Obstetrics; *Maternal Fetal Medicine and Perinatology*

Placental Vimentin Expression in Preeclampsia and Gestational Diabetes Mellitus

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ABSTRACT

OBJECTIVE: This study investigated vimentin expression in placentas of patients with preeclampsia and gestational diabetes mellitus (GDM).

STUDY DESIGN: Placentas of preeclamptic women (n=25), women with GDM (n=25), and control cases (n=25) were enrolled in this study. Placental samples were fixed in zinc-formalin and further processed for paraffin wax tissue embedding. Demographic and laboratory parameters of patients were recorded. Vimentin immune activity was analyzed in the placental sections with immunohistochemistry. Sections were imaged and analyzed under a light microscope. A semiquantitative measurement was done between groups by comparing the Vimentin signal and significance was calculated. Network construction and pathway enrichment analysis were conducted using Cytoscape (v3.10.1) and ShinyGO, respectively.

RESULTS: Vimentin expression was high in the placental sections of the control group. The preeclampsia group showed positive Vimentin expression in cytotrophoblast and syncytiotrophoblast cells and connective tissue of placental villi in the preeclampsia group. Vimentin expression was generally recorded as negative in placental villi, fibrinoid substances, and connective tissue cells in the GDM group. Bioinformatic analysis showed that the AGE-RAGE signaling pathway and cancer-related pathways were mainly observed in Vimentin-associated pathways, which finally activate inflammatory pathways in both preeclampsia and GDM.

CONCLUSION: Vimentin expression patterns in placental tissue sections reveal nuanced regulatory mechanisms, emphasizing the need for further exploration into the functional roles of vimentin in placental physiology and pathology.

Keywords: Gestational diabetes mellitus, Placenta, Preeclampsia, Vimentin

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Introduction

Preeclampsia is a dynamic, progressive, and multisystemic disease during pregnancy. It is one of the important causes of perinatal morbidity and mortality worldwide. Preeclampsia is characterized by new-onset hypertension that occurs after the 20th week of pregnancy, proteinuria, or hypertension and disorders in multiple organ functions (1,2). The etiology of preeclampsia is not fully understood. Preeclampsia causes vascular lesions, vasospasm, increased platelet activation, and vascular coagulation in many organs and systems. Preeclampsia generally causes complications at an average rate of 6-8% after the 24th week of pregnancy (3). Pregnant women with preeclampsia experience a decrease in blood vol-

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ume, a decrease/increase in cardiac output, an increase in arterial blood pressure with peripheral resistance, and a decrease in uteroplacental blood flow (4).

The most common metabolic disorder during pregnancy is diabetes mellitus (5,6). Those diagnosed with diabetes before pregnancy are defined as pregestational diabetes mellitus (PGDM), and those diagnosed with diabetes during pregnancy are defined as gestational diabetes mellitus (GDM). PGDM is observed in 0.2-0.3% of all pregnancies and GDM is observed in 2-5% of all pregnancies (7). Clinical symptoms of the disease occur due to different etiological reasons, deficiency in the hormone insulin, or peripheral resistance. GDM is characterized by the inability to maintain the balance of normal glucose and insulin supply required by tissues (8-10). In these patients, insulin sensitivity decreases and beta cell response increases (11). Insulin resistance remains normal in the first 3 months of pregnancy, but disorders begin to become evident in the 2nd and 3rd trimesters (12).

Vimentin is one of the most stable intermediate filaments of the cell skeleton. Vimentin is involved in many cellular processes such as migration, differentiation, proliferation, and signal transduction (13). Vimentin shows cell-specific localization and a distinct pathway of differentiation. Vimentin is mainly expressed by embryonic mesodermal cells, contributing formation of placental structures such as chorionic villi, placental connective tissue, and placental vascular structures. Also, mesodermal-derived endometrial stromal cells undergo transformation via a process called decidualization during pregnancy. Since mesoderm contributes to the formation of placenta and endometrial cells, differential expression of vimentin may play an important role in gestation (14,15). Norwitz et al showed that vimentin expression was high during the menstrual cycle in endometrial epithelium and glands however it was reduced after onset of pregnancy. Authors claimed that vimentin may play a role in decidualization (16). Koç et al studied the expression of vimentin in placenta with different intervals in rats. They revealed that vimentin expression was observed in trophoblasts and some decidual cells of the placenta (17). Another study recorded high expression of vimentin in trophoblastic giant cells at 7.5 days of gestation in mice. The authors stated that vimentin can be a prognostic tool for studying pathological pregnancies associated with defects in vascular trophoblast cells (18).

