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MACROPHAGE POLARIZATION IN PLACENTAS OF GESTATIONAL DIABETIC MOTHERS: AN IMMUNOHISTOCHEMICAL STUDY OF CD68 AND CD163 EXPRESSION IN A TERTIARY CARE CENTRE

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ABSTRACT

Introduction: Gestational diabetes mellitus (GDM) is associated with metabolic and inflammatory alterations that can significantly impact structure and function of placenta. Placental macrophages including hofbauer cells play a key role in maintaining immune homeostasis, alterations in density and polarization which may contribute to pathophysiology of GDM.

Materials and Methods: A case–control study was conducted at a tertiary care centre on 80 placenta (40 GDM, 40 controls). Placental tissues were processed for H&E staining and immunohistochemistry for CD68 and CD163 to quantify placental macrophages including hofbauer cells and assess macrophage polarization (CD163/CD68 ratio). Data were analyzed in SPSS version24 using Chi-square/Fisher's exact tests and t-test/ANOVA; $p < 0.05$ was considered significant.

Results: CD68-positive macrophages were significantly increased in GDM placentas compared to controls (23.1 ± 2.6 vs 10.2 ± 1.5 per 10 HPF; 64.0 ± 4.5 vs 29.8 ± 5.8 per 100 villi; $p < 0.001$). CD163-positive macrophages per 10 HPF were also significantly elevated in GDM cases (44.8 ± 5.3 vs 17.0 ± 2.7; $p < 0.001$). A significant positive correlation was observed between OGCT levels and macrophage markers (CD68 and CD163), indicating an association between maternal glycemic status and placental immune response.

Conclusion: GDM is associated with increased macrophage infiltration along with preservation of anti-inflammatory M2 polarization, suggesting a state of adaptive yet dysregulated immune response. These findings highlight the role of macrophage mediated immune modulation in placental dysfunction in GDM and suggest potential avenues for therapeutic targeting.

KEYWORDS: Gestational diabetes mellitus, Placenta, immunohistochemistry, CD68 and CD163.

INTRODUCTION

Gestational diabetes mellitus (GDM), a common metabolic disorder, is characterized by glucose intolerance with its first recognition during pregnancy. It is associated with significant maternal and fetal complications such as preeclampsia, cesarean delivery, fetal macrosomia, and long-term metabolic risk in offspring.[1] It is a state of metabolic dysregulation that extends beyond systemic glucose imbalance to influence placental structure and function.

The placenta plays a key role as the port between maternal and fetal systems, regulating nutrient transport, endocrine activity, and immune tolerance. In GDM, altered glucose metabolism leads to changes in placental physiology by increasing nutrient transfer and metabolic adaptations such as the fetal glucose steal phenomenon, which in turn contributes to fetal overgrowth and placental stress.[2] Within the placenta, these alterations are often accompanied by immune and inflammatory changes.

Placental macrophages which are known as Hofbauer cells are key mediators of immune regulation, tissue remodelling, and angiogenesis within the chorionic villi. These cells predominantly exhibit an anti-inflammatory M2 phenotype under normal physiological conditions, which supports immune tolerance and maintenance of pregnancy. But, a hyperglycemic and pro-inflammatory environment in GDM may influence macrophage behavior and functional polarization.[3]

Macrophage polarization is a dynamic process that is influenced by local microenvironmental signals. M1 macrophages are associated with pro-inflammatory responses and M2 macrophages are involved in anti-inflammatory activity, tissue repair, and immune regulation. Dysregulation of this balance has been connected in various placental pathologies, where altered macrophage-trophoblast interactions cause abnormal placental development and function.[4]

Recent evidence suggests that GDM is associated with significant alterations in placental immune responses, including changes in cytokine profiles and macrophage polarization patterns.[5] It has been reported that in GDM placentas, the increased expression of a M2 macrophage marker-CD163 indicates a potential compensatory anti-inflammatory response to metabolic stress.[6]The presence of an immunologically active and dysregulated placental environment in GDM is further supported by alterations in other immune cell populations and cytokine networks.[7]
The combined evaluation of macrophage density and polarization using CD68 and CD163 markers are limited. It is unclear whether GDM induces a classical shift toward a pro-inflammatory phenotype or results in a more complex pattern of immune adaptation and dysregulation. The present study aims to evaluate and compare macrophage density and polarization using CD68 and CD163 expression in GDM placentas and normoglycemic placentas.

