of Furman et al.[13] who highlighted the multifaceted nature of inflammatory responses in metabolic conditions.

In this study, serum high-sensitive C-reactive protein (hs-CRP) levels were significantly higher in pregnant women with gestational diabetes mellitus (GDM) compared to healthy controls. The median hs-CRP level in the GDM group was 11.4 mg/L (range: 0.3–45.2), while in the control group it was 4.7 mg/L (range: 0.3–40.5), with a statistically significant difference (p = 0.02). This elevation in hs- CRP underscores a state of systemic inflammation in women with GDM, a finding consistent with the role of chronic low-grade inflammation in the pathogenesis of insulin resistance and metabolic dysfunction during pregnancy. Our study identified a cut-off value of 7.75 mg/L for hs-CRP, above which inflammatory risk was considered elevated. At this threshold, hs-CRP demonstrated a moderate diagnostic performance, with a sensitivity of 66.7%, specificity of 62.7%, and area under the curve (AUC) of 64.2% (p = 0.02; 95% CI: 0.526 to 0.758). While these values indicate some discriminatory ability, they also suggest that hs-CRP alone may not be sufficient as a stand-alone diagnostic biomarker for GDM. Nonetheless, its performance is notable, particularly when interpreted in the broader context of metabolic and inflammatory interplay. Previous studies have shown that the hs- CRP level in the first and second trimesters is positively correlated with the risk of GDM, meanwhile, the hs-CRP level in the second trimester is also related to the adverse pregnancy outcomes [14,15].

Zhou et al.[16] and Li et al.[17] showed that metabolic syndrome is associated with inflammatory mediators, the most representative of which is hs-CRP: elevated serum hs-CRP level has been found in patients with diabetes, hypertension, obesity, hyperlipidemia, and even cardiovascular diseases.

In our study, the median HbA1c level was significantly higher in the case group (6.6%, range: 5.9–7.3) compared to controls (4.9%, range: 4.2–5.5), with p < 0.001, indicating poor glycemic control. Using a cut-off of 3.2%, HbA1c achieved 100% sensitivity and specificity, demonstrating perfect diagnostic accuracy. These findings confirm HbA1c as a highly reliable biomarker for distinguishing GDM cases from healthy pregnancies and support its use in clinical diagnosis of glucose intolerance or overt diabetes.

In conclusion, our study highlights the significant elevation of both serum Pentraxin-3 (PTX3) and high sensitive C-reactive protein (hs-CRP) in pregnant women with GDM, reflecting an underlying inflammatory state. While PTX3 demonstrated statistical significance, its limited diagnostic accuracy suggests its utility may lie in supporting rather than replacing established diagnostic methods. hs- CRP, with moderate diagnostic performance and significant correlation with glycemic markers, further reinforces the inflammatory component of GDM. The lack of correlation between HS-CRP and PTX3 is a noteworthy finding, implying they may represent distinct inflammatory pathways or provide unique prognostic insights. Notably, HbA1c emerged as the most reliable marker with perfect diagnostic accuracy. These findings suggest that incorporating inflammatory biomarkers alongside traditional glycemic parameters may improve early detection, risk assessment, and management strategies for GDM.

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Ethical approval

The study protocol was reviewed and approved by the Committee of Medical Ethics, Faculty of Medicine, Assiut University IRB no. 04-2023-200033.

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