ORIGINAL ARTICLE

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Immunohistochemical Evaluation of Placental NRG-1 Expression in Pregnant Women with Gestational Diabetes Mellitus

Gestasyonel Diabetes Mellitus Tanısı Alan Gebelerde Plasental NRG-1 Ekspresyonunun İmmünohistokimyasal Olarak İncelenmesi

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ABSTRACT

Aim: Gestational diabetes mellitus (GDM) is a significant pregnancy complication that threatens maternal and fetal health. Understanding the histopathological effects of GDM on the placenta and potential molecular markers is crucial for the diagnosis and treatment of the disease. Neuregulin-1 (NRG-1), which plays a role in cell proliferation, differentiation, and glucose metabolism, is one of the most promising candidate molecules in this context. This study aimed to compare histopathological findings and NRG-1 expression in the placentas of pregnant women diagnosed with GDM and healthy women using immunohistochemical methods and to identify potential micromorphological and molecular changes specific to GDM.

Materials and Methods: Study groups included Group 1 (control, n=22) healthy pregnant women and Group 2 (GDM, n=22) pregnant women diagnosed with GDM. Hematoxylin and eosin (H&E) and NRG-1 immunohistochemical staining were performed on all placental tissues. H&E sections were evaluated for villous edema, fibrinoid accumulation, fetal capillaries, and syncytial knots. Immunohistochemical staining intensity was classified as negative (-), weak (+), moderate (++), and strong (+++). Demographic data and vital signs were analyzed using SPSS.

Results: In the GDM group, compared to the control group, an increase in the number of villi, villous edema, dilatation of fetal capillary structures and an increase in the number of capillaries, an increase in syncytial knot formation, significant fibrinoid accumulation, and an increase in NRG-1 expression in the trophoblast cells around the villi, villous mesenchymal stromal cells, and vascular endothelial cells were detected in the examined placenta sections.

Conclusion: GDM has been shown to cause pathological alterations in the placenta at the light microscopic level while also increasing NRG-1 expression. This study is the first to investigate the role of NRG-1 in placental tissues obtained from GDM cases. NRG-1 appears to be a potential marker in the pathophysiology of GDM, underscoring the need for further studies in this field.

Keywords: Gestational diabetes mellitus, neuregulin-1, placenta, immunohistochemistry

ÖZ

Amaç: Gestasyonel diabetes mellitus (GDM), anne ve fetüs sağlığını tehdit eden önemli bir gebelik komplikasyonudur. GDM'nin plasenta üzerindeki histopatolojik etkilerini ve olası moleküler belirteçleri anlamak, hastalığın tanısı ve tedavisi için çok önemlidir. Hücre proliferasyonu, farklılaşması ve glikoz metabolizmasında rol oynayan nörogulin-1 (NRG-1), bu bağlamda en umut verici aday moleküllerden biridir. Bu çalışmada, GDM tanısı alan ve sağlıklı gebe kadınların plasentalarında histopatolojik bulgular ve NRG-1 ekspresyonunun immünohistokimyasal yöntemlerle karşılaştırılması ve GDM'ye özgü olası mikromorfolojik ve moleküler değişikliklerin belirlenmesi amaçlanmıştır.

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Gereç ve Yöntem: Çalışma grupları; Grup 1 (kontrol, n=22) sağlıklı gebeler ve Grup 2 (GDM, n=22) GDM tanılı gebelerden oluşmuştur. Tüm plasental dokulara hematoksilen ve eozin (H&E) ve NRG-1 immünohistokimyasal boyama uygulanmıştır. H&E kesitlerinde villöz ödem, fibrinoid birikim, fetal kapiller ve sinsityal düğümler değerlendirilmiştir. İmmünohistokimyasal kesitlerde boyanma yoğunluğu; negatif (-), düşük (+), orta (++), ve yüksek (+++) olarak sınıflandırılmıştır. Demografik veriler ve vital bulgular SPSS programı ile analiz edilmiştir.

Bulgular: GDM grubunda kontrol grubuna göre; incelenen plasenta kesitlerinde villus sayısında artış, villöz ödem, fetal kapiller yapılarında dilatasyon ve kapiller sayısında artış, sinsityal düğüm oluşumunda artış ile belirgin fibrinoid birikimi ve villus çevresindeki trofoblast hücrelerinde, villus mezenkimal bağ doku hücrelerinde ve damar endotel hücrelerinde NRG-1 ekspresyonunda artış tespit edilmiştir.