The placental pathogenesis in preeclampsia and GDM is not fully understood. However, three theories are proposed, which are placental dysfunction, endothelial dysfunction, and inflammatory response (19-21). Since vimentin is expressed in various cell types of the placenta (cytotrophoblasts, syncytiotrophoblasts, and endothelial cells), its role in placental development is crucial. Similarly, endothelial dysfunction led to increased vascular resistance in preeclampsia, suggesting potential dysregulation of vimentin expression. The involvement of vimentin in the inflammatory pathway is also another possible mechanism for the regulation of vimentin in preeclamptic patients (22,23). A similar pathogenesis of preeclampsia has been also associated with GDM. Considering the role of Vimentin in placentation, Vimentin may be a potential marker for the placental pathologies of GDM.

Therefore, considering the role of Vimentin in placental formation and decidualization, we aimed to compare vimentin expression levels in the placentas of pregnant women with preeclampsia and GDM.

Material and method

Ethical approval was taken from the Non-Interventional Clinical Research Ethics Committee, Siirt University (Date: 07.11.2022, approval number: 59214). A total of 25 preeclamptic pregnant women, 25 GDM women, and 25 uncomplicated control cases were included in the study. ACOG criteria were used for the diagnosis of preeclampsia (24). Patients was diagnosed with GDM according to the World Health Organization diagnostic criteria based on the 75 g oral glucose tolerance test (OGTT) (25). The control group included 25 healthy uncomplicated pregnant women who did not experience any adverse pregnancy throughout the pregnancy and had delivered at term.

The exclusion criteria included multiple pregnancies, ruptured amniotic membrane, preterm labor, infectious disease, hepatic disease, renal disorder, malignant tumor, and Cushing's syndrome. Patients with preexisting diabetes mellitus or a history of GDM were also excluded.

Demographics and laboratory parameters were recorded for study groups. Blood samples were collected before labor and further analyzed for laboratory parameters. All patients were informed and accepted to participate in the study. A patient consent form was signed by all participants.

Histological tissue processing: Placentas were acquired from the Department of Gynecology and Obstetrics, Siirt University Training and Research Hospital. Placental tissue processing was performed at the Immunohistochemistry Research Laboratory of Histology and Embryology Department, Dicle University. Placental samples for histologic analysis were excised and further analyzed for histological evaluation. Samples were fixed in zinc-formalin and dehydrated through grading alcohol series, immersed in xylene, and incubated in paraffin wax. 5 µm sections were cut from paraffin blocks and stained for immune staining of vimentin primary antibody. A graphical abstract of the major steps of the experiment is shown in Figure 1.

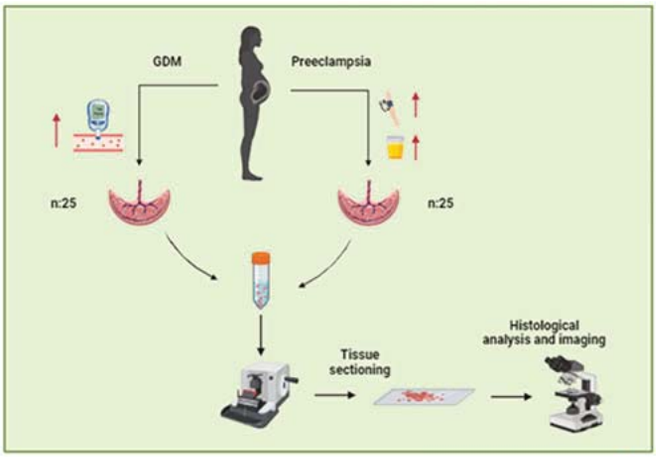

Figure 1: *Graphical abstract of major steps of material and methods (Courtesy of Biorender.com).*

Immunohistochemical examination: Placental sections were dewaxed, hydrated in grading alcohol series, and washed in distilled water. Slides were allowed to react with 3% hydrogen peroxide (H_2O_2) to block endogen peroxidase activity. After washing in PBS, sections were immersed in a blocking solution. Without washing, the previous solution was drained and sections were incubated with Vimentin (Catalog no: sc-6260, Santa Cruz, US) overnight at +4°C. Sections were biotinylated and then reacted with streptavidin peroxidase for 15 minutes. After PBS washing, diaminobenzidine (DAB) chromogen was used as a chromogen to observe color change. The reactions were stopped with PBS solution and sections were counter-stained with hematoxylin dye.

