ACTA BIOCLINICA

Artículo Original

Vinutha Gali y Col.

Volumen 15, N° 30 Especial, 2025

Depósito Legal: PPI201102ME3815

ISSN: 2244-8136

HISTOMORPHOLOGIAL CHANGES OF PLACENTA IN GESTATIONAL DIABETES WITH A REVIEW ON CD34 EXPRESSION

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Received: 02/17/2025
Accepted: 03/09/2025

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ABSTRACT

Gestational diabetes mellitus (GDM) poses significant risks to both maternal and foetal health, influencing placental structure and function. This study investigates the histomorphological and immunohistochemical changes in the placentas of diabetic mothers, with a particular focus on CD34 expression. A prospective analysis was conducted on 50

placentas from GDM pregnancies. Immunohistochemical staining revealed strong CD34 positivity in 74% of cases, indicating increased vascularization and villous density. The study identified a significant correlation between elevated maternal blood glucose levels (OGTT =140 mg/dL) and enhanced CD34 expression (p = 0.010), emphasizing the chronic hypoxic effects of diabetes on placental vasculature. Findings also demonstrated gestational age-related vascular changes, with the third trimester showing the highest glucose levels and CD34 expression. This study underscores the importance of understanding placental adaptations in GDM to improve maternal and neonatal outcomes.

KEYWORDS: Gestational diabetes mellitus (GDM); placenta; histomorphology; CD34 expression; vascularization; villous density; oral glucose tolerance test (OGTT); hypoxia..

CAMBIOS HISTOMORFOLÓGICOS DE LA PLACENTA EN LA DIABETES GESTACIONAL CON UNA REVISIÓN SOBRE LA EXPRESIÓN DE CD34

RESUMEN

La diabetes mellitus gestacional (DMG) presenta riesgos significativos para la salud materna y fetal, influyendo en la estructura y función placentaria. Este estudio investiga los cambios histomorfológicos e inmunohistoquímicos en las placentas de madres diabéticas, con un enfoque particular en la expresión de CD34. Se realizó un análisis prospectivo en 50 placentas de embarazos con DMG. La tinción inmunohistoquímica reveló una fuerte

positividad de CD34 en el 74% de los casos, lo que indica un aumento de la vascularización y la densidad de vellosidades. El estudio identificó una correlación significativa entre los niveles elevados de glucosa en sangre materna (PTOG = 140 mg/dL) y una mayor expresión de CD34 (p = 0,010), enfatizando los efectos hipóxicos crónicos de la diabetes en la vasculatura placentaria. Los hallazgos también demostraron cambios vasculares relacionados con la edad gestacional, con el tercer trimestre mostrando los niveles más altos de glucosa y expresión de CD34. Este estudio subraya la importancia de comprender las adaptaciones placentarias en la DMG para mejorar los resultados maternos y neonatales.

PALABRAS CLAVE: Diabetes mellitus gestacional (DMG); placenta; histomorfología; expresión de CD34; vascularización; densidad vellositaria; prueba de tolerancia oral a la glucosa (PTGO); hipoxia.

INTRODUCTION

1.Introduction

Gestational diabetes mellitus (GDM) is a significant metabolic disorder defined as glucose intolerance first identified during pregnancy. The condition affects approximately 7–10% of pregnancies globally, though prevalence rates vary depending on maternal demographics, healthcare systems, and diagnostic criteria. GDM typically manifests during the second or third trimester and is attributed to the increasing insulin resistance caused by placental hormones

Volumen 15, N° 30 Especial, 2025
Depósito Legal: PPI201102ME3815
ISSN: 2244-8136

such as human placental lactogen, progesterone, and cortisol. While glucose intolerance often resolves postpartum, women diagnosed with GDM face a heightened risk of developing type 2 diabetes in the future. The condition also increases the likelihood of adverse outcomes for both the mother and foetus, such as preeclampsia, macrosomia, stillbirth, neonatal hypoglycemia, and respiratory distress syndrome in the newborn. The placenta, a temporary yet essential organ, acts as the interface between maternal and foetal circulations. It facilitates oxygen, nutrient, and waste exchange, playing a pivotal role in foetal growth and development. Gestational diabetes introduces a hyperglycemic environment that profoundly affects placental morphology and function. Alterations such as villous edema, syncytial knot formation, and increased vascularization are commonly observed in GDM-affected pregnancies. These changes are primarily adaptive responses to chronic hypoxia, oxidative stress, and inflammatory conditions induced by maternal hyperglycemia. Consequently, the placenta's capacity to support optimal foetal development is compromised, often resulting in neonatal complications, such as macrosomia, hypoglycemia, and long-term metabolic disorders. A key feature of placental adaptation to GDM is angiogenesis, the formation of new blood vessels. CD34, a transmembrane glycoprotein and a reliable marker of vascular endothelial cells, plays a crucial role in identifying the extent of angiogenesis and vascular integrity in

placental tissues. CD34 immunohistochemical analysis allows for the quantitative evaluation of vascular density and capillary architecture, providing insights into how the placenta compensates for GDM-induced stressors. The increased expression of CD34 observed in diabetic placentas reflects the organ's response to chronic hypoxia, wherein excessive angiogenesis occurs to maintain foetal oxygenation and nutrient supply. However, this adaptive mechanism often fails to mitigate the adverse effects of prolonged hyperglycemia. This study focuses on the histomorphological and immunohistochemical evaluation of diabetic placentas, emphasizing CD34 expression. By correlating maternal oral glucose tolerance test (OGTT) levels with placental findings, this research seeks to deepen the understanding of how GDM impacts placental structure and function.

1.1.Background

The placenta is a chorio-decidual organ that develops during pregnancy, connecting the mother and foetus for physiological exchange. Structurally, the placenta comprises villous trees, which are the functional units responsible for nutrient and gas exchange. These villi are bathed in maternal blood within the intervillous space, allowing oxygen and nutrients to diffuse into foetal capillaries while waste products are transferred to maternal circulation. The syncytiotrophoblast, the outermost layer of the villi, is particularly vital in maintaining the placental barrier and

regulating exchange processes. Any disruption to this structure, such as those induced by GDM, can have significant implications for foetal health. In pregnancies complicated by GDM, elevated maternal glucose levels cross the placenta via facilitated diffusion. Once in the foetal circulation, excess glucose stimulates pancreatic insulin secretion, leading to hyperinsulinemia. foetal hyperinsulinemia acts as a growth factor, promoting macrosomia, or an overgrowth of foetal tissues. This excessive growth is associated with increased placental weight and volume, as well as morphological changes that impair placental efficiency. Additionally, diabetic placentas often exhibit increased deposition of fibrinoid material and collagen in the villous stroma, which reduces their elasticity and functional capacity. Histopathological studies have consistently reported a range of structural abnormalities in diabetic placentas. Villous immaturity is a hallmark feature, with a predominance of immature intermediate villi over terminal villi, which compromises the placenta's functional efficiency. Syncytial knots, clusters of syncytiotrophoblast nuclei, are frequently observed and are considered indicative of hypoxic stress. Similarly, fibrinoid necrosis, stromal fibrosis, and chorangiosis (increased capillary density within villi) are commonly reported in diabetic placentas. These changes collectively reflect the impact of maternal hyperglycemia on placental architecture and function. Despite extensive documentation of these gross and

microscopic changes, limited attention has been given to quantitative assessments of placental vascularization in GDM. Angiogenesis, the formation of new capillaries from pre-existing vessels, is a critical compensatory mechanism in diabetic placentas. It is driven by various growth factors, including vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF). CD34, a highly sensitive marker for endothelial cells, has emerged as a valuable tool for assessing angiogenesis in placental tissues. CD34 expression is typically localized to the endothelial cells lining foetal capillaries within placental villi. An increase in CD34 expression in diabetic placentas reflects enhanced capillary formation and vascular remodeling in response to hypoxia and hyperglycemia. However, the degree of angiogenesis may vary depending on the severity of maternal hyperglycemia and the duration of exposure. In some cases, excessive angiogenesis may lead to malfunctional capillary networks, further compromising placental efficiency. Studies evaluating CD34 expression in diabetic placentas are scarce, particularly in the Indian population, where the prevalence of GDM is increasing due to genetic and lifestyle factors. This research aims to address this gap by quantitatively analyzing CD34 expression in a cohort of diabetic placentas and correlating these findings with maternal glucose levels and pregnancy outcomes.