MATERIALS AND METHODS

The present case control study was carried out in the Department of Pathology at a tertiary care centre, from June 2025 to January 2026. In accordance with the sample size formula prescribed by the standards, 80 placental samples were collected, including 40 cases of gestational diabetes mellitus (GDM) and 40 control cases of normal pregnancy.
The inclusion criteria are placenta of women with Oral Glucose Challenge Test (OGCT) >140mg/dL, placenta of gestational diabetes mellitus women with live births, placenta of women with normal pregnancies as controls. Patients with pre-existing diabetes, hypertension, multiple pregnancies, infections, other systemic diseases, or those who refused to consent were excluded. A written informed consent form is obtained from all the participants.
Placental tissue samples were taken immediately after the delivery and fixed in 10% neutral buffered formalin. Besides determining the gross features and recording the placental weight, tissue bits were chosen for further processing, paraffin embedding, and cutting to 4 μm for staining with H&E to examine the histology. Immunohistochemistry was carried out on 4μm sections using peroxidase-anti peroxidase method after microwaving antigen retrieval. Monoclonal antibodies against CD68 and CD163 (PATHNSITU) were applied with DAB as the chromogen and hematoxylin as the counterstain. CD68 staining recognized the macrophages (Hofbauer cells) present in the villous stroma and was quantified as number of positive cells per 10 HPF and per 100 villi. Macrophages in the decidua, chorion and villi showed positivity for CD163 and were quantified as CD163 positive cells / 10 HPF after excluding chorion and decidua to avoid maternal macrophages. The CD163/CD68 ratio was also determined to highlight macrophage polarization, with greater values reflecting an M2 phenotype.
Charts were compiled and analyzed statistically using Microsoft Excel and IBM SPSS version 24. Qualitative data were described as frequency and percent and analyzed by Chi-square or Fisher's exact test. Quantitative data were represented as mean and standard deviation and analyzed using a paired t-test and one-way ANOVA. A p-value less than 0.05 was deemed statistically significant.

RESULTS

A total of 80 placental specimens were analyzed in this study, comprising 40 from pregnancies affected by gestational diabetes mellitus (GDM) and 40 from normoglycemic controls. Maternal age, gravida, gestational age at delivery, and mode of delivery, the baseline variables, were similar between the two groups, hence proper matching (**Table 1**).

Table 1: Baseline characteristics of study population.

Parameter	Variable	GDM n (%) / Mean ± SD	Control n (%) / Mean ± SD	P value
Age group (years)	21 to 25	12 (30.0%)	10 (25.0%)	0.893
	26 to 30	14 (35.0%)	17 (42.5%)	
	31 to 35	14 (35.0%)	13 (32.5%)	
Gravida	G1	13 (32.5%)	14 (35.0%)	0.967
	G2	17 (42.5%)	16 (40.0%)	
	G3	10 (25.0%)	10 (25.0%)	
Maternal anthropometrics	Weight – 1st trimester (kg)	67.2 ± 3.8	59.8 ± 2.9	0.028
	Weight – delivery (kg)	75.8 ± 3.0	72.0 ± 3.4	0.001
	BMI – 1st trimester (kg/m²)	27.4 ± 2.4	24.4 ± 1.4	0.001
	BMI – delivery (kg/m²)	30.9 ± 2.1	29.4 ± 1.9	0.048
OGCT	OGCT (mg/dL)	149.9 ± 4.6	94.1 ± 11.5	0.001

Maternal anthropometric measures and glycemic status, however, showed significant differences, confirming metabolic distinction between cases and controls(**Table 1**).
Placentae from GDM pregnancies showed greater placental weight, more syncytial knot formation, more fibrin deposition, and a higher frequency of central infarctions together with the features of villous immaturity (**Table 2**). **Figure 1 to 6** illustrate the H&E features of placentas of GDM and normoglycemic mothers.

Table 2: Placental morphological and histopathological features.

Parameter	GDM (n=32) n (%) / Mean ± SD	Control (n=32) n (%) / Mean ± SD	P value
Placental weight (g)	529.2 ± 81.8	443.7 ± 83.9	0.001

Feto-placental ratio	6.3 ± 1.2	6.8 ± 1.3	0.078
Syncytial knots (%/100 villi)	32.8 ± 12.7	20.5 ± 7.7	0.001
Villous immaturity – Diffuse	7 (17.5%)	0 (0.0%)	0.029
Villous immaturity – Focal	10 (25.0%)	8 (20.0%)	
Villous immaturity – Nil	23 (57.5%)	32 (80.0%)	
Fibrin deposition – Score 0	8 (20.0%)	15 (37.5%)	0.001
Fibrin deposition – Score 1	11 (27.5%)	17 (42.5%)	0.048
Fibrin deposition – Score 2	21 (52.5%)	8 (20.0%)	
Placental infarction – Central	10 (25.0%)	6 (15.0%)	

