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Research Article

Histopathological changes in the placentas of pregnant women with Gestational Diabetes Mellitus

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Abstract
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Objectives: Placental damage might be the primary underlying cause of the high rates of fetal adverse outcomes in cases with gestational diabetes mellitus (GDM). Histopathological changes were considered to be associated with GDM in the literature. However, there are also studies showing no changes in the placenta in some gestational diabetic women. The pathophysiology of these placental alterations remains elusive. Therefore, this study aimed to examine the histopathological changes in the placentas of patients with GDM and compare them with the placentas of the healthy control group.

Material and methods: This experimental non-randomized case-control study was conducted with 40 GDM patients and 40 healthy pregnant women at Dicle University Faculty of Medicine between March 2023 and December 2023. Placental tissues obtained following cesarean delivery were subjected to routine tissue processing. Placental samples were stained with hematoxylin-eosin and examined histopathologically using a light microscope.

Results: When histopathological findings were compared between the GDM and control groups, villous degeneration, congestion in blood vessels, fibrinoid accumulation, inflammation and intervillous hemorrhage were higher in the GDM group than in the control group (p<0.001).

Conclusion: The intricate relationship between GDM and placental pathology underscores the profound impact of metabolic changes on placental structure and function throughout pregnancy. Understanding these pathological changes is crucial for elucidating the progression of GDM and its implications for maternal and fetal health, highlighting the importance of comprehensive placental analysis in managing pregnancies complicated by GDM.

Keywords: Gestational diabetes, hematoxylin-eosin staining, histopathological examination

INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as glucose intolerance first identified during the second or third trimester of pregnancy that is not preexisting overt diabetes before pregnancy [1]. GDM is the most frequent metabolic disease during pregnancy and is considered to have a multifactorial origin [2,3]. The reported prevalence of GDM in the literature varied largely from clinic or hospital settings according to the screening procedure and the population, ranging between 6.8% and 40.3% [4]. Uncontrolled hyperglycemia during pregnancy causes recurrent interference in both maternal health as well as fetal well-being [5,6,7]. Also, women with GDM have a significantly increased risk of developing T2DM and cardiovascular disease (CVD) after pregnancy, while the offspring of these mothers are also at increased risk of developing obesity and T2DM later in life [8,9,10]. Therefore, strategies to optimize GDM management are essential during pregnancy. These should include preventing the development of GDM and correct diagnosis and treatment [11,12].

The placenta is an essential organ generated during gestation, one of its primary roles being the transfer of oxygen and macro- and micro-nutrients from the mother to the fetus and the removal of waste products from the fetus for fetal development and well-being [13]. Placentation was described as the 'approximation or combination of an embryo's tissues with those of its natural or surrogate parent for physiological interchange [14]. The placenta has a crucial immunoregulatory characteristics, which are clearly presented by the tolerance of the fetus by the maternal immune system in a process that includes excessive inflammation inhibition following blastocyst implantation, modulated by uterine natural killer cells and proliferation of regulatory T cells [15]. Alterations that occur in the placenta during pregnancy should be compatible with the functions that the placenta undertakes during pregnancy. Defective implantation and placentation have been shown to be associated with adverse pregnancy outcomes [16]. Moreover, placentas have antibacterial, anti-inflammatory, and anti-scarring activities. All these features have encouraged an interest in utilizing placentas for applications in cell therapy and regenerative medical treatment modalities [15].

Mostly, the placental weight is higher in GDM cases, and early aging of the placenta is more commonly detected. Placental damage might be the primary underlying cause of the high rates

of fetal adverse outcomes in cases with GDM [17]. Histopathological changes that were associated with GDM in previous reports, including villous edema, villous immaturity, syncytial knots, fibrinoid necrosis, and fibrin thrombus, have not been demonstrated in totality in every placenta [18]. There are also studies showing no changes in the placenta in some gestational diabetic women, or only minimal changes are detected compared to the control group [19]. The pathophysiology of these placental alterations remains elusive but possibly is associated not only with the severity of hyperglycemia [20].

Therefore, in this study, we aimed to examine the histopathological changes in the placentas of patients with GDM and compare them with the placentas of the healthy control group.