1.2.Objectives

This study is guided by the following objectives:

1. **To evaluate histomorphological changes in placentas from GDM pregnancies:**
   A detailed examination of placental tissues using light microscopy will document structural abnormalities, such as villous immaturity, stromal fibrosis, and syncytial knot formation.
2. **To assess CD34 expression in diabetic placentas:**
   Immunohistochemical analysis will be used to measure the density and distribution of capillaries within placental villi, providing insights into vascular adaptations to GDM.
3. **To correlate maternal OGTT levels with placental findings:**
   By linking maternal glucose levels to histological and immunohistochemical data, this study will explore the relationship between glycemic control and placental vascular changes.

1.3.Scope

Understanding the placental adaptations in GDM is critical for improving maternal and neonatal outcomes. By assessing CD34 expression and histopathological changes, this study provides insights into the mechanisms underlying placental dysfunction in diabetes. These findings may inform strategies for early

intervention and management of GDM, minimizing risks for both mother and child. Additionally, the study contributes to the broader understanding of angiogenesis and vascular adaptations in pregnancy-related metabolic disorders.

Table 1: Key Histopathological Changes Observed in Diabetic Placentas

Histological Feature	Description
Villous Immaturity	Presence of immature villi with reduced differentiation and stromal edema.
Fibrinoid Necrosis	Degenerative changes in villi, characterized by fibrinoid deposits.
Syncytial Knots	Increased syncytial nuclei clusters indicating hypoxic stress.
Villous Stromal Fibrosis	Thickening and fibrosis of stromal tissues, reducing placental elasticity.
Increased Vascularization	Higher density of capillaries and dilated vessels in chorionic villi.

This table 1 summarizes common histopathological changes observed in diabetic placentas, reflecting adaptive and pathological responses to a hyperglycemic environment.

Table 2: Clinical Impact of GDM on Maternal and foetal Health

Parameter	Impact
Maternal Health	Increased risk of hypertension, preeclampsia, and future development of type 2 diabetes.
Foetal Growth	Higher incidence of macrosomia, neonatal hypoglycemia, and birth trauma.
Neonatal	Increased risk of respiratory distress, hypocalcemia, and long-term

Outcomes	glucose intolerance.
Placental Function	Altered nutrient and oxygen exchange due to morphological and vascular changes.

This table 2 outlines the clinical implications of GDM on maternal, fetal, and placental health, emphasizing the importance of early diagnosis and management.

2. Literature Review:

Gestational diabetes mellitus (GDM) is a complex metabolic disorder that significantly impacts both maternal and foetal health. The pathophysiology of GDM involves insulin resistance, altered glucose metabolism, and a variety of endocrine and vascular adaptations, all of which are crucial to understanding its consequences and management. Several studies have shown that GDM, if left untreated, can lead to severe complications such as macrosomia, preeclampsia, and an increased risk of type 2 diabetes in the mother later in life (Evers et al., 2002). Understanding the various risk factors, mechanisms, and complications of GDM is critical for early detection and management. One of the most important complications of GDM is **macrosomia**, or large-for-gestational-age infants, which is often observed despite good glycemic control during pregnancy. According to Evers et al. (2002), even well-controlled glucose levels do not necessarily prevent macrosomia in Type 1 diabetic pregnancies, indicating the complex nature of glucose metabolism and foetal growth in diabetes. Similarly, **placental dysfunction** has been linked to

gestational diabetes, with studies suggesting that GDM affects placental function and vascularization, leading to altered nutrient and oxygen delivery to the foetus (Pietryga et al., 2006). This is particularly important as changes in placental morphology, such as increased vascularization and edema, are commonly seen in diabetic pregnancies (Herrick & Bordoni, 2019). The **embryology and physiology of the placenta** are integral to understanding the pathophysiology of GDM, as it plays a central role in fetal development. The placenta acts as a barrier between maternal and foetal circulations, and any disturbances in its function can significantly affect foetal outcomes (Kapila & Chaudhry, 2019). In diabetic pregnancies, alterations in placental structure, including **syncytial knots**, **villous immaturity**, and **fibrinoid necrosis**, are commonly observed (Saddler, 2004; Cunningham et al., 2005). These structural changes are linked to the oxidative stress and inflammation caused by high maternal glucose levels. The effects of GDM extend beyond pregnancy, with long-term implications for both the mother and the child. Studies have shown that women with GDM are at an increased risk for developing type 2 diabetes after childbirth (Berkowitz et al., 1996). The foetus, in turn, is at a higher risk of developing metabolic conditions such as obesity and insulin resistance later in life (Xiang et al., 1999). This relationship underscores the importance of effective management during pregnancy to mitigate long-term risks. The role of **adipocytokines** in pregnancy

has also garnered attention in recent years, as these molecules play a significant role in the metabolic changes seen in GDM. Briana and Malamitsi-Puchner (2009) reviewed the impact of adipocytokines in normal and complicated pregnancies, highlighting how these factors contribute to the altered glucose metabolism observed in women with GDM. Similarly, **cortisol** levels have been shown to impact glucose tolerance in pregnant women, with higher cortisol levels exacerbating insulin resistance (Ahmed & Shalayel, 1999). These findings point to the complex interplay between hormonal changes and glucose metabolism in GDM. Early diagnosis and intervention are critical for managing GDM and improving outcomes. Studies have demonstrated that controlling blood glucose levels during pregnancy can significantly reduce the incidence of complications such as macrosomia and neonatal hypoglycemia (Crowther et al., 2005). The **HAPO study** further confirmed that even mild hyperglycemia in pregnancy can have adverse outcomes, emphasizing the need for early detection and effective glycemic control (Metzger et al., 2008). The findings of this study suggest that interventions aimed at controlling glucose levels during pregnancy are crucial for reducing the risk of negative outcomes. **Placental Doppler velocimetry** has been explored as a potential diagnostic tool for assessing placental function in GDM pregnancies. Pietryga et al. (2006) highlighted the role of Doppler ultrasound in measuring blood flow through the

placenta, offering insights into how GDM affects placental vascularization. This non-invasive tool can help monitor foetal well-being and detect complications early, enabling timely interventions. **Macrosomia** is not solely a result of poor glycemic control, as some studies suggest that even with optimal glucose management, GDM pregnancies may still result in large babies. This phenomenon was explored by Langer et al. (2005), who discussed the consequences of untreated gestational diabetes, including the risks of macrosomia and other complications such as preterm delivery and stillbirth. Effective management strategies include lifestyle interventions, pharmacological treatments, and careful monitoring throughout the pregnancy (Plows et al., 2018). Furthermore, **hyperglycemia and adverse pregnancy outcomes (HAPO)** have been extensively studied, with findings indicating that even mild forms of hyperglycemia can lead to significant complications for both the mother and the child (Metzger et al., 2008). This highlights the importance of understanding the nuances of glucose metabolism and its effects on pregnancy. The long-term implications of GDM have been discussed in various studies, emphasizing the importance of monitoring women after delivery to prevent the development of type 2 diabetes. Crowther et al. (2005) found that women diagnosed with GDM had a higher likelihood of developing type 2 diabetes, underscoring the need for continued monitoring and intervention after pregnancy. This long-term

management strategy is crucial for reducing the incidence of chronic diseases in this high-risk group. Overall, the research on **gestational diabetes** and its implications underscores the complexity of the disorder and its impact on both maternal and foetal health. Effective management of GDM through early detection, lifestyle interventions, and appropriate pharmacological treatments can significantly improve pregnancy outcomes and reduce the risk of long-term health issues for both the mother and the child. By further exploring the molecular and hormonal mechanisms underlying GDM, future research can contribute to more effective strategies for diagnosis, treatment, and prevention.