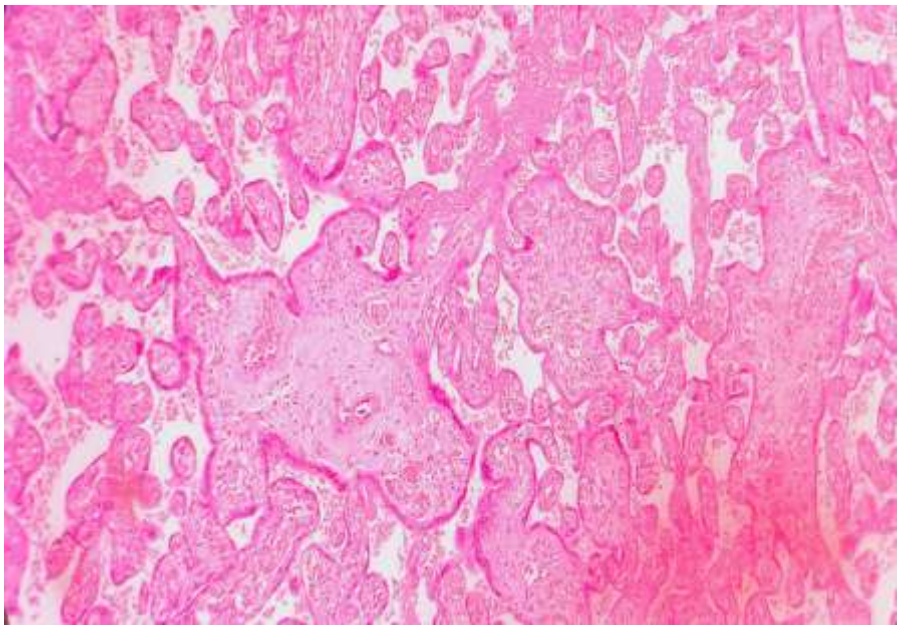

Figure 1: Villous immaturity (H&E, 100x)

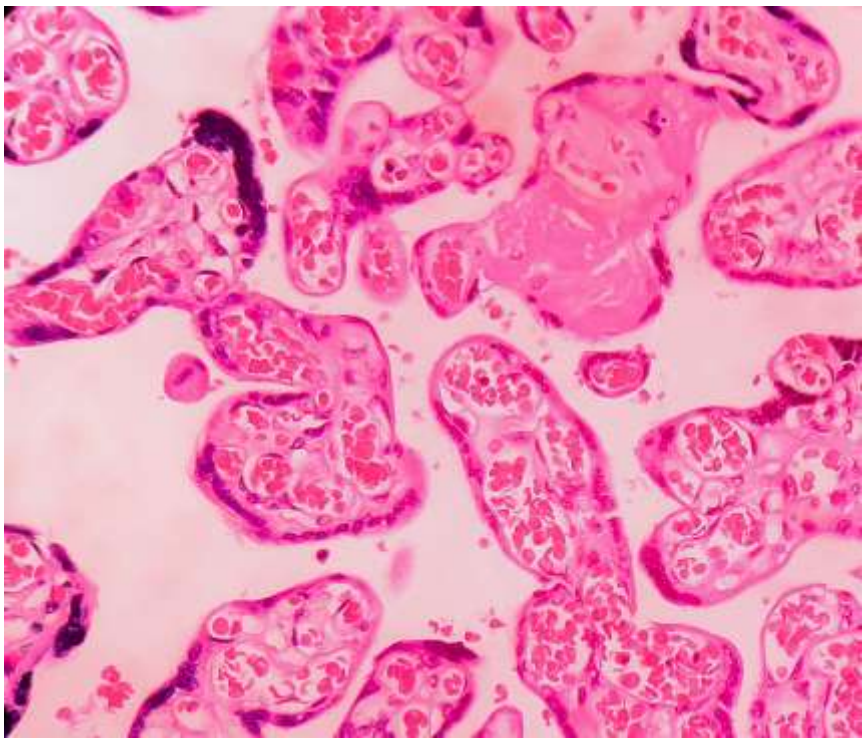

Figure 2: Syncytial knots in placenta of normoglycemic mother (H&E, 400x)