Sonuç: GDM, plasentada ışık mikroskobik düzeyde patolojik değişikliklere neden olmakla birlikte NRG-1 ekspresyonunu artırmıştır. Bu çalışma, GDM tanılı olgulara ait plasental dokularda NRG-1 rolünü inceleyen literatürdeki ilk çalışmadır. NRG-1, GDM patofizyolojisinde potansiyel bir belirteç görevi görebilmekte ve bu alanda daha fazla araştırma gerekmektedir.

Anahtar Kelimeler: Gestasyonel diabetes mellitus, nörogulin-1, plasenta, immünohistokimya

INTRODUCTION

Gestational diabetes mellitus (GDM) refers to glucose intolerance that begins during pregnancy or is first detected during pregnancy[1]. According to the 2017 guidelines of the International Diabetes Federation, hyperglycemia during pregnancy is estimated to occur in 16.2% of pregnancies, or approximately 21.3 million live births. Of these cases, an estimated 86.4% are due to GDM, 6.2% to pre-gestational diabetes (diagnosed before pregnancy), and 7.4% to other types of diabetes first diagnosed during pregnancy (including type 1 and type 2 diabetes)[2]. Risk factors for GDM include overweight/obesity, advanced maternal age, Western-style diet[3], ethnic background[4], polycystic ovary syndrome, and a family history of insulin resistance and/or diabetes[1,5]. It is noted that each of these risk factors is directly or indirectly associated with impaired pancreatic β-cell function and/or insulin sensitivity[6].

GDM increases the risk of pregnancy complications such as preterm birth and preeclampsia[7]. Exposure to maternal hyperglycemia during pregnancy leads to hyperinsulinemia in the fetus, and it has been shown that this condition increases the risk of macrosomia, neonatal hypoglycemia, hyperbilirubinemia, etc.[8]. Babies born to mothers with GDM are at high risk for obesity, type 2 diabetes mellitus, cardiovascular disease, and related metabolic disorders in the long term[9-11]. It has been reported that women with a history of GDM have an increased risk of developing type 2 diabetes mellitus later in life[12,13], and that their risk of cardiovascular disease also increases due to changes in their vascular structures[14-16].

The placenta functions as a specialized organ that facilitates the exchange of substances between the mother and the fetus during pregnancy. It is responsible for transferring maternal nutrients and oxygen to the fetus and for transporting waste products accumulated in the fetus back into the maternal circulation. Additionally, the placenta produces hormones that regulate maternal physiology during pregnancy and acts as a barrier against the maternal immune system. It separates the maternal and fetal circulations, which are in contact through various surfaces. Because the placenta is situated between the maternal and fetal circulations, it is exposed to both the mother's and the fetus's diabetes-related endocrine and metabolic disorders[17-19]. It has been reported that maternal hyperglycemia can affect fetal development by influencing placental structure and vascularization[20], and may increase the risk of maternal and fetal complications as well as perinatal morbidity and mortality[21].

In the presence of GDM, the placenta is thought to play a triggering role in inflammatory processes. It has been noted that the interaction between adipose tissue and the placenta may contribute to the development of both systemic inflammation and insulin resistance. These two tissue types produce high levels of various pro-inflammatory mediators such as interleukin (IL)-6, IL-8, tumor necrosis factor-alpha, resistin, and leptin[22,23]. In GDM, the balance between pro-inflammatory and anti-inflammatory cytokines in the placenta is disrupted[24]. It has been reported that GDM increases the risk of perinatal mortality and morbidity by causing abnormal development of the placenta and umbilical cord, and is therefore a significant public health issue[25].

Neuregulin-1 (NRG-1) is a member of the neuregulin family. Encoded and produced by the *NRG-1* gene, it undergoes various modifications both within and outside the cell. Different isoforms of the NRG-1 molecule play roles in various signaling pathways. It is noted that NRG-1-related signaling pathways are essential for the proper functioning of many systems in the body. Within the cell, NRG-1 plays a role in numerous mechanisms, including growth, proliferation, differentiation, and cell death. Studies have shown that NRG-1 and its associated signaling pathways play a role in the pathophysiology of neurological, psychiatric, cardiovascular, and many other diseases[26-28]. Glucose metabolism is also among the physiological mechanisms in which NRG-1 is involved. It has been reported that NRG-1 plays a role in the pathophysiology of GDM, a condition characterized by impaired glucose metabolism[29]; however, the exact mechanism by which NRG-1 exerts its effects in GDM remains unclear.