3.Methodology

3.1.Study Design

This study was a **prospective observational analysis** aimed at evaluating histomorphological changes and CD34 expression in placentas from diabetic pregnancies. Conducted between **November 2018 and September 2020**, the research was performed in the Department of Pathology at Sree Balaji Medical College and Hospital, Chennai.

Study Population

- **Inclusion Criteria:** Placentas from gestational diabetes mellitus (GDM) pregnancies diagnosed using the 75 g oral glucose tolerance test (OGTT) as per International Association of

Diabetes and Pregnancy Study Groups (IADPSG) criteria.

- **Exclusion Criteria:** Placentas from patients whose GDM resolved after treatment or were diagnosed with preexisting diabetes mellitus.

Sample Size

A total of **50 placentas** from GDM pregnancies were included in the study, ensuring a representative cohort for statistical and morphological analysis.

Clinical Data Collection

The clinical data were recorded for each participant, including:

1. **Maternal Age:** Stratified into age groups to analyze the relationship between age and placental changes.
2. **Trimester at Delivery:** Second and third trimesters were included to observe gestational age-related differences.
3. **OGTT Values:** Classified into two groups: <140 mg/dL and ≥140 mg/dL, to correlate glucose levels with histological and immunohistochemical findings.
4. **Gravida Status:** Divided into primigravida and multigravida categories.

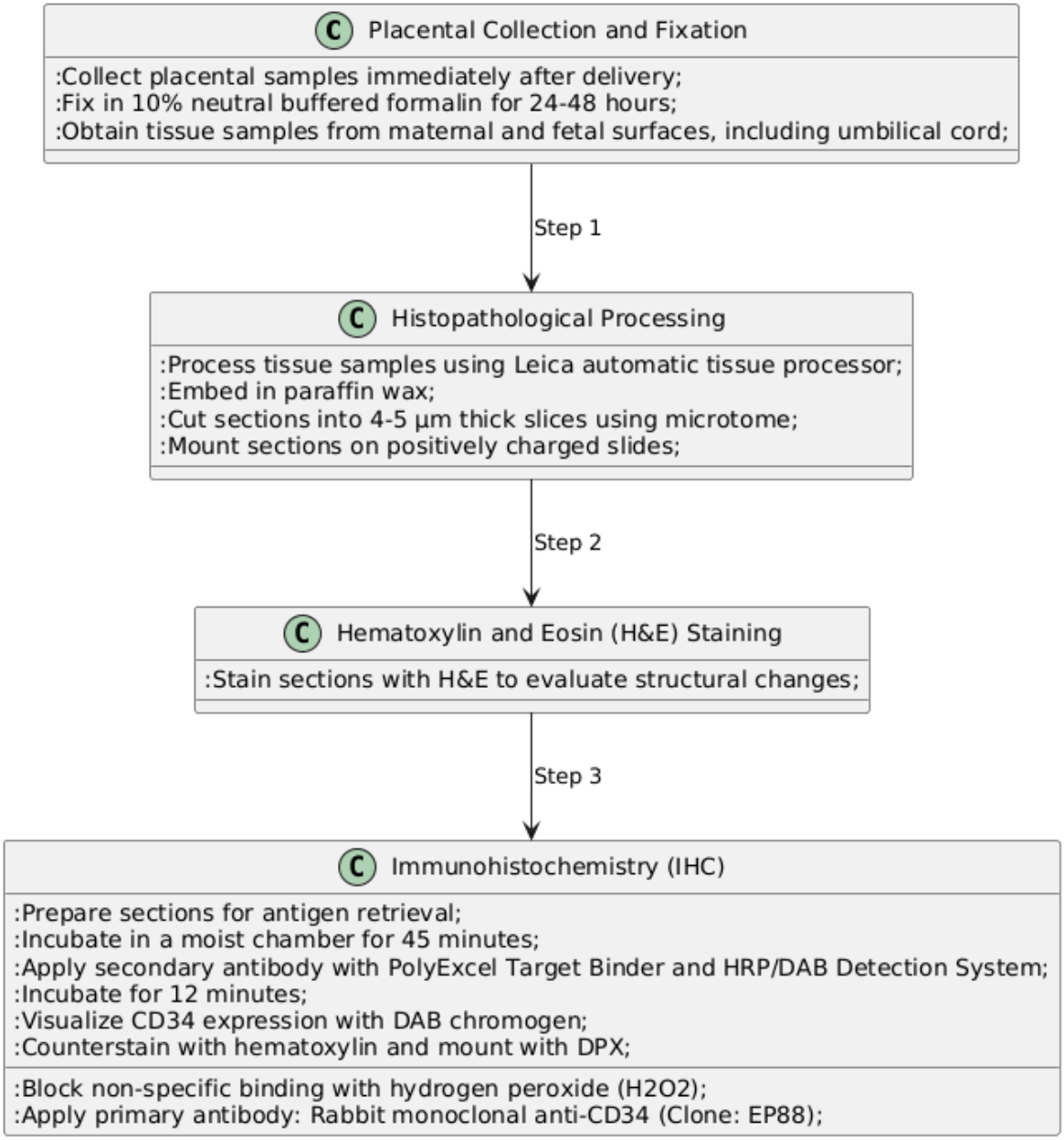

Figure 1: Methodology

Figure 1 shows a flowchart of the methodology used in this study, outlining the key steps involved in sample processing and preparation, including placental collection, histopathological processing, H&E staining, and immunohistochemical analysis for CD34 expression. The diagram provides a clear visual representation of the sequential steps taken to evaluate the placental changes in response to gestational diabetes mellitus.

3.2.Sample Processing and Preparation

Placental samples were collected immediately after delivery to ensure the integrity of the tissue for histological and immunohistochemical analysis. Each placenta was carefully inspected and promptly fixed in 10% neutral buffered formalin for a period of 24–48 hours to preserve its structural and cellular integrity. Representative tissue samples were then obtained from both the maternal and foetal surfaces, ensuring comprehensive coverage of the placenta's structural regions. Additionally, sections of the umbilical cord were included to evaluate potential vascular changes in this vital connection between the mother and foetus. Following fixation, the samples underwent histopathological processing using an automatic tissue processor (Leica). This standardized process ensured consistent dehydration, clearing, and infiltration of tissues with paraffin wax, which facilitated long-term preservation and ease of sectioning. The processed tissue blocks were then embedded in paraffin wax and sliced into sections measuring 4–5 µm in thickness using a microtome. This thickness was chosen to provide optimal resolution under light microscopy. The sections

were subsequently mounted onto positively charged slides to enhance tissue adherence, reducing the risk of detachment during staining procedures. For the evaluation of structural changes, the mounted tissue sections were stained with hematoxylin and eosin (H&E). Hematoxylin highlighted nuclear components, while eosin stained the cytoplasmic and extracellular matrix, allowing for a detailed assessment of cellular architecture and histopathological alterations. This staining method provided critical insights into structural changes such as villous immaturity, syncytial knots, stromal fibrosis, and fibrinoid necrosis. To investigate vascular changes, immunohistochemical (IHC) analysis was performed on the placental sections using CD34, a specific marker for vascular endothelial cells. The IHC process involved several sequential steps to ensure specificity and accuracy. After antigen retrieval, which was conducted to unmask epitopes trapped during formalin fixation, the sections were treated with a primary antibody specific to CD34. This antibody selectively binds to endothelial cells, marking areas of vascularization. The antigen-antibody complexes were then visualized using a chromogen system, resulting in a distinct yellow-brown membranous staining for CD34-positive cells. This methodological approach combined routine histopathology with advanced immunohistochemistry, enabling the identification of both gross morphological and specific vascular changes in the placental tissues. By integrating H&E

staining and CD34 IHC, this study provided a comprehensive assessment of the structural and functional adaptations of the placenta in response to gestational diabetes mellitus. Figure 2 illustrates the detailed process of sample processing and preparation, from the collection and fixation of placental samples to histopathological processing, H&E staining, and immunohistochemical analysis for CD34 expression. This figure provides a step-by-step overview of the methods used to evaluate the structural and vascular changes in the placenta associated with gestational diabetes mellitus.