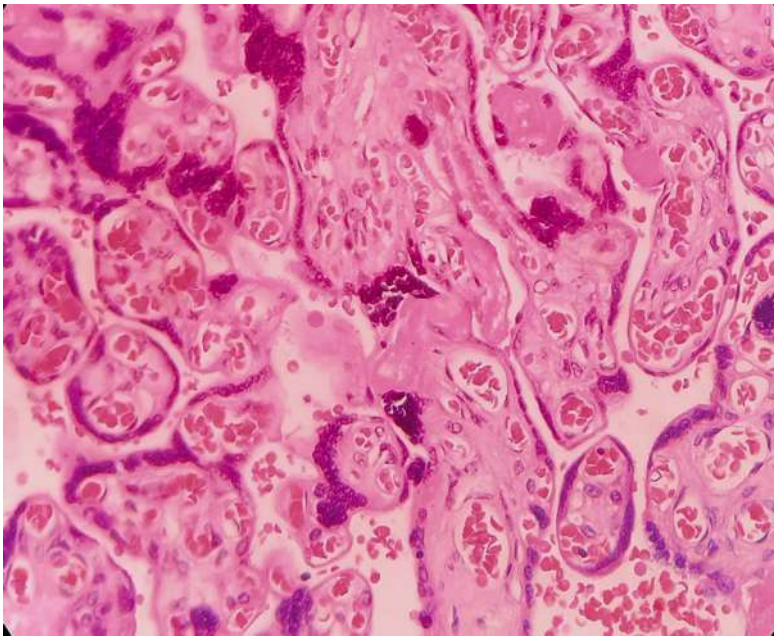

Figure 3: Syncytial knots in placenta of GDM mother (H&E, 400x)

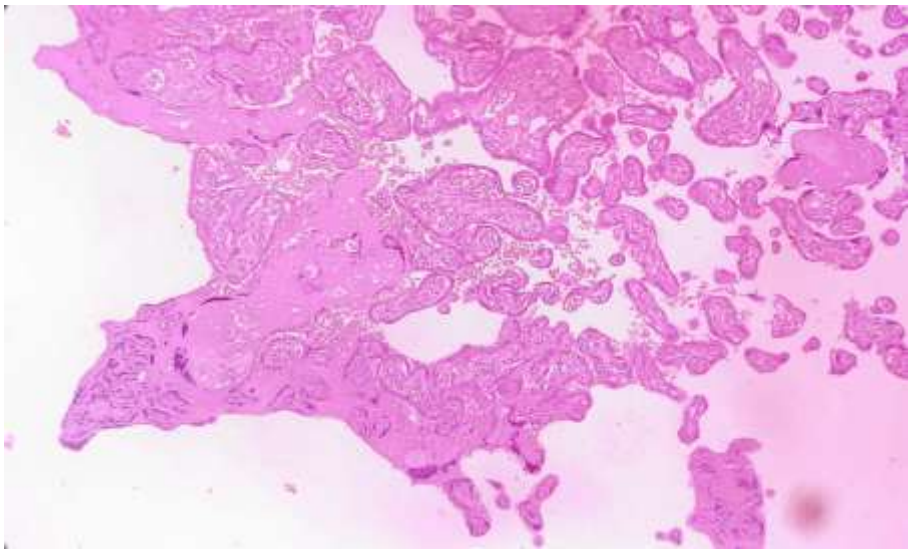

Figure 4: Intervillous and perivillous fibrin deposition in placenta of GDM mother (H&E,100x)

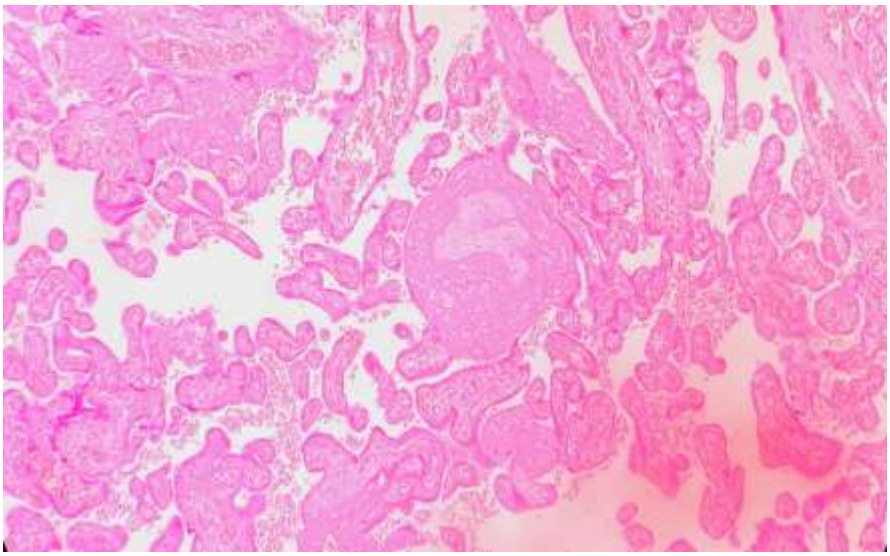

Figure 5: Areas of infarction in placenta of normoglycemic mother (H&E. 100x)