MATERIAL AND METHODS

This experimental non-randomized case-control study was conducted with 80 patients at the Department of Histology and Embryology, Dicle University Faculty of Medicine (Diyarbakır, Turkey) between March 2023 and December 2023. The study group included 40 singleton pregnant women who had not previously been diagnosed with diabetes mellitus and who were diagnosed with GDM between 24-28 weeks of gestation. Forty healthy singleton pregnant women who had no history of GDM or any complications in their current or previous pregnancies were included in the control group. The diagnosis of GDM was made by applying a 75-g OGTT between 24-28 weeks of pregnancy according to the 2010 IDPSG criteria [21]. A written informed consent was obtained from all participants. Ethical approval for this study was obtained from the Dicle University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee. The placentas used as materials in the study were obtained from patients delivered at Dicle University Faculty of Medicine, Gynecology and Obstetrics Clinic. Tissue preparation and histological evaluation of the study was carried out at Dicle University Faculty of Medicine Histology and Embryology Research Laboratory.

Routine histological tissue preparation

Placentas obtained following cesarean delivery were transferred to the laboratory under appropriate conditions. Placental tissues taken in small pieces were subjected to routine tissue preparation. Tissues were placed in a Zinc Formalin fixative (catalogue no: Z2902-3.75L, Sigma, Germany) solution and kept for 24 hours to be fixed. Then, the tissues were kept under running water overnight and dehydrated by passing through increasing alcohol series (50%, 70%, 80%, 90%, 96% and absolute ethyl alcohol). Samples were kept in xylene for 3x30 minutes and the alcohol was removed. The tissues were incubated within paraffin wax at 58°C for 3x45 minutes and embedded in paraffin blocks. Four µm thick sections from the blocks were taken on a positively charged slide with the help of a microtome (catalogue number: Leica RM2265, Wetzlar, Germany) and histochemical staining was performed [a22,a23].

Hematoxylin-Eosin staining

Placental tissue sections taken from paraffin blocks were placed in a boiler set at 37°C. The sections were kept in an oven at 58-62°C for 6 hours to melt excess paraffin on the slide. After the sections were deparaffinized in xylene for 3x15 minutes, they were passed through decreasing alcohol series (100%, 96%, 90%, 70%, 50% ethyl alcohol) for 10 minutes each and were brought to distilled water and kept for 5 minutes. Hematoxylin-eosin (H&E) stain was applied to the sections. After the staining phase, the sections were quickly immersed in increasing alcohol series (passing through 80%, 90%, and 96% ethyl alcohol series) and kept in absolute alcohol for 2 minutes. Finally, the sections were kept in xylene for 3x15 minutes and the tissue was covered with a coverslip by dropping mountain medium on it.

RESULTS

Hematoxylin-eosin staining findings

In the placental sections of the control group patients, placental structures were observed normal. Chorionic villi were oval and regular, with syncytiotrophoblasts on the outside and cytotrophoblasts on the inside. Syncytial knots were few in number and little fibrin deposition was observed. Endothelial cells were squamous and thin in villous capillaries, fibers in the connective tissue were in the regular organization in the root villi, and stromal cells were regularly located. No hemorrhage or lymphocyte infiltration was observed in the intervillous area (Figures 1 and 2).