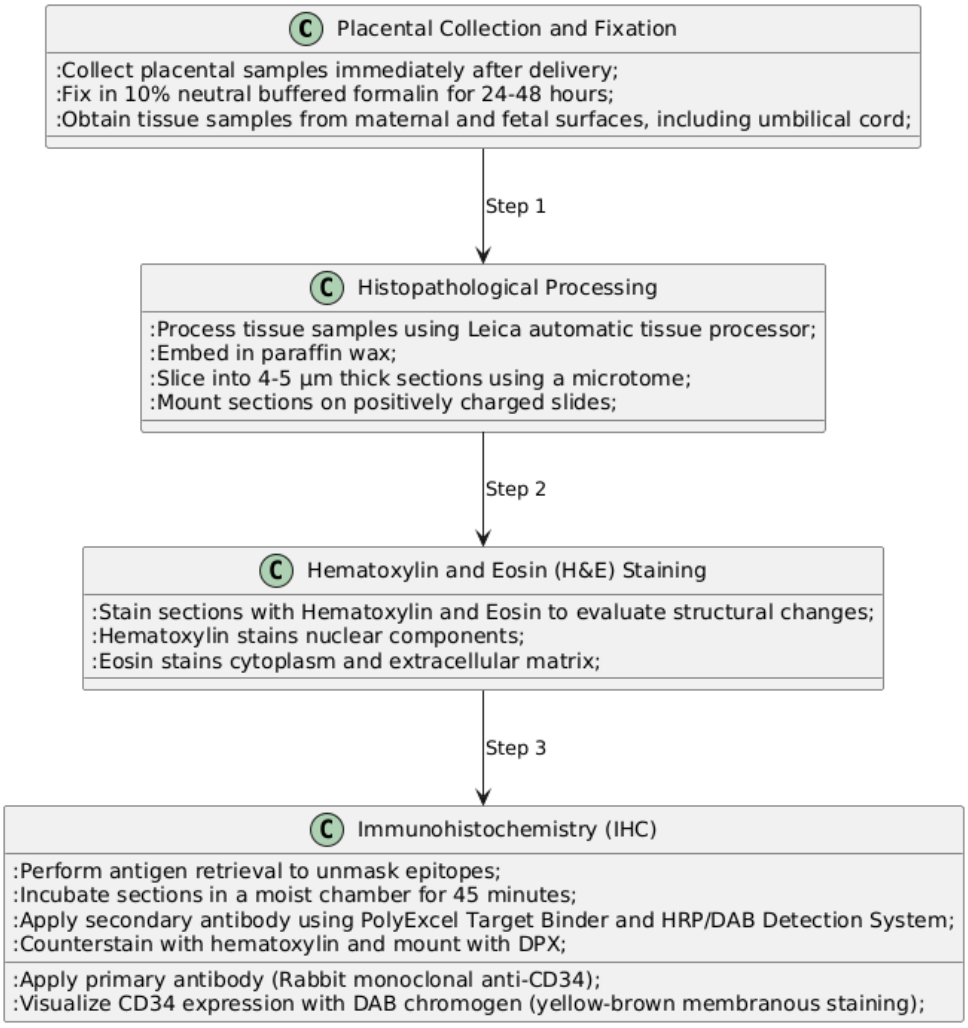

Figure 2: Sample Processing and Preparation

3.3.Immunohistochemical Procedure

The immunohistochemistry (IHC) technique was employed to evaluate CD34 expression in placental tissues, providing insights into vascular changes associated with gestational diabetes

mellitus (GDM). The process began with slide preparation, during which tissue sections mounted on positively charged slides were incubated at 60–70°C for 30 minutes. This step ensured optimal adherence of the tissue to the slides, preventing detachment during subsequent procedures. The sections were then deparaffinized using two xylene washes, each lasting 10 minutes, to remove embedding medium. To restore the tissue's hydrophilic state, sections were rehydrated by sequential immersion in decreasing concentrations of ethanol (absolute ethanol, 95%, and 70%), followed by rinsing in distilled water to prepare them for antigen retrieval. Antigen retrieval was performed to unmask the epitopes that might have been masked during formalin fixation and tissue processing. This step was carried out using TRIS-EDTA buffer (pH 9.0), a solution specifically designed for high-temperature antigen retrieval. The slides were placed in a pressure cooker and heated for 15 minutes, which facilitated the breaking of cross-links formed by formaldehyde, thereby exposing antigenic sites for antibody binding. To minimize non-specific binding and ensure the specificity of staining, endogenous peroxidase activity was blocked by incubating the sections with hydrogen peroxide (H2O2) for 10 minutes. This step prevented background staining caused by endogenous enzymes that could interfere with the interpretation of results. Following blocking, the primary antibody was applied to the sections. A rabbit monoclonal anti-CD34 antibody

(Clone: EP88), specific for endothelial cells, was used to detect vascularization in the placental tissues. The sections were incubated with the primary antibody in a moist chamber for 45 minutes to allow optimal antigen-antibody binding. This step marked the CD34-expressing endothelial cells, providing a foundation for visualization. The next phase involved the application of a secondary antibody system to enhance the signal and make it visible under a microscope. The PolyExcel Target Binder was applied first, followed by the PolyExcel HRP/DAB Detection System, each incubated for 12 minutes. This system used horseradish peroxidase (HRP) to amplify the antigen-antibody complex, enabling precise detection of the target antigen. For visualization, sections were treated with DAB (diaminobenzidine) chromogen for 5 minutes. This reagent reacts with HRP to produce a yellow-brown precipitate at the site of antigen-antibody binding, highlighting CD34 expression in the endothelial cells. The intensity and distribution of this yellow-brown membranous staining were assessed to determine the vascular density and changes in the placental tissues. Finally, the sections were counterstained with hematoxylin, which provided contrast by staining the nuclei blue, making it easier to distinguish between different cellular components. After counterstaining, the slides were dehydrated, cleared, and mounted using DPX (Dibutyl Phthalate Polystyrene Xylene) mounting medium. This ensured the preservation of the slides for long-

term analysis and facilitated clear visualization under the microscope. The interpretation of CD34 staining involved the identification of yellow-brown membranous staining localized to the endothelial cells lining placental capillaries. Strong CD34 positivity indicated increased vascularization and capillary density, often observed in placental tissues subjected to chronic hypoxia, as seen in GDM. This staining pattern was crucial for correlating vascular changes with maternal glucose levels and histological findings, offering valuable insights into the impact of GDM on placental structure and function. This comprehensive IHC process not only ensured the accuracy and specificity of results but also provided a reliable framework for studying angiogenesis and vascular adaptations in diabetic pregnancies.

3.4.Data Analysis

The following histological and immunohistochemical parameters were evaluated:

1. **Histological Features:**
    - Villous immaturity, stromal fibrosis, syncytial knots, fibrinoid necrosis, and villous edema.
2. **CD34 Expression:**
    - **Strong Positivity:** Defined as intense and widespread CD34 staining.
    - **Weak Positivity:** Focal and mild staining of CD34.
3. **Correlation with Clinical Data:**

- OGTT levels, maternal age, gravidity, and gestational age were statistically analyzed against histological findings and CD34 expression.

Statistical Tools: Data were analyzed using SPSS software. Fisher's Exact Test and Chi-square tests were used to assess statistical significance ($p < 0.05$ was considered significant).

Table 3: Materials Used for Histopathological and Immunohistochemical Analysis

Material	Description
Fixative	10% Neutral Buffered Formalin
Paraffin Wax	Embedding medium for tissue sections
H&E Stain	Used for routine histopathological analysis
Primary Antibody (CD34)	Rabbit monoclonal anti-CD34 antibody, clone EP88
DAB Chromogen	Visualizing reagent for detecting antibody-antigen complexes
TRIS-EDTA Buffer (50X)	Alkaline buffer for antigen retrieval
PolyExcel HRP/DAB System	Kit for secondary antibody application and chromogen development
Mounting Medium	DPX (Dibutyl Phthalate Polystyrene Xylene) for slide preservation

This table 3 summarizes the key materials used for sample preparation, histological staining, and immunohistochemistry in this study.