ImageJ analysis: The staining intensity of vimentin expression was measured by Image J software (version 1.53, http://imagej. nih.gov/ij). Measurement was calculated by the method of Crowe et al. (26). Quantification was recorded by analyzing ten fields from each specimen per group (27). In specimens, the brown color stands for the positive expression of the antibody of interest while the blue color represents a negative expression of the antibody of interest. Signal intensity (expression) from a field was calculated by dividing the intensity of the antibody of interest by the whole area of the specimen. A value for staining area/whole area was calculated for each specimen from ten fields. An average value was measured for groups and analyzed for semi-quantitative immunohistochemistry scoring. Slides were imaged with Zeiss Imager A2 light microscope. All images were processed and quantified using ImageJ software.

Statistical analysis

Statistical analysis was done using the IBM SPSS 25.0 software (IBM, Armonk, New York, US). The data were recorded as median (Quartile 1 - Quartile 3). Statistical distribution was evaluated with the Shapiro-Wilk test. Non-normal distributed data was analyzed by the Kruskal Wallis test (post hoc Dunn's test) for group comparisons. Significance was considered for p-values <0.05. The number of patients for each group was calculated by G Power analysis (version 3.1). Cohen's criteria were defined according to the study of Alviggi et al. (28).

Network construction and enrichment analysis: Protein-protein interaction (PPI) networks for the diseases-preeclampsia and GDM-and vimentin, were constructed using Cytoscape software (v3.10.1) through Search Tool for the Retrieval of Interacting Genes/Proteins (STRING): disease and STRING: protein modules, respectively. A cutoff value of 0.4 was applied for all analyses, and the maximum additional interactions were set at 200 for the diseases and 100 for vimentin. Subsequently, the networks of diseases and vimentin were intersected. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of these intersected pathways was performed using the ShinyGO 0.80 online platform (http://bioinformatics.sdstate.edu/go/). Box plots were generated for 10 significant KEGG pathways with a False Discovery Rate (FDR) less than 0.05.

Results

Laboratory parameters: Demographic and biochemical parameters of patients with preeclampsia and GDM are shown in Tables I and II, respectively.

Table I: *Demographics and laboratory records of pregnant women with preeclampsia*

Parameters (n=25)	Preeclampsia Median (Q1-Q3)
Maternal age, years	31.4 (25.6-36.0)
Body mass index, kg/m^2	29.2 (25.5-35.7)
Pregnancy week	34.5 (29.7-39.8)
Labor week	37.1 (35.3-39.2)
Gravida	2.0 (1.0-3.0)
Parity	1.0 (0.0-1.0)
Systolic blood pressure, mmHg	155.0 (148.0-165.0)
Diastolic blood pressure, mmHg	98.0 (94.0-125.0)
24-h proteinuria	893.5 (462.6-2173)
AST, U/L	21.2 (14.5-48.3)
ALT, U/L	10.1 (7.4-56.5)
LDH, U/L	305.1 (297.4-361.8)
Platelet count, mm^3 ×10^3	210.5 (123.3-289.4)

AST: aspartate dehydrogenase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase

Table II: *Demographics and laboratory records of pregnant women with gestational diabetes mellitus*

Parameters (n=25)	Gestational diabetes mellitus Median (Q1-Q3)
Maternal age, years	30.3 (23.5-38.43)
Body mass index, kg/m^2	28.2 (24.72-44.51)
Labor week	36.2 (34.5-38.8)
Gravida	2.0 (1.0-3.0)
Parity	2.0 (0.0-4.0)
Systolic blood pressure, mmHg	112.0 (88.0-138.0)
Diastolic blood pressure, mmHg	77.0 (60.0-90.0
HbA1c (%)	5.3 (4.2-6.6)
75 g OGTT (fasting)	88 (71-113)
75 g OGTT (1-hour)	163 (90-216)
75 g OGTT (2-hours)	135 (68-228)

OGTT: oral glucose tolerance test, g: gram, GDM: gestational diabetes mellitus

Histological staining: Vimentin immune staining of placental tissue sections is shown in Figures 2-4. The control group showed mainly positive vimentin expression in placental villi, villous mesoderm, and vascular endothelial cells (Figure 2A and 2B). In the images of the preeclampsia group, Vimentin expression was high in cytotrophoblast and syncytiotrophoblast cells, the connective tissue of placental villi and capillaries, while some villi and endothelial cells showed negative Vimentin expression (Figures 3A and 3B). Compared to the control group, the Vimentin expression was slightly decreased in the preeclampsia group.