In the histopathological examination of the placentas in the GDM group, degeneration in the villi, pyknosis and apoptosis in the cells in the trophoblastic layer, vascular dilatation and congestion in the villi, and hyperplasia in the endothelial cells were observed. Dense hemorrhage and immune cells were observed in the intervillous area. An increase in fibrin accumulation was observed. Hoffbauer cells were observed in the villous stroma (Figures 3 and 4).
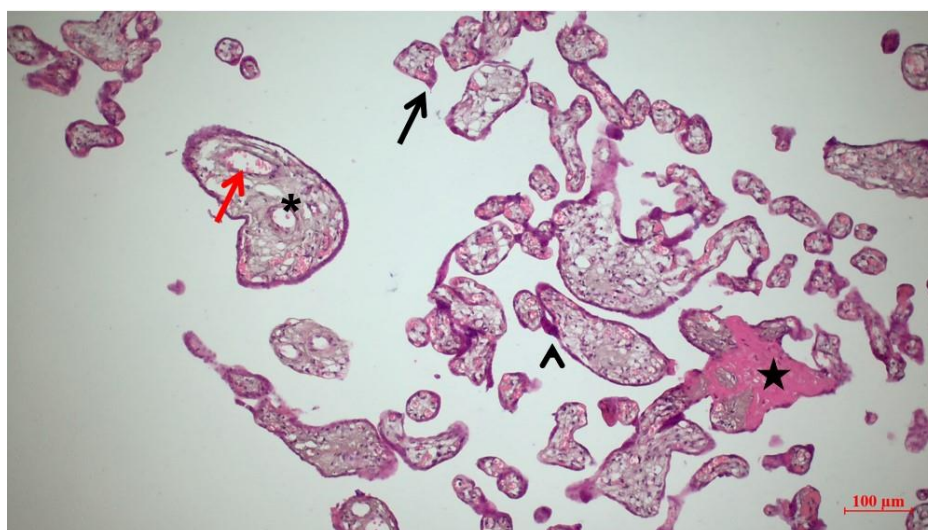

Figure 1: In the placenta sections of control group patients, normal chorionic villi (black arrow), syncytial knots (arrowhead), mesenchymal area (asterisk) and fibrinoid accumulation (star), chorionic capillaries (red arrow) (Magnification: 10x, scale bar: 100 µm).
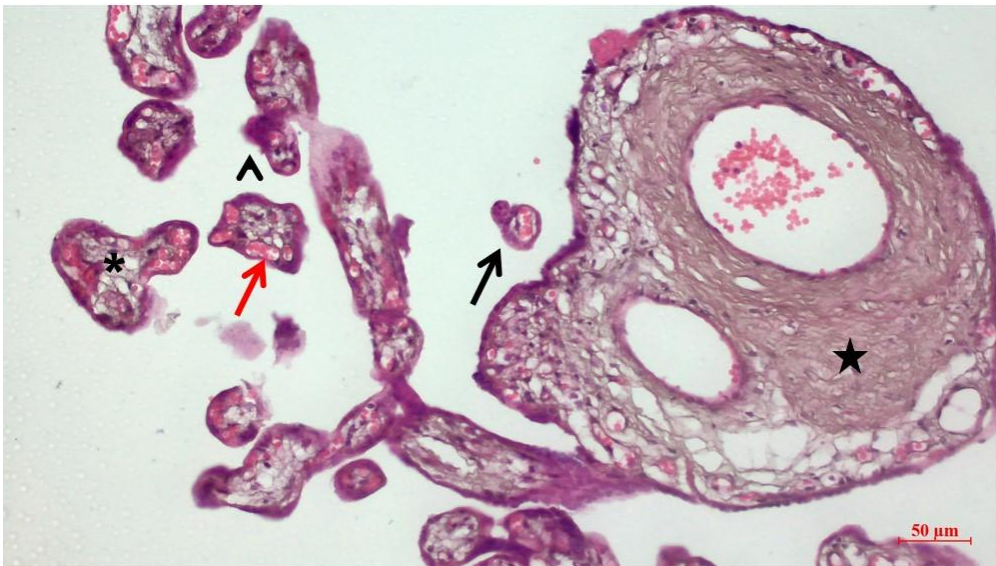
Figure 2: Close-up image of placenta sections of control group patients. Normal chorionic villi (arrow), syncytial knots (arrowhead), mesenchymal area (asterisk) and root villi (star) (Magnification: 20x, scale bar: 50 μm).
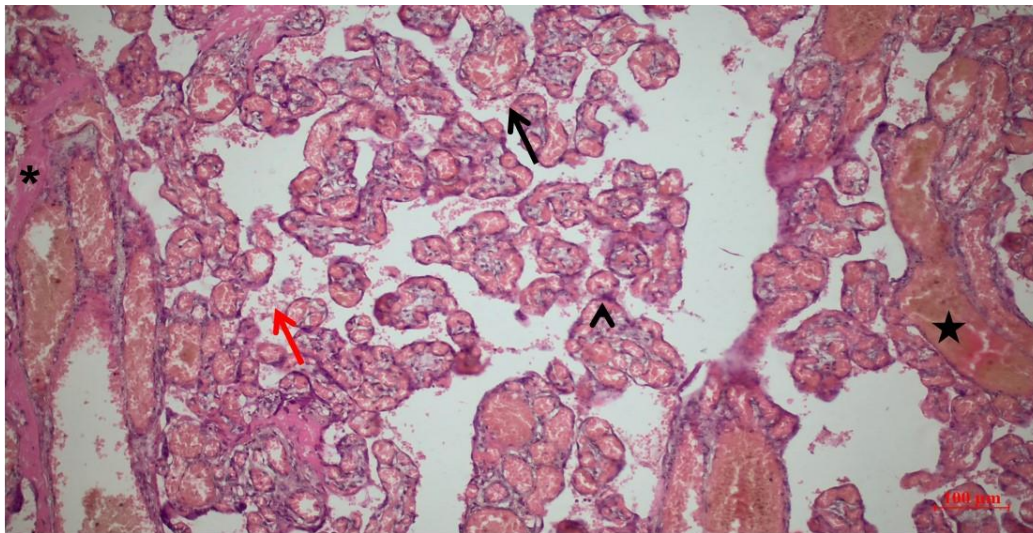