Table 4: Staining Protocol for CD34 Immunohistochemistry

Step	Reagent/Procedure	Duration	Purpose
Slide Incubation	Heat (60–70°C)	30 minutes	Fix tissue sections to slides
Deparaffinization	Xylene	2 × 10 minutes	Remove paraffin
Rehydration	Graded ethanol series	5 minutes each	Prepare tissue for antigen retrieval
Antigen Retrieval	TRIS-EDTA buffer	15 minutes	Unmask epitopes
Blocking Endogenous Activity	H2O2	10 minutes	Prevent non-specific peroxidase activity
Primary Antibody Incubation	Anti-CD34 antibody	45 minutes	Bind antibody to target antigen
Secondary Antibody	PolyExcel Target Binder	12 minutes	Enhance primary antibody signal
Visualization	DAB Chromogen	5 minutes	Develop yellow-brown color for positive staining
Counterstaining	Hematoxylin	15 seconds	Provide contrast
Mounting	DPX	—	Preserve slide for microscopic examination

This table 4 provides a step-by-step summary of the immunohistochemical staining protocol for CD34, detailing the reagents and their functions.

3.5.Ethical Considerations

The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Institutional ethical clearance was obtained before initiating the research. Written informed consent was obtained from all participants for the collection of placental samples and associated clinical data. This detailed methodology ensured standardized sample collection, processing, and analysis, enabling reliable evaluation of histological and immunohistochemical parameters in diabetic placentas. The combination of routine H&E staining and CD34 immunohistochemistry provided a comprehensive understanding of the morphological and vascular changes associated with GDM.

4.Results

This study evaluated the histomorphological and immunohistochemical changes in 50 placentas obtained from diabetic pregnancies. The findings included maternal demographics, histological observations, and the expression of CD34 as a marker of vascularization. The results were analyzed in relation to maternal age, gravidity, OGTT levels, and gestational age.

1. Maternal Demographics

The study cohort consisted of 50 pregnant women diagnosed with gestational diabetes mellitus (GDM). The mean age of the participants was **27.28 years** (SD: 3.63).

- **Age Distribution:** Most participants (54%) were aged between **26–30 years**, while 34% were aged ≤25 years. Only 4% of the participants were aged ≥36 years.
- **Gravidity:** More than half of the participants (54%) were primigravida, while 46% were multigravida as shown in Table:5.

Table 5: Age Distribution of Study Participants

Age Group (Years)	Frequency	Percentage (%)
≤25	17	34.0
26–30	27	54.0
31–35	4	8.0
≥36	2	4.0
Total	**50**	**100.0**

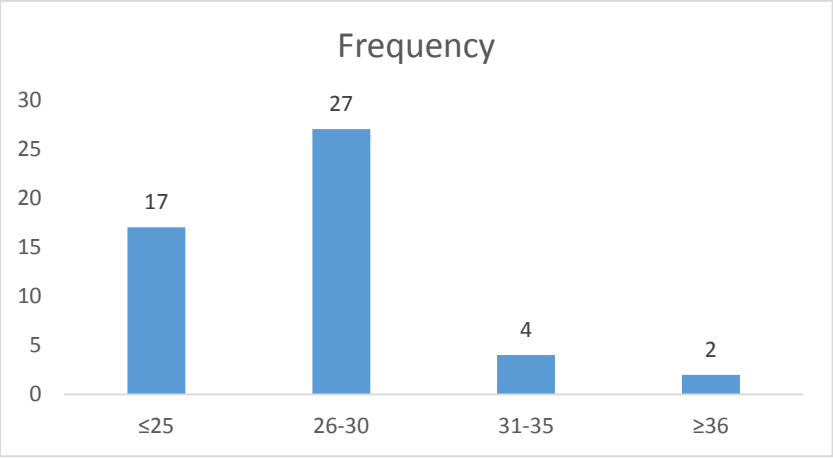

Figure 3: Graphical representation of age distribution.

Figure 3 shows the graphical representation of the age distribution of study participants. It visually illustrates the proportion of participants across different age groups, highlighting that the majority (54%) were between the ages of 26-30 years, followed by 34% aged ≤25 years, and a smaller percentage (8%) in the 31-35 age group. The graph provides a clear overview of the maternal age range in the study cohort

2. Gestational Age and OGTT Levels

The study primarily included participants in their third trimester (**90%**), with a smaller proportion in the second trimester (**10%**) as shown in table:6.

- **OGTT Levels:** The majority of participants (**94%**) had OGTT levels ≥140 mg/dL, indicating poorly controlled glucose levels, while only 6% had levels <140 mg/dL.

Table 6: Trimester and OGTT Distribution

Parameter	Category	Frequency	Percentage (%)
Trimester	2nd Trimester	5	10.0
	3rd Trimester	45	90.0
OGTT Levels	<140 mg/dL	3	6.0
	≥140 mg/dL	47	94.0

Figure 4 illustrates the distribution of participants by trimester, showing that 90% of the placental samples were obtained from the third trimester, while 10% were from the second trimester. This distribution emphasizes the predominance of third-trimester cases in the study, which provided

insights into the long-term effects of gestational diabetes on placental structure and function. Figure 5 depicts the distribution of participants based on gravidity. It shows that 54% of the participants were primigravida (first-time pregnant), while 46% were multigravida (women with more than one pregnancy). This distribution is important for understanding the potential influence of gravidity on placental changes and gestational diabetes outcomes.

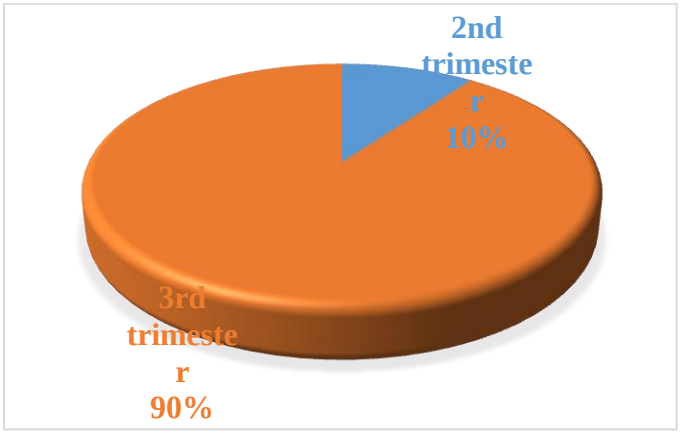

Figure 4: Distribution of Trimester

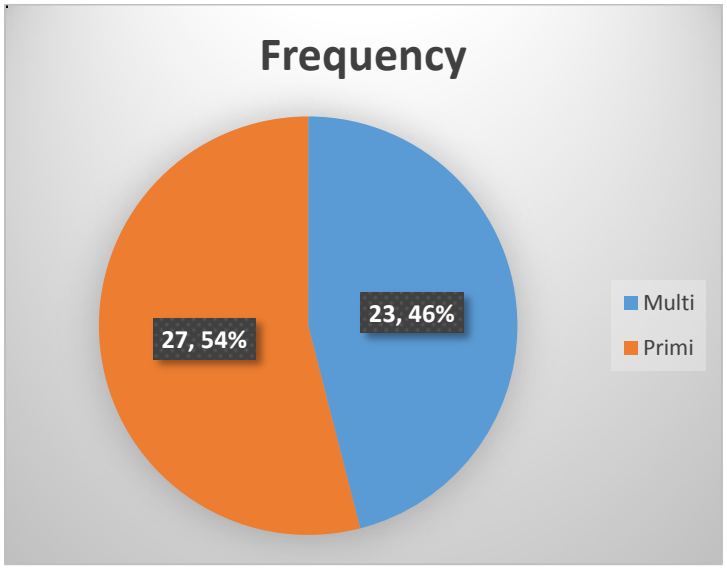

Figure 5: Gravida Distribution

3. Histomorphological Observations

Histopathological examination of placental tissues revealed the following changes:

1. **Villous Immaturity:** Predominant in diabetic placentas, characterized by a high proportion of immature intermediate villi.
2. **Syncytial Knots:** Increased syncytial knots were observed in response to hypoxic stress (Figure 10).