Compared to the control and preeclampsia groups, Vimentin expression generally was negative in placental villi, fibrinoid substances, and connective tissue cells in the GDM group (Figures 4A and 4B).

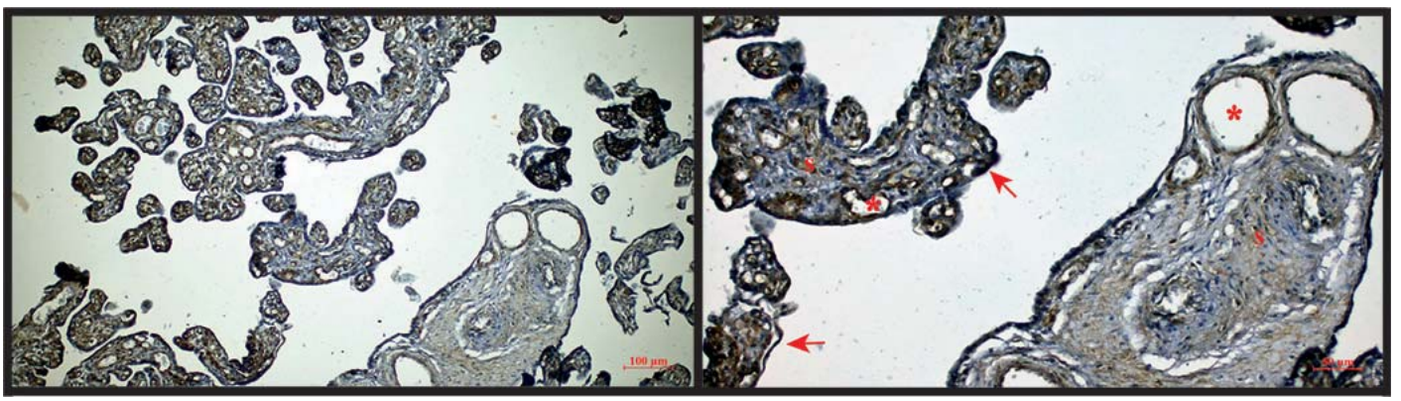

Figure 2: *Low (A) and high (B) magnification of placental sections belonging to the control group. High Vimentin expression was observed. Arrow: Placental villi, asterisk (*): capillary, s: villous stroma (mesoderm), A: 100 µm, magnification: 10X; B: 50 µm, magnification: 20X.*