The aim of this study is to evaluate the histopathological effects of GDM on the placenta and changes in NRG-1 expression. Placental samples obtained from healthy and GDM-affected pregnant women were examined histopathologically and for NRG-1 expression using immunohistochemical methods.

MATERIALS AND METHODS

Groups

This study was conducted using placental tissue samples obtained from volunteer pregnant women who presented to the Obstetrics and Gynecology Outpatient Clinic at Muğla Sıtkı Koçman University Faculty of Medicine and who agreed to participate by reading and signing the informed consent form. For this study, approval was obtained from the Muğla Sıtkı Koçman University Clinical Research Ethics Committee on January 16, 2020, under decision no. 01/VI, confirming that the study was ethically appropriate. Human rights were protected in accordance with the principles of the "Declaration of Helsinki as revised in 2013." A total of 44 pregnant women were included in the study. Study groups: The study consisted of two groups: Group 1 (Control group), comprising healthy pregnant women with no underlying conditions (n=22), and Group 2 (GDM group), comprising pregnant women diagnosed with GDM (n=22). The control group consisted of healthy pregnant women who presented to the outpatient clinic and had no history of coronary artery disease, liver disease, kidney disease, or preeclampsia. The pregnant women underwent routine prenatal examinations performed by an obstetrician-gynecologist. The GDM diagnosis was made based on the results of a single-step 75 g oral glucose tolerance test performed between the 24th and 28th weeks of gestation, following at least an 8-hour overnight fast, in women without a known history of diabetes. Fasting, one-hour, and two-hour blood glucose levels were measured; a diagnosis of GDM was made if any of these values were equal to or higher than the threshold values. Threshold values were determined in accordance with the 2010 IADPSG recommendations as fasting ≥92 mg/dL, first hour ≥180 mg/dL, and second hour ≥153 mg/dL[30].

Inclusion Criteria

For the control group, healthy pregnant women aged 18-40 years who were in their 38th-40th week of gestation were included. For the GDM group, pregnant women aged 18-40 years who were in their 38th-40th week of gestation, had been diagnosed with GDM, were managed with diet, and were not receiving insulin therapy were included.

Exclusion Criteria

Participants with severe physical illness, alcohol, tobacco, or illicit drug use; preeclampsia; liver or kidney failure; any endocrine disorder; those with a history of gastric or intestinal surgery, those who had received medical treatment for any reason within the past three months, cases with chronic inflammation or infection, cases with fetal biometry outside the 10th-90th percentile on obstetric ultrasound, and cases diagnosed with GDM who were receiving insulin therapy were excluded from the study.

Histopathological Analysis

Placenta samples obtained from women who had given birth were washed with physiological saline solution. The tissue samples were placed in a 10% formalin solution for fixation and left to stand for 24 hours. Subsequently, the tissues were rinsed under running water for 24 hours to remove the fixative. Following dehydration and clearing using a tissue processor, the tissues were embedded in paraffin. Sections 5 μm thick were cut using a microtome (Thermo Scientific Finesse Me+), stained with hematoxylin and eosin (H&E), and evaluated using a light microscope (Nikon Eclipse 80i).

Immunohistochemical Analysis

Following deparaffinization, the sections were placed in a series of decreasing alcohol concentrations (96%, 96%, 80%, 70%) to initiate the hydration process. Hydration was completed by soaking the sections in distilled water for 5 minutes. For antigen retrieval, the sections were boiled in citrate buffer for 5-10 minutes and then left to cool without removing the citrate buffer. The cooled sections were washed with Phosphate-Buffered Saline (PBS). The borders of the sections were outlined with a hydrophobic pen PAP pen. Endogenous peroxidase was blocked (3% H_2O_2), followed by washing with PBS. Protein blocking solution was applied to the sections and left for 5 minutes, after which the blocking solution was removed without washing. The anti-NRG-1 primary antibody (Santa Cruz Biotechnology, cat: sc-393006) was applied to the sections at the dilution recommended by the manufacturer, and the sections were incubated at 4 °C in a humid environment for 24 hours. Staining procedures were performed in accordance with the manufacturer's protocol. Negative control sections were prepared to assess staining specificity; in the negative control, PBS was used instead of the primary antibody, and no specific staining was observed. The sections were then washed with PBS solution. A biotinylated secondary antibody compatible with the primary antibody was added and incubated at room temperature for 20 minutes. In the next step, horseradish peroxidase was applied and incubated for 10 minutes. After washing with PBS, the chromogenic substrate 3,3′-diaminobenzidine was added and incubated until a color change was observed. After washing with PBS, the nuclei were stained with Mayer's hematoxylin. Following these procedures, the sections were sealed with Entellan.