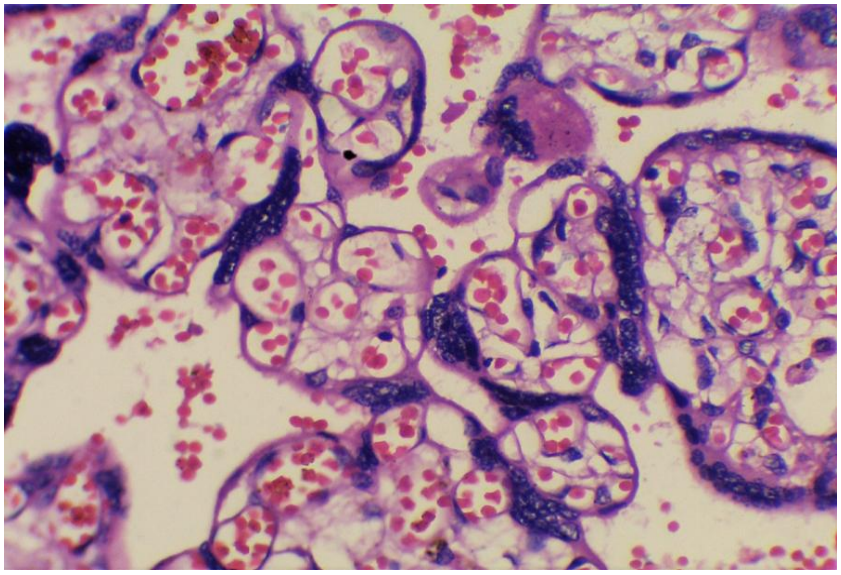

Figure 10: Microphotograph(400x) showing dilated blood vessels and syncytial knots

Volumen 15, N° 30 Especial, 2025
Depósito Legal: PPI201102ME3815
ISSN: 2244-8136

3. **Fibrinoid Necrosis:** Identified in terminal villi, indicative of placental ischemia (Figure 11).

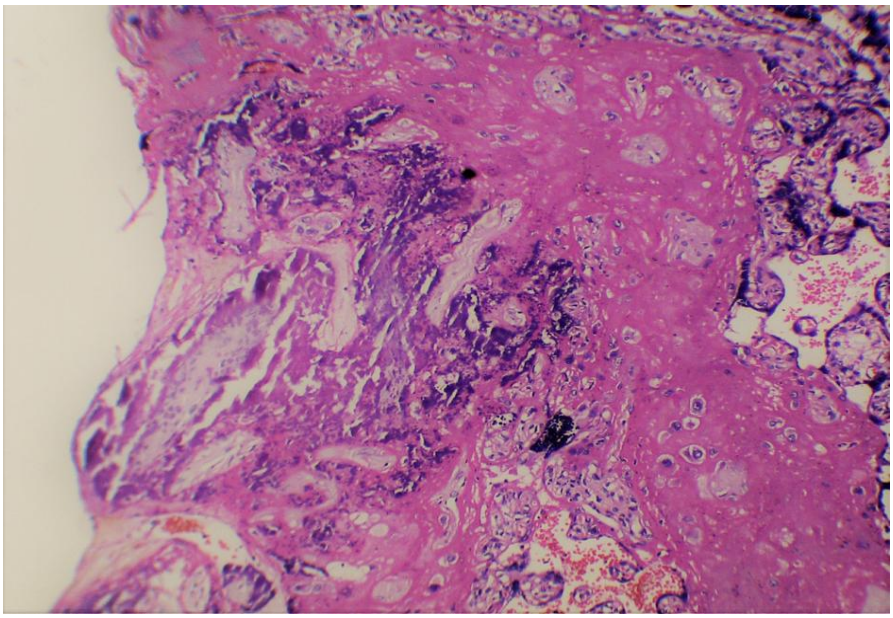

Figure 11: Microphotograph(100x) showing areas of necrosis and calcification

4. **Villous Edema:** Common in cases with higher OGTT levels, suggesting fluid accumulation due to impaired vascular function (Figure 12).

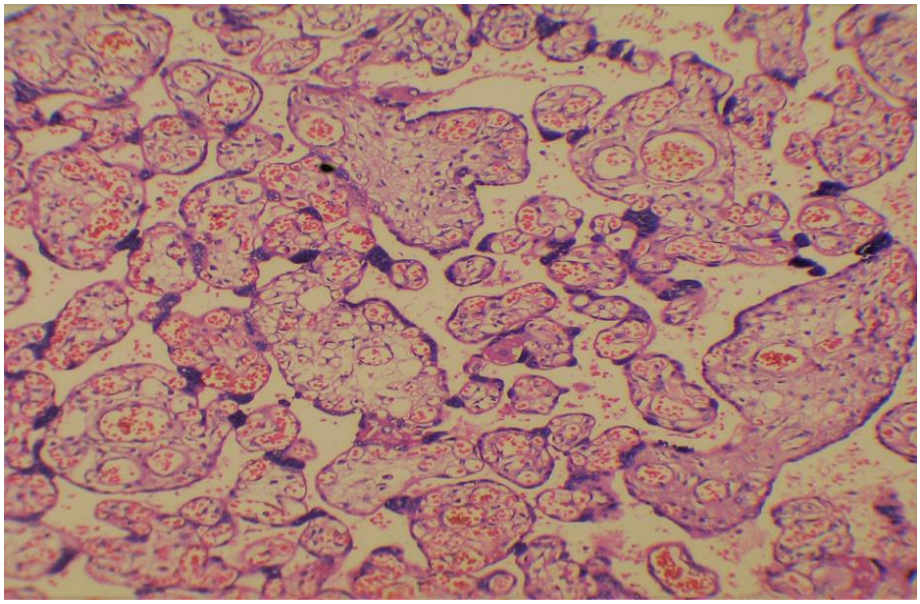

Figure 12: low power view showing villous edema with increased vascularity

5. **Stromal Fibrosis:** Observed in a significant number of cases, impairing villous elasticity and nutrient exchange. The Table:7 shows the clear summary.

Table 7: Summary of Histopathological Changes in Diabetic Placentas

Histological Feature	Frequency (n)	Percentage (%)
Villous Immaturity	42	84.0
Syncytial Knots	40	80.0
Fibrinoid Necrosis	35	70.0
Villous Edema	28	56.0
Stromal Fibrosis	24	48.0

4. CD34 Expression and Vascular Changes

Immunohistochemical analysis using CD34 as a marker revealed increased vascularization in diabetic placentas as shown in table:8. CD34 expression was categorized as:

- **Strong Positivity:** Intense staining observed in 74% of cases, indicative of increased vascular density (Figure 13).
- **Weak Positivity:** Focal staining noted in 24% of cases, often associated with lower OGTT levels as shown in Table 8.

Table 8: CD34 Expression in Diabetic Placentas

CD34 Expression	Frequency (n)	Percentage (%)
Strongly Positive	38	74.0
Weakly Positive	12	24.0
Total	**50**	**100.0**

Interpretation: Strong CD34 expression correlated significantly with OGTT levels ≥140 mg/dL ($p = 0.010$), reflecting the adaptive angiogenic response to chronic hypoxia.