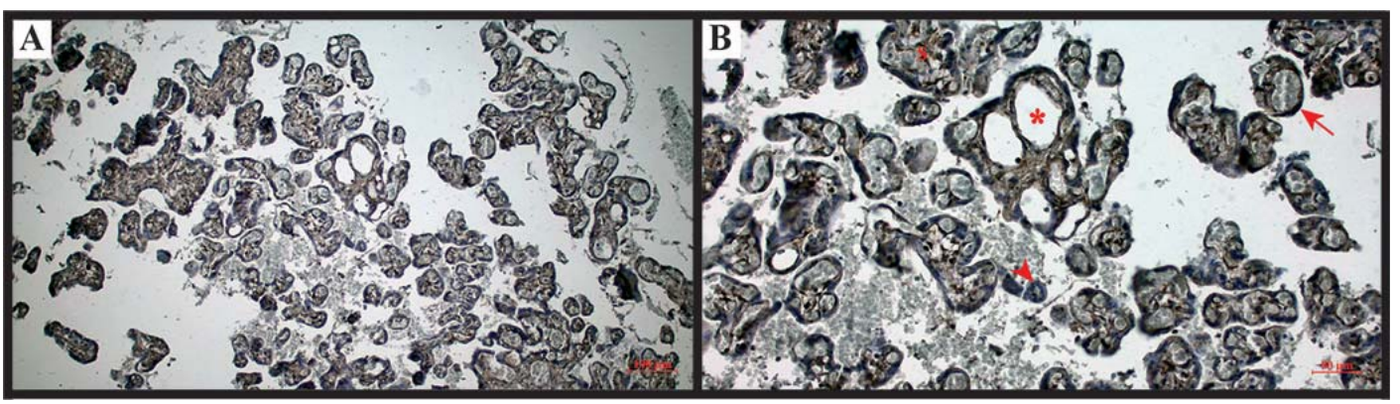

Figure 3: *Low (A) and high (B) magnification of placental sections belonging to the preeclampsia group. Arrow: Placental villi with positive Vimentin expression, arrowhead: placental villi with negative Vimentin expression, asterisk (*): capillary, s: villous stroma (mesoderm), A: 100 µm, magnification: 10X; B: 50 µm, magnification: 20X.*

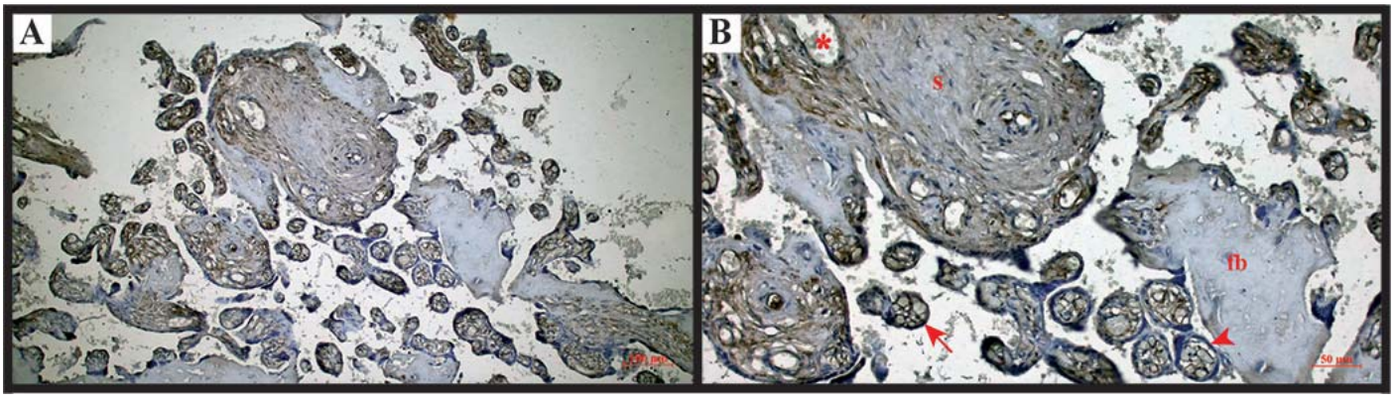

Figure 4. *Low (A) and high (B) magnification of placental sections belonging to the GDM group. Arrow: Placental villi with positive vimentin expression, arrowhead: placental villi with negative vimentin expression, asterisk (*): capillary, s: villous stroma (mesoderm), fb: fibrinoid, A: 100 µm, magnification: 10X; B: 50 µm, magnification: 20X.*

Semi-quantitative measurements of Vimentin: Semiquantitative measurement of vimentin immune staining was shown in Table III. A graphical illustration of signal intensity is shown in Figure 5 with significance. The Vimentin signal is significantly lower in the GDM group compared to the control and preeclampsia groups, indicating there is a distinct difference in Vimentin expression between these two pregnancy-related conditions.