For immunohistochemistry (IHC) analysis, the sections were evaluated by two independent and blinded histologists using a light microscope (Nikon Eclipse 80i) at 20X magnification. Based on staining intensity, the sections were classified as unstained (–), weakly stained (+), moderately stained (++), and strongly stained (+++). Inter-observer agreement for IHC scoring was assessed using Cohen's kappa test, and the kappa value was found to be 0.82.

Statistical Analysis

The required sample size for the statistical analyses planned in the study was calculated using the G* Power 3.1.9.7 software. For the analysis, the t-test for independent samples was selected to compare the difference between the means of two independent groups. The power analysis was conducted a priori (predefined); the effect size (Cohen's d) was set at 0.78, the α error level at 0.05, and the statistical power (1–β) at 0.80. A one-tailed test was assumed. Based on these parameters, the calculation resulted in a minimum required sample size of 22 for each group (44 in total for both groups).

Statistical analysis of the data was performed using the SPSS 14 statistical software (SPSS Inc., Chicago, IL, USA). Continuous variables are presented as mean ± standard deviation or median and 25th to 75th percentiles. The normality of the data was assessed using the Kolmogorov-Smirnov or Shapiro-Wilk test. Mean differences between the control and study groups were compared using the Student's t-test. Continuous variables that did not follow a normal distribution were evaluated using the Mann-Whitney U or Kruskal-Wallis tests, depending on the number of independent groups. Variables that followed a normal distribution (gravidity, number of living children, number of miscarriages, gestational age at delivery, birth weight, systolic blood pressure, diastolic blood pressure, and hemoglobin level) were compared between groups using Student's t-test, whereas variables that did not follow a normal distribution (fasting glucose level) were analyzed using the Mann-Whitney U test. NRG-1 expression was assessed semi-quantitatively (– / + / ++ / +++), and differences in distribution between groups were evaluated using the chi-square test. A p-value of <0.05 was considered statistically significant.

RESULTS

The clinical and demographic characteristics of a total of 44 pregnant women are presented in Table 1. No statistically significant differences were observed between the control and GDM groups in terms of gravidity, number of living children, number of miscarriages, gestational age at delivery, birth weight, systolic blood pressure, diastolic blood pressure, and hemoglobin levels. Blood glucose levels were significantly higher in the GDM group compared to the control group ($p<0.05$).

Histopathological Findings

Placentas from the control and GDM groups were examined in H&E-stained sections. Sections from the control group demonstrated normal histological architecture of stem villi, terminal villi, fetal capillaries within the villous stroma, and the syncytiotrophoblast layer. In the control group, syncytial knots were observed, and fetal capillaries were identified within the villous stroma. The intervillous spaces showed normal histological architecture, with no evidence of pathological dilatation or narrowing. Minimal fibrinoid deposition was observed in the intervillous and perivillous areas (Figure 1). Sections from the GDM group showed an increased number of villi and villous edema. Dilatation and increased number of fetal capillaries within the villous stroma were observed. An increased number of syncytial knots was noted. The intervillous spaces were generally narrowed; however, dilatation was observed in areas with fibrinoid deposition. Prominent fibrinoid deposition was identified in the intervillous, perivillous, and intravillous areas (Figures 1-3).

Immunohistochemical Findings

Placental NRG-1 expression, as previously described in the literature, was observed in both the nucleus and cytoplasm of the cells[31]. In the control group, placental NRG-1 expression showed weak staining (+) in trophoblast cells surrounding the villi, whereas no staining (–) was detected in villous mesenchymal stromal cells and vascular endothelial cells (Table 2, Figure 4). In the GDM group, placental NRG-1 expression showed strong staining (+++) in trophoblast cells surrounding the villi, while

Table 1. Demographic and clinical characteristics and laboratory data of the groups