Figure 3: Microscopic image of free villi in hematoxylin-eosin staining of placenta sections of patients in the GDM group. Degeneration in chorionic villi (black arrow), intense hemorrhage in the intervillous area (red arrow), dilatation and congestion in villous capillaries (star), significant increase in the number of syncytial knots (arrowhead), increase in fibrinoid accumulation (asterisk) (Magnification: 10x, scale bar: 100 μm).
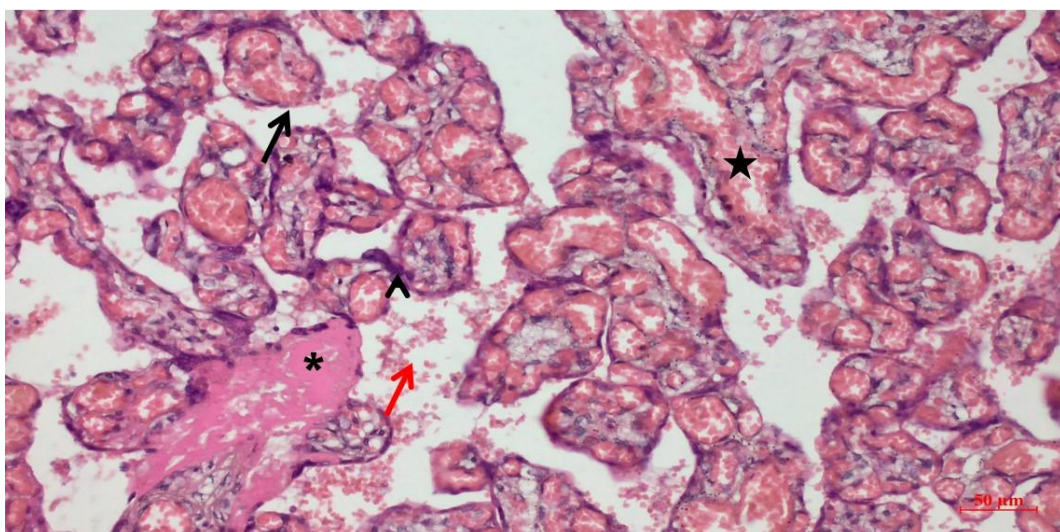

Figure 4: Close-up image of hematoxylin-eosin staining of placenta sections of GDM group patients. Degeneration in chorionic villi (black arrow), intense hemorrhage in the intervillous area (red arrow), dilatation and congestion in villous capillaries (star), significant increase in the number of syncytial knots (arrowhead), increase in fibrinoid accumulation (asterisk) (Magnification 20x, scale bar : 50 μm).