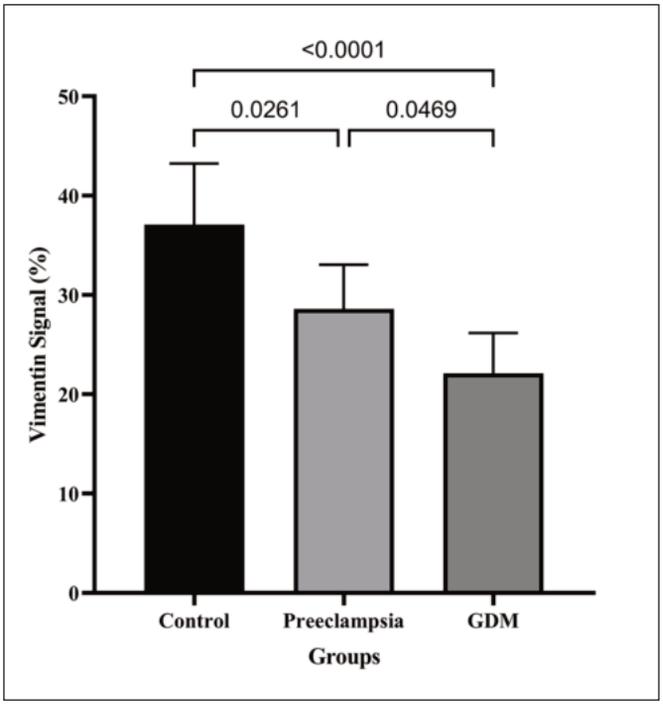

Figure 5: *Graphical illustration of vimentin signal intensity and significance between groups.*

Intersected PPI networks and KEGG pathway enrichment analysis: The PPI network of Vimentin intersected with that of target diseases to identify common proteins implicated in the mechanisms of preeclampsia and GDM. The 14 proteins shared between the PPI network of vimentin and preeclampsia, along with the 8 common proteins in the PPI network of Vimentin and GDM were depicted in Figure 6. Utilizing these proteins, KEGG pathway analysis was conducted to explore the Vimentin-associated cellular signaling pathways in both target diseases. Vimentin was frequently observed in association with Advanced Glycation End Products (AGE)-Receptor for AGE (RAGE) signaling pathway in diabetic complications, cancer-related pathways, toxoplasmosis, and hepatitis B for preeclampsia and GDM. Moreover, the Vimentin-associated hypoxia-inducible factor (HIF)-1 signaling pathway and relaxin signaling pathway were identified as signaling pathways specifically effective in GDM.

Discussion

Vimentin is recognized as a crucial intermediate filament protein densely present within the cell, playing a vital role in stabilizing the intracellular structure. Furthermore, the Vimentin promoter exhibits a complex structure and is thought to be regulated by a combination of positive and negative regulatory elements. Additionally, there is speculation that Vimentin may be released outside the cell, either on the surface of the plasma membrane or under various physiological and pathological conditions (13). Shirakawa et al. evaluated the expression of Vimentin in the placental tissue through immunohistochemical staining in 19 patients diagnosed with adherent placenta after hysterectomy. According to the study's

Table III: *Vimentin signal level in study groups*

Signal	Control group	Preeclampsia group Median (Q1-Q3)	GDM group Median (Q1-Q3)	p
Vimentin	36 (33-43)	28 (25-33)	23 (18-27)	**<0.001***