Groups	Gravidity*	Parity*	Number of miscarriages**	Gestational week**	Infant weight**	Systolic blood pressure*	Diastolic blood pressure**	Blood glucose level**	Blood hemoglobin level**	Maternal age**
Control (n=22)	2 (1-2)	2 (1-2)	0.40±1.14	38 (34-41)	3229.09±623.63	113.13±16.65	72.40±10.41	90.86±12.61	11.82±0.95	27.13±4.38
GDM (n=22)	2 (1-3)	1 (1-2)	0.45±0.73	38 (35-41)	3300.90±386.52	116.36±16.19	71.50±10.04	104.31±17.37[a]	11.78±1.10	28.22±4.38
p-values	0.682	0.399	0.252	0.666	0.622	0.300	0.852	0.015	0.906	0.778

*Expressed as median and 25th-75th percentiles, **Data are presented as frequency (percentages in parentheses) or mean ± standard deviation, except for descriptive statistics. [a]Control vs. gestational diabetes mellitus p=0.015. GDM: Gestational diabetes mellitus

weak staining (+) was detected in villous mesenchymal stromal cells and vascular endothelial cells (Table 2, Figure 4).

DISCUSSION

GDM is defined as glucose intolerance with onset or first recognition during pregnancy[1]. Histopathological changes in placental tissue associated with GDM have been demonstrated in numerous studies, with largely consistent findings reported in the literature. In particular, an increase in syncytial knots, villous stromal edema, and fibrinoid deposition have been

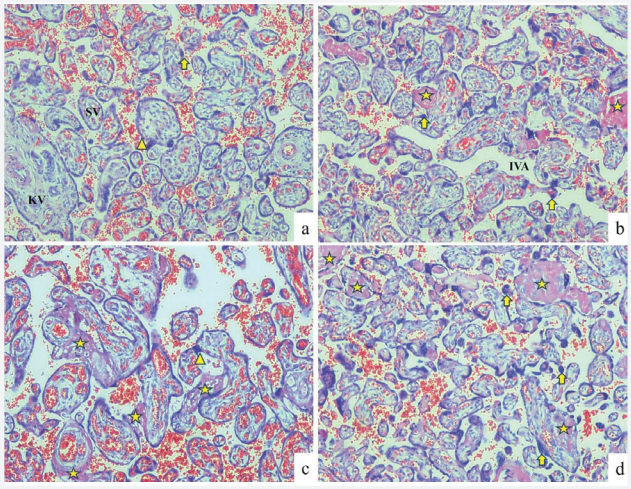

Figure 1. Placental sections from all groups

(a) In the placental section of the control group, stem villus, free villus, syncytial knot (arrow), and syncytial bridge (arrowhead) were observed (H&E; X100). (b) In the placental section of the control group, chorionic villi, syncytial knot (arrow), intervillous space, and fibrinoid material deposits (asterisk) were observed (H&E; X100). (c) In the placental section of the GDM group, chorionic villi, increased fibrinoid material deposition (asterisk), and syncytial bridge (arrowhead) were observed (H&E; X100). (d) In the placental section of the GDM group, chorionic villi, increased fibrinoid material deposition (asterisk), and an increased number of syncytial knots (arrow) were observed (H&E; X100).

GDM: Gestational diabetes mellitus, H&E: Hematoxylin and eosin". No other changes are required

Table 2. Anti-NRG-1 immunoreactivity

Groups	Trophoblast cells around the villi	Villus mesenchymal connective tissue cells	Endothelial cells in the villus mesenchymal connective tissue
Group 1 (Control group)	+	-	-
Group 2 (GDM group)	+++	+	+

GDM: Gestational diabetes mellitus, NRG-1: Neuregulin-1

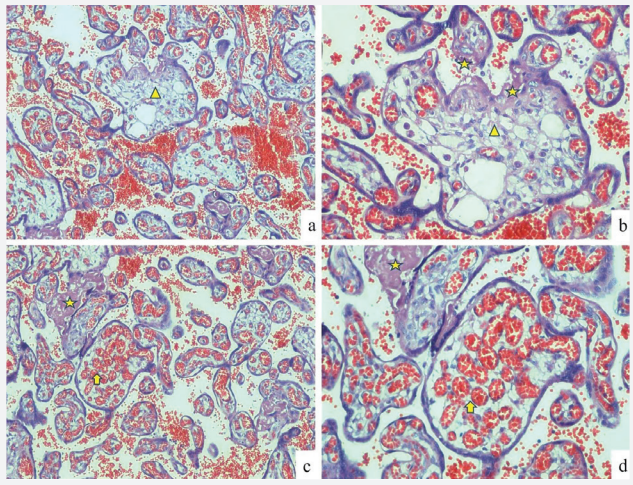

Figure 2. Placental sections from the GDM group

(a) Villous edema (arrowhead) was observed (H&E; X100). (b) Villous edema (arrowhead) and fibrinoid material deposits (asterisk) were observed (H&E; X200). (c) Fetal capillary structures in the villous stroma (arrow) and fibrinoid material deposits (asterisk) were observed (H&E; X100). (d) Fetal capillary structures (arrow) and fibrinoid material deposits (asterisk) were observed (H&E; X200)