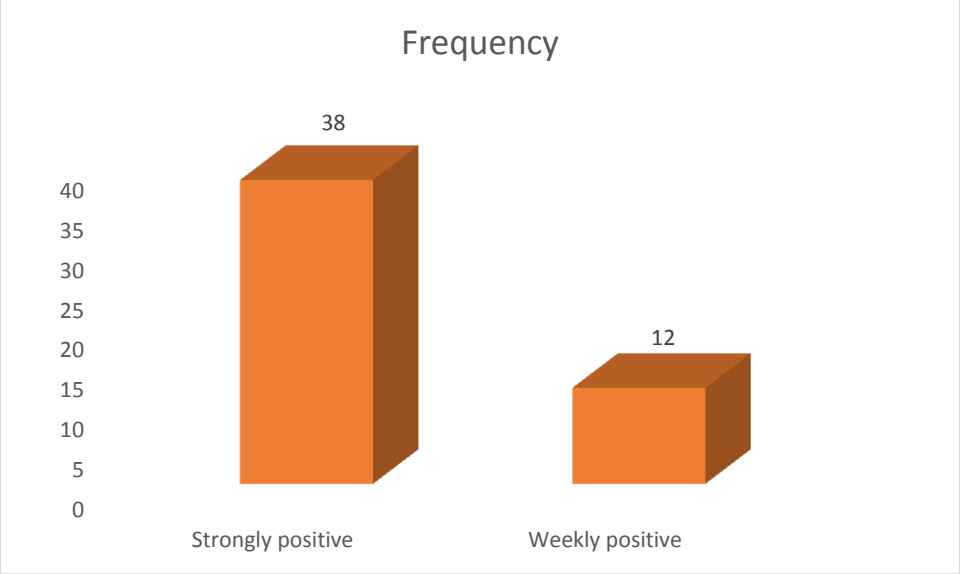

Figure 6: CD34 Distribution among study group

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5. Relationship Between Clinical Parameters and Histological Changes

5.1 OGTT Levels and Histological Changes

High OGTT levels (≥140 mg/dL) were strongly associated with advanced histological changes, including increased syncytial knots, villous edema, and stromal fibrosis. Figure 6 shows the distribution of CD34 expression in the study group, with 74% of placental samples exhibiting strong CD34 positivity, indicating increased vascularization. The remaining 26% showed weak CD34 positivity. This distribution highlights the significant angiogenic response in placental tissues, which is associated with chronic hypoxia induced by gestational diabetes mellitus. Figure 7 compares the oral glucose tolerance test (OGTT) levels with parity, showing the relationship between maternal glucose levels and the number of pregnancies (primigravida vs. multigravida). The figure demonstrates that higher OGTT levels (≥140 mg/dL) were more common in multigravida participants, suggesting a potential association between multiple pregnancies and higher maternal glucose levels in gestational diabetes.

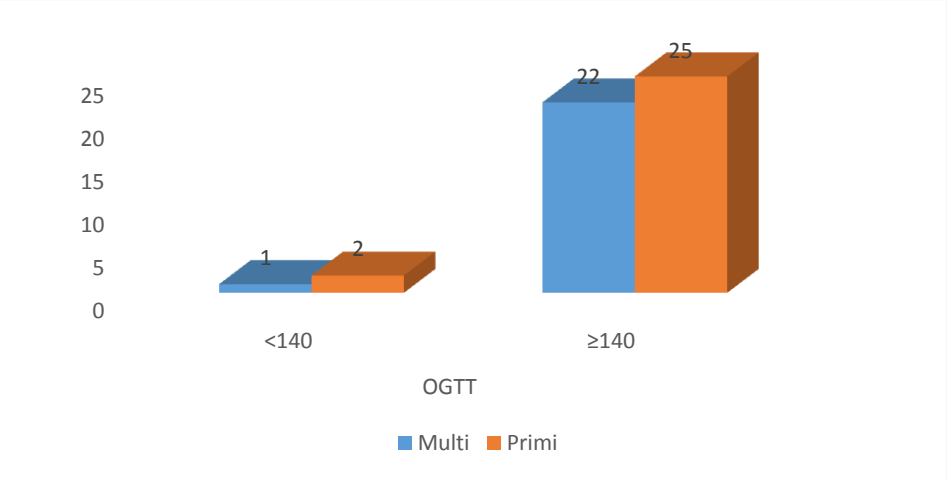

Figure 7: Comparison of OGTT level and parity

Table 9 highlights the relationship between maternal oral glucose tolerance test (OGTT) levels and the presence of key histological changes in the placenta, specifically in cases of gestational diabetes mellitus (GDM).

Table 9: Correlation of OGTT Levels with Histological Features

Histological Feature	OGTT <140 mg/dL	OGTT ≥140 mg/dL	p-value
Villous Immaturity	1	41	<0.001
Syncytial Knots	1	39	<0.001
Villous Edema	1	27	0.005
Stromal Fibrosis	0	24	<0.001

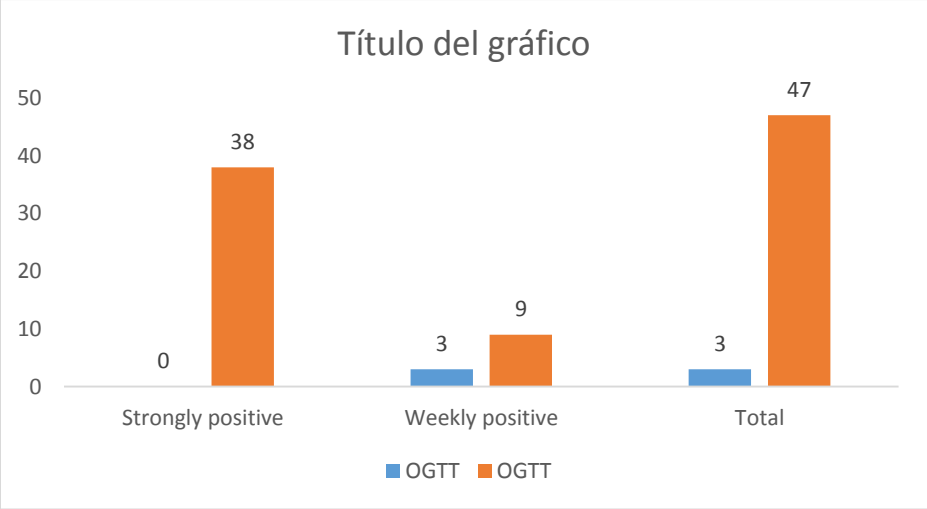

Figure 8: Distribution of expression of CD34 in placenta of diabetic mother and level of OGTT

5.2 Gravidity and Histological Changes

Multigravida cases showed a slightly higher prevalence of advanced histological changes compared to primigravida cases, though this difference was not statistically significant.