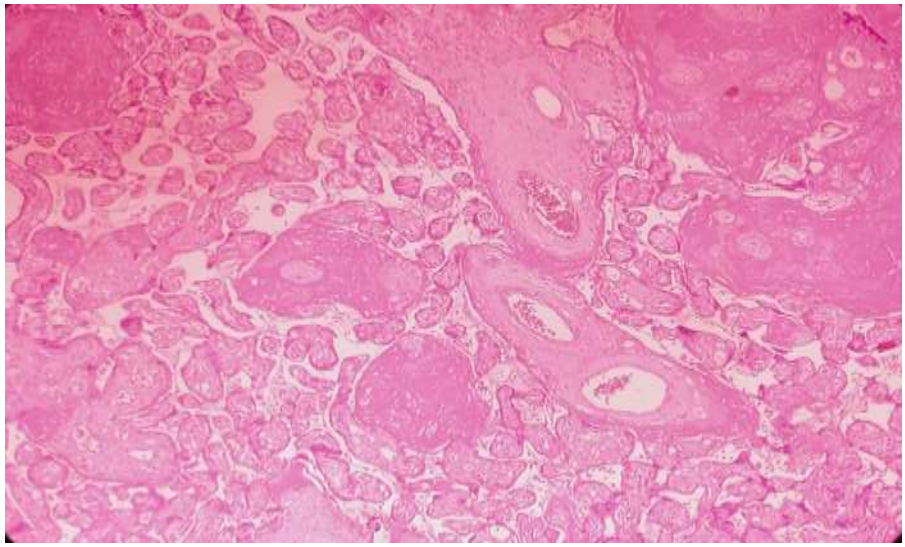

Figure 6: Areas of infarction in placenta of GDM mother (H&E. 100x)

Immunohistochemical studies showed a significant rise in both CD68-positive and CD163-positive macrophages in the GDM placentae. A positive correlation was found between OGCT values and macrophage markers which means that increased maternal glycemia is associated with greater macrophage infiltration and altered macrophage polarization in the placenta (**Table 3, 4**). **Figure 7, 8** illustrate the CD68+ cells per villi in mothers of normoglycemic and GDM placentas respectively. **Figure 9, 10** show placenta from a normoglycemic and GDM mothers showing CD163+ cells per villi respectively.

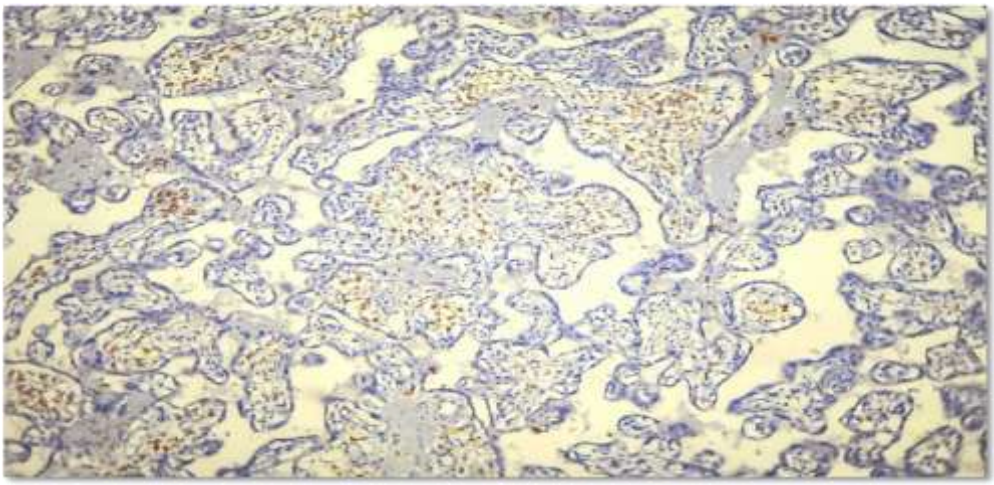

Figure 7: Placenta showing 12 CD68+ cells per villi in normoglycemic mother (CD68 IHC, 100x)