GDM: Gestational diabetes mellitus, H&E: Hematoxylin and eosin

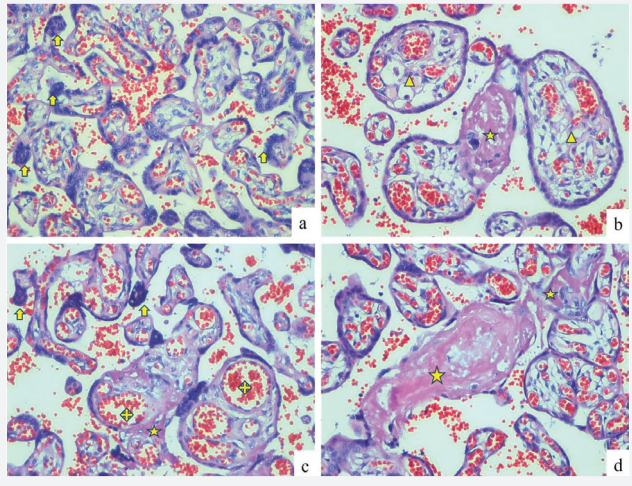

Figure 3. Placental sections from the GDM group (a) Syncytial knot structures (arrow) were observed (H&E; X200). (b) Villous edema (arrowhead) and fibrinoid material deposition (asterisk) were observed (H&E; X200). (c) Syncytial knot structures (arrow), fibrinoid material deposition (asterisk), and dilatation of fetal capillaries within the villous stroma (plus sign) were observed (H&E; X200). (d) Fibrinoid material deposits (asterisk) were observed (H&E; X200)