** Kruskal Wallis test; GDM: Gestational diabetes mellitus*

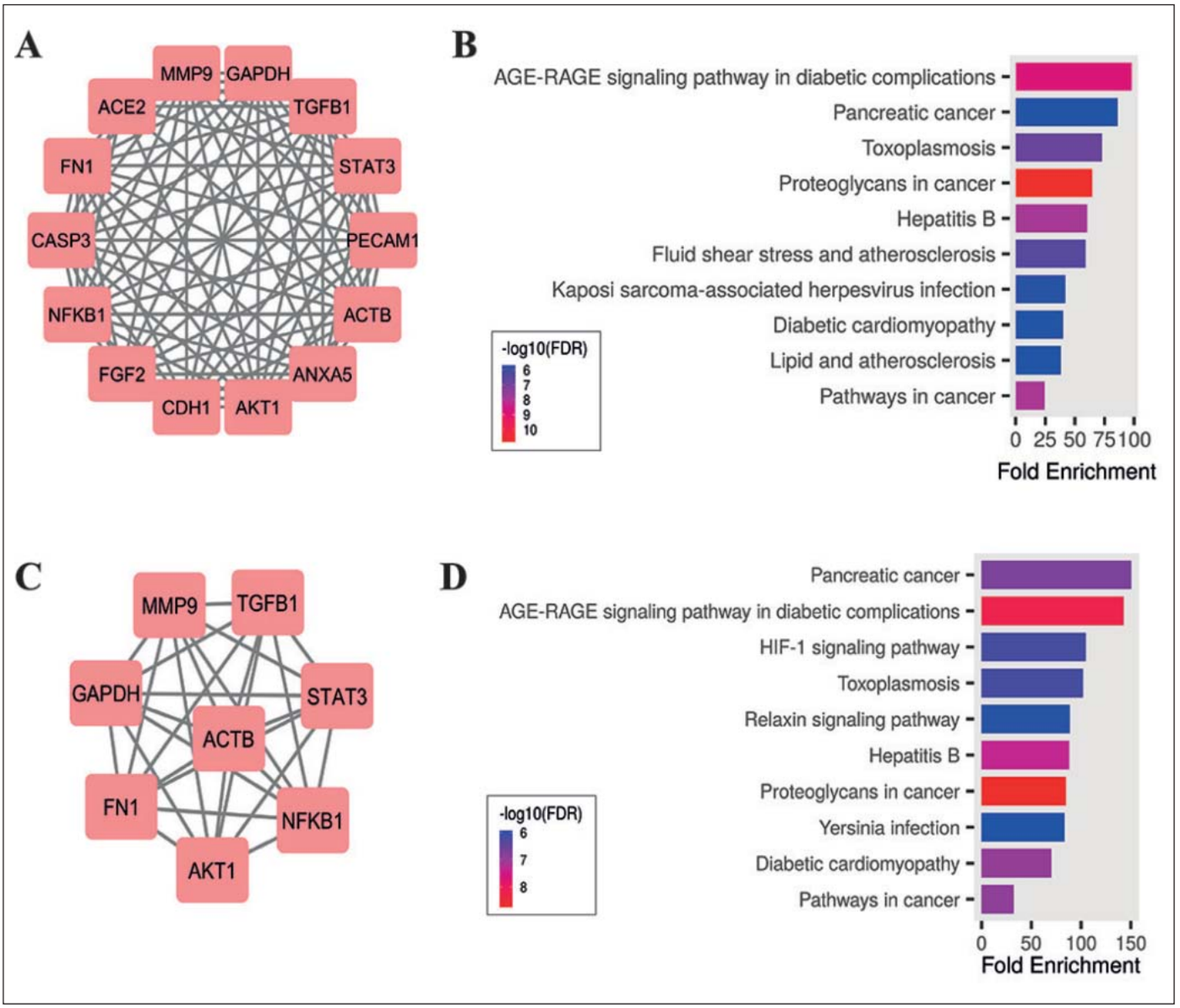

Figure 6: *Intersected PPI networks and KEGG enrichment analysis. A. The intersected PPI network uncovered 14 proteins shared between the PPI network of Vimentin and preeclampsia. B. The significant top 10 pathways associated with Vimentin in preeclampsia. C. The intersected PPI network uncovered 8 proteins shared between the PPI network of Vimentin and GDM. D. The significant top 10 pathways associated with Vimentin in GDM. The color transition from red to blue signifies a shift in the FDR value of the pathway, with a transition from larger to smaller values. Additionally, the greater the surface area, the higher the degree of enrichment.*

results, the researchers reported that the Vimentin promoter has a highly complex structure, regulated by a combination of positive and negative regulatory elements. In this context, they have stated that Vimentin is essential for trophoblast invasion (29). A study conducted by Korgun et al. reported the significant role of Vimentin protein in the process of decidualization. According to their research, on the 6th day of pregnancy, Vimentin protein was localized in the decidual area beneath the luminal epithelium and around the implanting embryo (30). In similar experimental studies, a positive immunoreaction for Vimentin has been reported in the examination of the placental decidua towards the end of pregnancy (31). In a study by Khong et al., placentas at different stages of pregnancy were examined. Vimentin positivity was detected in the chorionic villus stroma, while Vimentin expression was observed to be negative in trophoblasts. Additionally, in the same study, Vimentin expression was noted in the connective tissue of the myometrium, decidual cells, and stroma (32). In the study conducted by Irtegün et al., an increase in Vimentin observed in preeclamptic placenta has been reported to potentially lead to a reduction in vascular permeability (33). Our results revealed that positive Vimentin expression was observed in various components of the placenta including placental villi, villous mesoderm, and vascular endothelial cells in the control group, suggesting that Vimentin was normally expressed in placental structures and likely played a role in their structural integrity and function. Findings of the preeclampsia group showed high Vimentin ex-

pression in cytotrophoblast and syncytiotrophoblast cells, connective tissue of placental villi, and capillaries. Some villi and endothelial cells showed negative expression compared to the control group. The observed changes in Vimentin expression in preeclamptic placentas could indicate alterations in placental structure and function associated with preeclampsia. The increased Vimentin expression in trophoblast cells might suggest a compensatory mechanism in response to the pathological changes associated with preeclampsia.