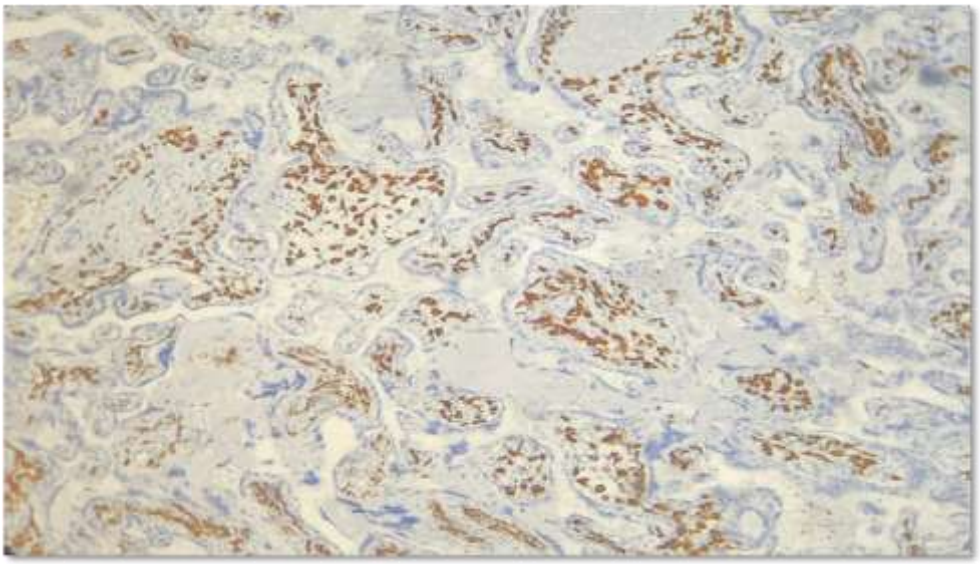

Figure 8: GDM Placenta showing 30 CD68+ cells per villi (CD68 IHC, 100x)

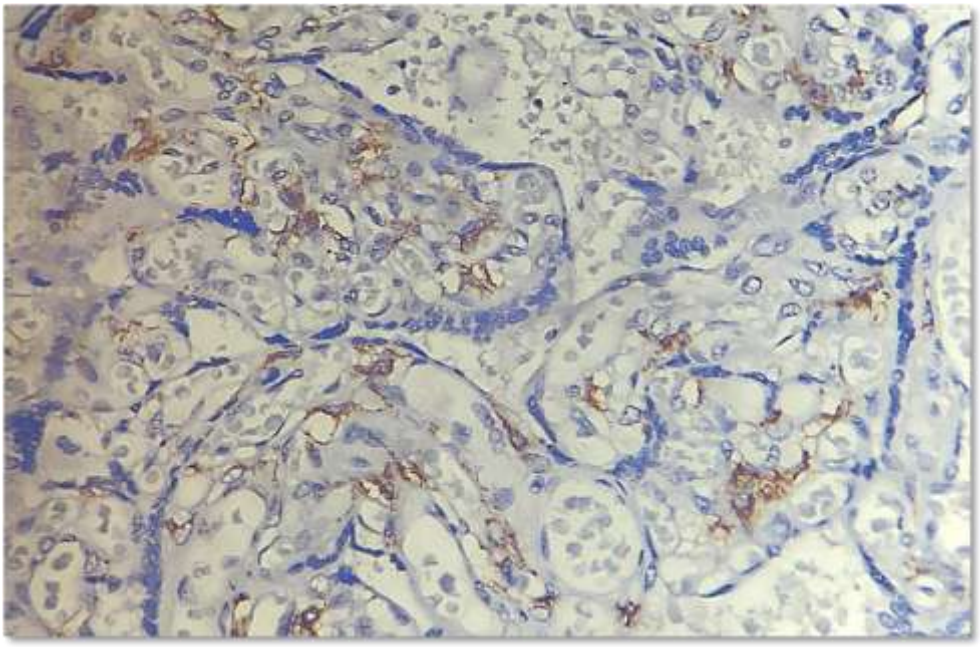

Figure 9: Placenta from a normoglycemic mother showing 6 CD163+ cells per villi (CD163 IHC, 400x)