GDM: Gestational diabetes mellitus, H&E: Hematoxylin and eosin

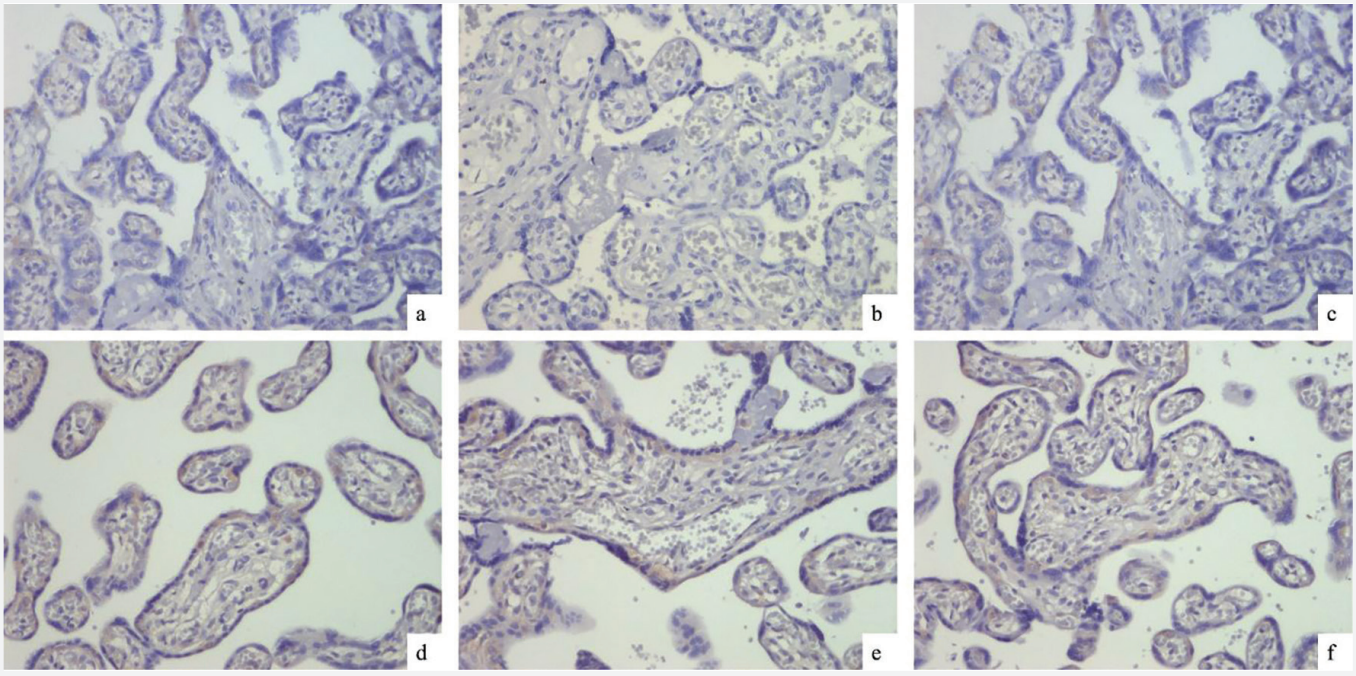

Figure 4. NRG-1 immunoreactivity (a-c) anti-NRG-1 immunoreactivity in placental sections from the control group (X200). (d-f) anti-NRG-1 immunoreactivity in placental sections from the GDM group (X200)

GDM: Gestational diabetes mellitus, NRG-1: Neuregulin-1

consistently reported in multiple studies[32-37]. In addition to these common findings, several specific histopathological alterations have also been reported: villous agglutination and chorangiosis by Aldahmash et al.[32]; increased villous number and villous vascularity by ElSawy et al.[33]; chronic inflammation and villous infarction by Lai et al.[34]; proliferation of fetal capillaries by Verma et al.[35]; fibrinoid deposition in perivillous areas by Meng et al.[36]; and villous immaturity by Gheorman et al.[37]. These differences suggest that GDM exerts complex effects on placental micromorphology not only at the structural level but also at the immunological and vascular levels.

In our study, placental samples obtained from cases diagnosed with GDM were histopathologically evaluated using H&E staining and compared with those of the control group. An increased number of villi, villous edema, dilatation and increased number of fetal capillaries, a marked increase in syncytial knots, and widespread fibrinoid deposition in the intervillous, perivillous, and intravillous areas were identified. A substantial proportion of these findings is consistent with previous studies investigating placental changes associated with GDM. In particular, increased syncytial knots, villous stromal edema, and fibrinoid deposition have been frequently reported in earlier studies[32-37], and these changes are among the characteristic features of placental remodeling associated with GDM. However, some findings observed in our study, particularly prominent dilatation of stromal capillaries and increased capillary density, have been reported to a limited extent in the literature[33,35]; in our study, these vascular changes were more widespread and pronounced. In addition, the accumulation of fibrinoid material not only in the perivillous area but also in the intervillous and intravillous areas represents a noteworthy finding that has been rarely emphasized in the existing literature[36,37]. In this respect, our study may provide novel contributions to the understanding of placental micromorphology in GDM.

NRG-1, a member of the neuregulin family, is thought to play an important role in pathological conditions characterized by impaired glucose metabolism, such as diabetes, obesity, and metabolic syndrome; however, the mechanisms by which NRG-1 contributes to dysregulated glucose metabolism remain unclear. *In vitro* studies have demonstrated that NRG-1 treatment increases the expression of the GLUT4 transporter in muscle cells and activates glucose uptake. These effects have been reported to be mediated through signaling pathways such as PI3K–PDK1–PKCζ and ERK1/2–Akt[29]. These molecular mechanisms suggest that NRG-1 may contribute to glucose homeostasis through insulin-like pathways. Further studies are needed to clarify the mechanisms by which NRG-1 is involved in the pathophysiology of diabetes and to determine whether NRG-1 has potential therapeutic effects.

In a study by Zhang et al.[29], serum NRG-1 levels were evaluated in 36 pregnant women with GDM at 24-28 weeks of gestation; serum NRG-1 levels were found to be higher in the GDM group compared to the control group. In a study by Lee et al.[38] investigating the genetic relationship between pre-pregnancy obesity and GDM, no statistically significant difference in *NRG-1* gene expression was reported between GDM patients with and without pre-pregnancy obesity. In another study by Lee et al.[39], psychiatric conditions such as anxiety, depression, and stress were found to be more prevalent in patients with GDM compared to healthy pregnant women, and one of the genes associated with these conditions was reported to encode the NRG-1 protein.

As previously discussed in the literature, several studies have examined the association between GDM and NRG-1; however, to the best of our knowledge, no study to date has assessed NRG-1 expression in placental samples from patients with GDM.