Studies on the placentas of GDM patients related to vimentin are quite limited. However, it is known that the pathophysiology of GDM, gestational diabetes mellitus, involves irregularities in immune regulation (34). Immune activity of Vimentin was generally negative in placental villi, fibrinoid substances, and connective tissue cells in the GDM group. Compared to both the control and preeclampsia groups, there was a significant reduction in Vimentin expression in various placental structures in the GDM group. This suggests a significant alteration in the Vimentin expression pattern in GDM-affected placentas. The relatively less Vimentin expression may indicate impairment in structural support and cellular dynamics within the placenta in the GDM. To explore the potential regulatory role of Vimentin in preeclampsia and GDM, KEGG analysis was conducted and we observed that Vimentin is mainly enriched in pathways such as AGE-RAGE signaling and cancer-related pathways in these diseases. Previous studies revealed that AGE-RAGE signaling (35,36), and cancer-related pathways (37,38) played important roles in both target diseases. However, in GDM, unlike in preeclampsia, Vimentin was found to modulate the HIF-1 and relaxin signaling pathways. Li et al. demonstrated that the HIF-1a and TWIK-1-related potassium channel 1 (TREK1) proteins are involved in the alterations of uterus contractility in GDM (39). Moreover, some studies have suggested the regulatory effects of relaxin levels in GDM (40). Thus, the reduced Vimentin expression identified in our GDM samples might regulate the HIF-1 and relaxin correlated signaling pathways. The bioinformatic analysis also showed that Vimentin is differentially regulated in preeclampsia and GDM, which were consistent with immunohistochemical findings.

Limitations of this study: The study may be limited by a small sample size, potentially affecting the statistical power and the ability to generalize findings to a broader population. Variability in staining protocols or subjective interpretation of results could impact the reliability and reproducibility of the findings. It may be helpful to include additional complementary methods, such as molecular assays or quantitative techniques, to strengthen the results and provide a more comprehensive understanding. Moreover, experimental approaches are needed to validate the impact of the vimentin-associated pathways presented through bioinformatical analyses.

Strengths of this study: Previous studies have shown limited investigation of Vimentin in preeclampsia and GDM. This study is the first to show the significance of Vimentin in pregnancy complications with the inclusion of immunohistochemical and bioinformatical analysis. The study also potentially has clinical implications by shedding light on the molecular mechanisms underlying placental complications like preeclampsia and GDM.

Conclusion

These findings suggest a complex and dynamic regulation of Vimentin expression in the placenta, indicating potential involvement in specific functions within distinct cell types or stages of placental development. The observed variability in Vimentin expression patterns between the two pregnancy-related conditions may imply differential roles of Vimentin in response to the pathophysiological processes associated with preeclampsia and GDM. Specifically, the AGE-RAGE signaling pathway has the potential to be associated with Vimentin in both diseases, which eventually leads to the activation of inflammatory mediators, one of the causes of both diseases. Further research is needed to elucidate the specific functions and regulatory mechanisms of vimentin in the context of placental development and its implications for pregnancy-related complications.

Declarations

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Ethics approval and consent to participate: All participants signed informed written consent before being enrolled in the study. The study was reviewed and approved by the ethics committee of the Non-Interventional Clinical Research Ethics Committee, Siirt University (Ethics approval reference number: 59214, date 07.11.2022). All procedures were performed according to the Declaration of Helsinki.

Availability of data and materials: The data supporting this study is available through the corresponding author upon reasonable request.

Competing interests: The authors declare that they have no competing interests.

Authors' contributions: Conceptualization: FA, SCO; Data duration: FA, TK, FT; Formal analysis: FA, MY, FE; Funding acquisition: FA, SCO, EZY, GB; Investigation:FA, SCO, TK, FŞ, HA; Methodology: FA, TK, FT, FE; Project administration:FA; Resources: FA, SCO, EZY, GB; Software: FA, TK; Supervision: FA, SCO; Validation: FA, SCO, TK, HA; Visualisation: FA, TK, FT, FE, FŞ; Writing -original draft: FA, SCO; Writing - review & editing:FA, SCO, TK.

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