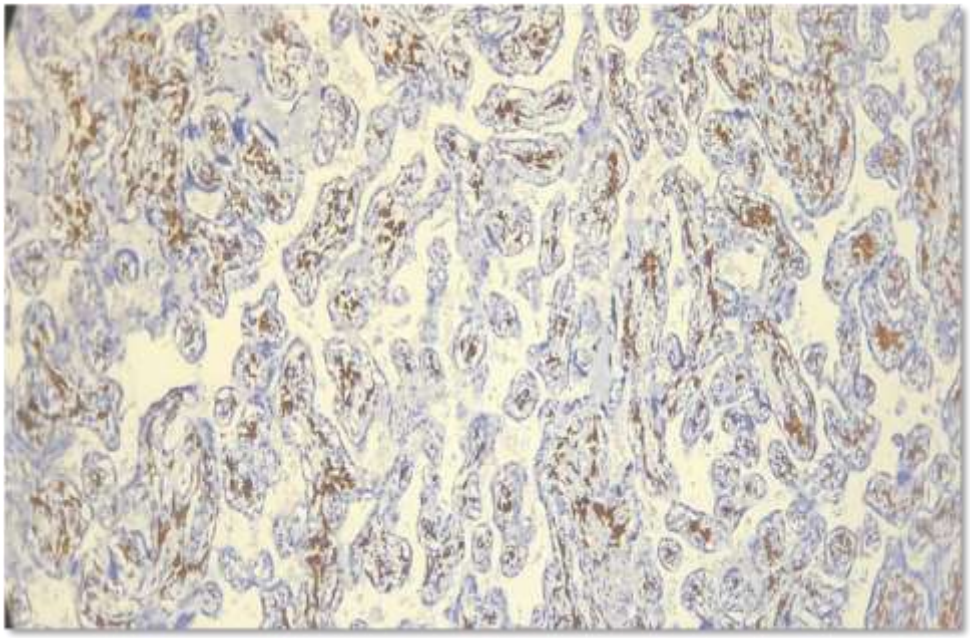

Figure 10: Placenta from a GDM mother showing 20 CD163+ cells per villi (CD163 IHC, 100x)

Table 3: Comparison of CD68 and CD163 Expression (Macrophage Markers)

Parameter	GDM (Mean ± SD)	Control (Mean ± SD)	P value
CD68+ cells / 10 HPF	23.1 ± 2.6	10.2 ± 1.5	0.001
CD68+ cells / 100 villi	64.0 ± 4.5	29.8 ± 5.8	0.001
CD163+ cells / 10 HPF	44.8 ± 5.3	17.0 ± 2.73	0.001
CD163/CD68 Ratio	1.94	1.67	0.001

Table 4: Macrophage markers and correlation with OGCT

Parameter	r value	P value
OGCT vs CD68+ cells / 10 HPF	0.402	0.031
OGCT vs CD68+ cells / 100 villi	0.395	0.034
OGCT vs CD163+ cells / 10 HPF	0.399	0.032

DISCUSSION

Pregnancy-induced diabetes, or gestational diabetes mellitus (GDM), is a frequently occurring metabolic pregnancy disorder characterized by glucose intolerance first developing or identified during pregnancy. Besides the metabolic

effects, GDM is viewed as a condition of chronic moderate inflammation and immune imbalance at the maternal, fetal interface. Being a highly active immunological organ, the placenta reacts to the mother's metabolic stress by changing its cellular makeup and immune communication. Located among the main immune cells in the placental villous stroma are Hofbauer cells, which have a crucial function in keeping immune tolerance, controlling trophoblast activity, and assisting placental growth. Hence, it is important to understand how these macrophages react to a high glucose environment to uncover the mechanism of placental alterations in GDM.[8,9]

The present study demonstrated significantly higher expression of macrophage-associated markers, including CD68 and CD163, in placentas from the study group compared to controls. Increased densities of CD68-positive and CD163-positive cells suggest enhanced macrophage presence and altered immune activation within the placental villous stroma in gestational diabetes mellitus. These findings indicate an immunologically activated placental microenvironment in metabolically compromised pregnancies.

High blood glucose levels in pregnant women create a pro-oxidant environment for the placenta and stimulate the production of advanced glycation end products as well as cytokines, which all lead to an increase in placental inflammatory signaling. Earlier research and clinical data have confirmed that oxidative and inflammatory mechanisms, especially those related to IL-1β and Toll-like receptor pathways, are more active in placentas of GDM women, resulting in trophoblast impairment and disruption of placental functions. These events quite likely account for the rise in CD68 macrophage infiltration found by us and go along with other studies reporting placental inflammation in GDM[10].