In our study, NRG-1 expression was evaluated in placental tissue samples from patients with GDM using the IHC method. NRG-1 expression was found to be higher in placental samples obtained from patients with GDM compared to the control group. This finding is consistent with a clinical study reporting elevated serum NRG-1 levels in patients with GDM[29], as well as with findings from diabetic animal models[40]. The observed endogenous increase suggests a compensatory response to oxidative stress, inflammation, and cellular damage associated with hyperglycemia. However, increased NRG-1 expression does not necessarily indicate an improvement in the underlying pathophysiology. Despite this increase, the biological activity of endogenous NRG-1 may remain limited due to impaired receptor sensitivity, dysregulation of intracellular signaling pathways, and insufficient levels of active NRG-1 reaching target tissues. In contrast, recombinant NRG-1 administration provides a pharmacologically potent signal that exceeds physiological levels, thereby enhancing glucose uptake, more robustly activating PI3K/Akt and ERK pathways, and alleviating vascular and mitochondrial damage[40-42]. Therefore, the coexistence of increased endogenous NRG-1 levels and the need for recombinant NRG-1 therapy does not represent a contradiction; rather, it reflects the insufficiency of the endogenous compensatory response and the superior therapeutic potential of exogenous treatment.

In addition to GDM, NRG-1 has also been reported to play a role in other pregnancy-related disorders. In a study by Kilis et al.[31], in which placental NRG-1 expression was evaluated by IHC in pregnancies complicated by preeclampsia, the intensity of anti–NRG-1 staining around the villi was reported to be lower in the preeclampsia group compared with controls. In an experimental study by Arutjunyan et al.[43], decreased placental NRG-1 levels were observed in pregnant rats with prenatal hyperhomocysteinemia. These differences suggest that NRG-1 expression may be subject to distinct regulatory mechanisms depending on the pathophysiological processes occurring during gestation. While the increase observed in GDM may reflect a compensatory response to hyperglycemia, the decrease observed in conditions such as preeclampsia or hyperhomocysteinemia may be related to endothelial dysfunction and placental oxidative stress suppressing NRG-1 expression. Therefore, alterations in NRG-1 levels may be associated with distinct pathways specific to the etiopathogenesis of each condition. In this context, the changes in placental NRG-1 expression observed in GDM may be linked to disease-specific compensatory and pathophysiological mechanisms; however, the clinical significance of this association requires further elucidation through advanced molecular and functional studies.

Study Limitations

Although this study achieved its intended objectives, it has certain limitations. The most notable limitation is the absence of biochemical analyses, given that GDM is a multisystem disorder. In addition, patients receiving insulin therapy were excluded from the GDM group to ensure a homogeneous study population. Future studies are planned to compare patients with and without insulin requirements. Another significant limitation is the absence of body mass index (BMI) data. Given the potential effects of obesity on both the development of GDM and NRG-1 expression, the inability to include BMI in the analyses may limit the interpretation of the findings. Finally, the absence of advanced imaging techniques, such as electron microscopy and fluorescence microscopy, which could enhance the accuracy and interpretability of the data, constitutes another limitation of this study.

CONCLUSION

In conclusion, it appears that diabetes occurring during pregnancy may lead to abnormalities in NRG-1 expression as well as placental defects. It is thought that this condition may stem from the effects of NRG-1 on embryogenesis and angiogenesis. This study is the first to demonstrate histopathological changes in the placenta in cases of GDM and to address the role of NRG-1. In this regard, it will make a significant contribution to the literature at a foundational level.

Ethics

Ethics Committee Approval: Approval for this study was obtained from the Muğla Sıtkı Koçman University Clinical Research Ethics Committee via decision no. 01/VI dated January 16, 2020, confirming that the study is ethically acceptable.

Human rights were protected in accordance with the principles of the "Declaration of Helsinki as revised in 2013."

Informed Consent: Written informed consent was obtained from all participants.

Footnotes

Authorship Contributions

Surgical and Medical Practices: B.K., M.N.A. Concept: H.E., B.G.Ö., Design: H.E., B.G.Ö., F.Ö. Data Collection or Processing: B.G.Ö., A.Y., K.D. Analysis or Interpretation: H.E., D.Ç., K.D., A.Y. Literature Search: B.G.Ö., H.E., A.Y., K.D. Writing: B.G.Ö., A.Y., K.D., D.Ç.

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