DISCUSSION

Metabolomic changes in glucose metabolism in the early weeks of gestation influence the placental structure and placental function in the advanced weeks of pregnancy [24]. Various histopathological phenotypes have been published in the placentas of GDM cases, including maternal vascular malperfusion lesions, vasculopathies, and histopathological phenotypes [25]. Therefore, the severity and adverse pregnancy outcomes of GDM are frequently reflected in the placental pathology of these cases. The critical analysis of placental pathologies is crucial for an increased knowledge of the onset and progression of GDM and its impacts on maternal and fetal well-being [26].

Villous immaturity is a typical histopathological outcome in GDM. In term placentas of GDM cases, there is a decrease in the number and total surface area of terminal villi and a thickening of the basement membranes. Additionally, capillaries are commonly centrally located. Also, immature villi in GDM are related to abnormally formed and fewer microvilli on the syncytiotrophoblast surface. All these changes increase the obstacle to mother-fetus oxygen and nutrient exchange [27]. Therefore, degenerative changes in the chorionic villi in GDM placentas indicate deteriorations in the structural integrity of the placenta and can be said to negatively affect the healthy functioning of the placenta. In a review of the histopathological examination of the placentas of pregnant women with GDM, changes such as the proliferation of stromal fibroblast cells and Hofbauer cells in the connective tissue with immature villi, degeneration of cytotrophoblast cells and basement membrane thickening, as well as an increase in syncytial knots and villous edema were identified [18]. Villous edema, one of the important villous structural abnormalities observed in GDM, has been reported to be associated with decreased placental function [27]. The incidence of placental vascular lesions (histopathological changes due to blood flow changes) increases in the third trimester of pregnancies with GDM. These lesions are most commonly seen in the root villus blood vessels. These lesions include fetal thrombotic vasculopathy (occlusion of arteries and veins in the placental vasculature of the fetal side), high fibrinoid deposition in the intervillous spaces, and fibrinoid necrosis [28,29]. The increased rate of intervillous thrombus in placentas with GDM has been suggested to be related to localized bleeding, but the exact cause is unknown [30] In the placentas of pregnant women with GDM, intense hemorrhage in the intervillous area and enlargement and congestion of villous capillaries indicate disruptions in placental circulation. Therefore, we consider that it affects the nutrient and oxygen intake of the fetus. Additionally, an increase in fibrinoid accumulation in the placentas of women with GDM indicates pathological changes in the tissue structure and vascular system of the placenta. Vascular lesions affect the fetoplacental circulation, blood flow in the degenerated umbilical cord with ischemic histiocytic villitis, and placental infarction. They also trigger syncytial necrosis and perivillous fibrin deposition with chronic fetal hypoxia [31]. It has been reported that there is an increase in the number of chorionic villus branching and syncytial knots in diabetic pregnancies [32]. With a significant increase in syncytial knots in the placentas of women with GDM, it can be said that there are changes in the cellular components of the placenta and the metabolic and endocrine functions of the placenta are affected. It is also suggested that these changes develop as a mechanism against fetal hypoxemia and increase the surface area where oxygen exchange occurs between the fetus and the mother [31]. In our study, consistent with the literature, we found increased degeneration in chorionic villi, intense hemorrhage in the intervillous area, occasional dilatation and congestion in villous capillaries, a significant increase in the number of syncytial knots and an increase in

fibrinoid accumulation in placental sections of patients with GDM compared to pregnant women without GDM. Our findings confirm the previous studies and showed distinctive pathological alterations in GDM placentas. These changes in GDM placentas are important to elucidate mechanisms contributing to the adverse outcomes associated with GDM.

CONCLUSION

The intricate relationship between GDM and placental pathology underscores the profound impact of metabolic changes on placental structure and function throughout pregnancy. Our results suggest that histopathological alterations such as villous immaturity, vascular lesions, and syncytial knot formations in GDM placentas contribute to compromised placental integrity and function. Understanding these pathological changes is crucial for elucidating the progression of GDM and its implications for maternal and fetal health, highlighting the importance of comprehensive placental analysis in managing pregnancies complicated by GDM.

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