While CD163 indicates alternatively activated, anti-inflammatory macrophages engaged in tissue remodeling and immunological control, CD68 functions as a pan-macrophage marker reflecting the total macrophage burden. In GDM, chronic metabolic stress and low-grade inflammation promote recruitment and activation of placental macrophages. Although Hofbauer cells typically maintain an M2-like phenotype, sustained hyperglycaemic and oxidative stress can alter macrophage functional profiles, leading to increased cytokine production, extracellular matrix remodeling, and modulation of angiogenic signaling within the placenta[3,6].

Barke et al. reported increased CD163 expression and iron storage in placentas from GDM pregnancies, suggesting enhanced M2 macrophage activity in response to metabolic stress[6]. Schliefsteiner et al. demonstrated that although Hofbauer cells in GDM maintain an M2 phenotype, their functional responses to inflammatory stimuli are altered, highlighting subtle immunological dysregulation in diabetic placentas[3]. **Gosain et al.** also reported increased CD68-positive macrophage infiltration in GDM placentas, particularly in regions adjacent to fetal vasculature[11]. These findings align with the present observations of heightened macrophage marker expression in GDM placentas.

Variations in reported macrophage marker expression across studies may result from differences in placental sampling sites, gestational age at delivery, and methods used to quantify macrophage density. Discrepancies in defining macrophage subsets, antibody specificity, and immunohistochemical scoring criteria can also contribute to heterogeneity. Additionally, heterogeneity in maternal metabolic control and the presence of coexisting inflammatory or infectious conditions may modulate placental macrophage profiles[12].

This study reveals quite a pronounced and highly statistically significant positive association between maternal OGCT values and the levels of both macrophage markers, which was a clear demonstration of the fact that the glycemic condition exerts a direct effect on the immune responses of the placenta. The idea of the "fetal glucose steal, " as put forward by DesoyeG and Nolan CJ, not only complements these data but also highlights the fact that continuous maternal hyperglycemia is responsible for the alteration of placental metabolic and inflammatory pathways leading to changes in immune cell behavior within the villous stroma[2].

Collectively, comparison with existing literature indicates that GDM placentae exhibit increased macrophage infiltration while simultaneously maintaining anti-inflammatory polarization characteristics. This dual response represents an adaptive yet dysregulated immune mechanism aimed at preserving placental homeostasis under metabolic stress. Studies have unequivocally supported the notion that the placental cytokine milieu and the macrophage polarization patterns are altered in GDM.[13] Rather than a purely inflammatory shift, the placental immune environment in GDM appears to involve a complex interplay between inflammatory activation and compensatory regulation[5].

The present study has demonstrated that the two markers CD68 and CD163 can coexist at elevated levels, which lends support to the idea of macrophage functional plasticity rather than strict conformity to the classical M1/M2 dichotomy. In fact, the two reviews by ZuluM. Z. et al[14]., and Reyes L et al[15]., put a spotlight on the fact that Hofbauer cells are extremely versatile and can radically change their phenotypes in response to different environmental factors. In other words, a recent study on GDM and abnormal fetal growth (taking into account the placental tissue also) showed that under metabolic stress, Hofbauer cells are capable of functionally hybrid states, which alludes to the mixed macrophage profile seen in this study[16].

Increasing maternal hyperglycaemia intensifies oxidative stress, inflammatory signaling, and endothelial dysfunction within placental tissues. Elevated glucose exposure promotes macrophage recruitment and activation. These responses likely represent adaptive attempts to maintain placental perfusion and nutrient transfer under metabolic stress, but may become maladaptive with sustained hyperglycaemic exposure, contributing to placental dysfunction and altered fetal programming[17,18].**Lappas et al.** demonstrated that increasing maternal glucose levels were associated with amplified oxidative stress and inflammatory mediator expression in placental tissues from GDM pregnancies[17].

CONCLUSION

Gestational diabetes mellitus is associated with significant placental immune alteration characterized by increased macrophage infiltration and modified polarization, evidenced by elevated CD68 and CD163 expression. This pattern indicates that placental macrophages in GDM exhibit adaptive immune dysregulation rather than a purely pro-

inflammatory shift. The positive correlation between maternal glycemic levels and macrophage markers further confirms the influence of metabolic stress on placental immune responses. These findings highlight the crucial role of macrophage dynamics in the pathophysiology of placental dysfunction in GDM and suggest their potential as targets for therapeutic intervention to improve maternal and fetal outcomes.

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