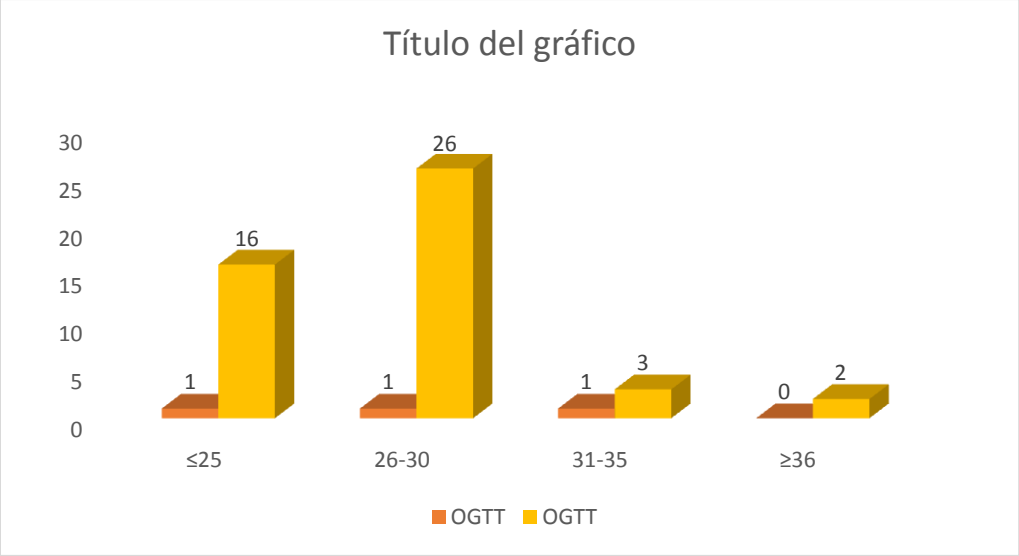

Figure 9: Distribution of age with blood glucose level of diabetic patients

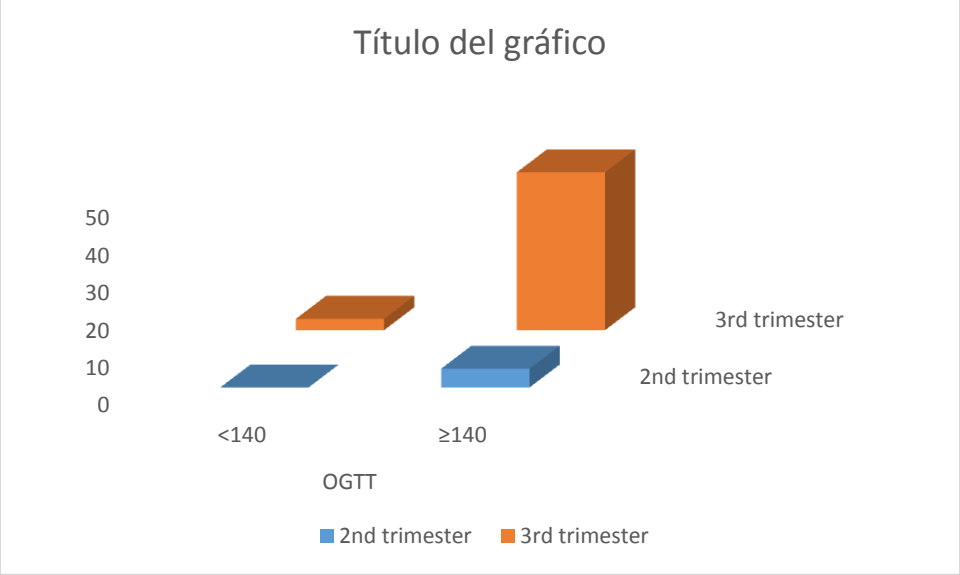

Figure 10: Stage of trimester and blood glucose level of the diabetic patients

Table 10: Gravidity and Histological Changes

Histological Feature	Primigravida (n=27)	Multigravida (n=23)	p-value
Villous Immaturity	21	21	0.500
Syncytial Knots	22	18	0.420

Figures 8-10 provide critical insights into the relationship between maternal glucose levels and various factors in gestational diabetes. Figure 8 shows a significant correlation between strong CD34 expression in the placenta and OGTT levels ≥140 mg/dL, indicating increased vascularization due to chronic hypoxia. Figure 9 reveals that older age groups (26–30 years) tend to have higher blood glucose levels, suggesting age-related glucose intolerance. Figure 10 highlights that third-trimester pregnancies exhibit higher blood glucose levels compared to second-trimester cases, emphasizing the impact of gestational age on glucose control in diabetic patients. These findings underscore the importance of age, trimester, and glucose levels in understanding placental changes and diabetes management. Table 10 presents the relationship between gravidity (primigravida vs. multigravida) and the occurrence of histological changes in the placenta. It shows that while histological alterations such as villous immaturity and syncytial knots were observed in both groups, the severity was slightly more pronounced in multigravida pregnancies, although the difference was not statistically significant.

6. Combined Analysis of CD34 Expression and OGTT Levels

CD34 expression was closely linked to maternal glucose levels. Cases with OGTT ≥140 mg/dL demonstrated significantly stronger CD34 expression.

Table 11: Distribution of CD34 Expression Based on OGTT Levels

CD34 Expression	OGTT <140 mg/dL	OGTT ≥140 mg/dL	p-value
Strongly Positive	0	38	0.010
Weakly Positive	3	9	0.010

Table 11: shows the Elevated OGTT levels are a critical determinant of increased vascularization in placental tissues, as evidenced by strong CD34 positivity.

7. Maternal Age and Glucose Levels

A significant association was observed between advanced maternal age and higher OGTT levels.

Table 12: Age Distribution and OGTT Levels

Age Group (Years)	OGTT <140 mg/dL	OGTT ≥140 mg/dL
≤25	1	16
26–30	1	26
31–35	1	3
≥36	0	2

Table 12: shows the older age groups tended to have higher glucose levels, which correlated with severe placental changes.

5.Summary of Key Findings

This study provided significant insights into the impact of gestational diabetes mellitus (GDM) on placental morphology and vascular adaptations, emphasizing the critical role of maternal glucose levels in shaping placental structure and function. One of the most notable findings was the correlation between maternal oral glucose tolerance test (OGTT) levels and histomorphological changes in the placenta. Elevated OGTT levels, particularly those ≥140 mg/dL, were

strongly associated with pathological changes, including villous immaturity, increased syncytial knots, and fibrinoid necrosis. These changes reflect the placenta's response to the hyperglycemic and hypoxic environment induced by GDM, which disrupts normal placental development and function. Villous immaturity, characterized by the persistence of immature intermediate villi, compromises the placenta's ability to support adequate nutrient and oxygen exchange. Similarly, the observed increase in syncytial knots and fibrinoid necrosis indicated hypoxic stress and placental ischemia, both of which impair placental efficiency and foetal development. The immunohistochemical evaluation of CD34 expression further revealed the adaptive angiogenic responses of the placenta to the hypoxic conditions induced by GDM. CD34, a marker of vascular endothelial cells, demonstrated strong positivity in 74% of cases, signifying a substantial increase in vascular density and angiogenesis. This heightened angiogenic activity likely represents a compensatory mechanism aimed at maintaining oxygen and nutrient delivery to the foetus despite the adverse metabolic environment. However, while increased vascularization is adaptive, excessive or aberrant angiogenesis can result in malfunctional capillary networks, further complicating placental efficiency. The significant correlation between strong CD34 expression and elevated OGTT levels underscores the critical impact of maternal hyperglycemia on placental vascular remodeling.

Gestational age also played a significant role in determining the severity of histopathological changes and glucose levels. The majority of placentas analyzed in this study were from third-trimester pregnancies, where the effects of prolonged exposure to hyperglycemia were more pronounced. Pregnancies in the third trimester exhibited severe histopathological alterations, including increased villous stromal fibrosis and villous edema, along with higher levels of maternal glucose. This indicates that as the pregnancy progresses, the placenta's ability to adapt to the hyperglycemic environment becomes increasingly compromised, leading to more pronounced structural and functional dysfunction. These findings highlight the importance of early detection and management of GDM to minimize its adverse effects on placental development and function. The clinical relevance of these findings cannot be overstated. The study emphasizes the urgent need for strict glycemic control in pregnancies affected by GDM. Uncontrolled maternal hyperglycemia not only increases the risk of adverse outcomes, such as preeclampsia and preterm delivery, but also has long-term implications for foetal health, including an elevated risk of obesity and type 2 diabetes later in life. From a placental perspective, mitigating hyperglycemia could reduce hypoxic stress and limit the extent of pathological changes, thereby improving maternal-foetal outcomes. Furthermore, the insights gained from this study highlight the potential of histopathological and

immunohistochemical analyses, particularly the use of CD34 as a biomarker, to serve as diagnostic tools for assessing placental health in GDM pregnancies. In conclusion, the findings of this study underscore the profound impact of maternal glucose levels on placental morphology and vascular function. The observed changes, including increased CD34 expression and histomorphological alterations, illustrate the placenta's adaptive responses to the challenges posed by GDM. However, these adaptations are often insufficient to counteract the detrimental effects of prolonged hyperglycemia, necessitating proactive measures to achieve glycemic control. These results not only advance the understanding of placental pathology in diabetic pregnancies but also provide a foundation for future research and clinical strategies to improve pregnancy outcomes.

6. Conclusion

In conclusion, this study highlights the significant impact of gestational diabetes mellitus (GDM) on placental structure and function, particularly in terms of vascular adaptations. The observed increase in CD34 expression and vascularization within the placental tissues underscores the chronic hypoxic environment induced by elevated maternal blood glucose levels. The correlation between higher OGTT levels and enhanced CD34 positivity further emphasizes the role of glycemic control in placental health. Additionally, the findings suggest that gestational age plays

Volumen 15, N° 30 Especial, 2025
Depósito Legal: PPI201102ME3815
ISSN: 2244-8136

a critical role in the severity of these vascular changes, with the third trimester showing the most pronounced alterations. This research provides valuable insights into the mechanisms underlying GDM-related placental dysfunction and calls for targeted interventions to improve maternal and neonatal outcomes by managing glucose levels throughout pregnancy.

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