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Evaluation of serum neopterin, periostin, Tenascin-C, tissue inhibitor of metalloproteinase-1 and matrix metalloproteinase-2 levels in obese pregnant women

Obez gebelerde serum neopterin, periostin, Tenascin-C, metalloproteinaz-1 doku inhibitörü ve matriks metalloproteinaz-2 düzeylerinin değerlendirilmesi

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Abstract

Objective: To investigate the role of extracellular matrix proteins in the molecular mechanism of inflammatory response in obese pregnant women by comparing serum levels of neopterin, periostin, Tenascin-C, tissue inhibitor of metalloproteinase-1, and matrix metalloproteinase-2 between obese and normal weight pregnant women in the third trimester.

Materials and Methods: A prospective cross-sectional study was conducted between April 2021 and December 2021. A total of 84 pregnant women were included and three groups were formed with 28 participants in each group.

Results: Serum levels of neopterin, periostin, Tenascin-C and tissue inhibitor of metalloproteinase-1 were significantly higher in class II-III obese pregnant women than in class I obese and normal-weight women ($p=0.002$, $p<0.001$, $p<0.001$, and $p<0.001$, respectively). There was no significant difference in serum matrix metalloproteinase-2 levels between the groups ($p=0.769$). Receiver operating characteristic curve analysis showed that Tenascin-C and periostin were effective in predicting pre-eclampsia [area under the curve (AUC)=0.82, 95% confidence interval (CI), 0.72-0.90, $p<0.001$ and AUC=0.71, 95% CI, 0.60-0.80, $p=0.007$, respectively].

Conclusion: This study demonstrated that class II-III obese pregnant women had significantly higher serum levels of neopterin, periostin, Tenascin-C, and tissue inhibitor of metalloproteinase-1 in the third trimester. These higher serum levels may be associated with the adverse perinatal effects of obesity during pregnancy.

Keywords: Neopterin, obesity, periostin, pregnancy, Tenascin-C, tissue inhibitor of metalloproteinase-1

Öz

Amaç: Bu çalışmanın amacı, obez gebe kadınlarda ve normal kilolu gebe kadınlarda üçüncü trimester serum neopterin, periostin, Tenascin-C, metalloproteinaz-1 doku inhibitörü ve matriks metalloproteinaz-2 düzeylerini karşılaştırarak, ekstrasellüler matriks proteinlerinin obez gebe kadınlarda enflamatuvar yanıtın moleküler mekanizmasındaki rolünü araştırmaktır.

Gereç ve Yöntemler: Nisan 2021 ile Aralık 2021 tarihleri arasında yürütülen bu prospektif kesitsel çalışmaya toplam 84 hasta dahil edildi ve her grupta 28 katılımcı olmak üzere üç grup oluşturuldu.

PRECIS: Serum neopterin, periostin, tenascin-C, and TIMP-1 levels are significantly higher in class II-III obese pregnant women than in class I obese and normal weight women.

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Bulgular: Sınıf II-III obez gebelerde serum neopterin, periostin, Tenascin-C ve metalloproteinaz-1 doku inhibitörü seviyeleri, sınıf I obez ve normal kilolu kadınlara göre anlamlı olarak daha yüksek saptanırken (sırasıyla p=0,002, p<0,001, p<0,001, ve p <0,001), gruplar arasında serum matriks metalloproteinaz-2 seviyeleri açısından anlamlı fark izlenmedi (p=0,769). İşlem karakteristik eğrisi analizi, Tenascin-C ve periostinin preeklampsiyi öngörmede etkili olduğunu gösterdi [sırasıyla eğri altındaki alan (AUC)=0,82, %95 güven aralığı (GA), 0,72-0,90, p<0,001 ve AUC=0,71, %95 GA, 0,60-0,80, p=0,007].

Sonuç: Bu çalışma, sınıf II-III obez gebelerin üçüncü trimester serum neopterin, periostin, Tenascin-C ve metalloproteinaz-1 doku inhibitörü düzeylerinin anlamlı derecede yüksek olduğunu göstermiştir. Bu yüksek serum seviyeleri, gebelikte obezitenin olumsuz perinatal etkileri ile ilişkili olabilir.

Anahtar Kelimeler: Neopterin, obezite, periostin, gebelik, Tenascin-C, metalloproteinaz-1 doku inhibitörü

Introduction

Obesity, which is characterized by an excess of adipose tissue in the body, is a condition that affects an increasing number of people globally. Because of the numerous alterations in adipose tissue brought on by obesity, including significant changes in the structure and growth of adipocytes, the differentiation of preadipocytes into adipocytes, and the accumulation of inflammatory cells, obesity is thus considered a chronic inflammatory condition. As a result of obesity and pregnancy, pregnant obese women are prone to an increased inflammatory response with increased macrophage accumulation and synthesis of inflammatory mediators in maternal plasma[1]. Obesity during pregnancy is thought to alter metabolic, neuroendocrine, microbiological, and immunological pathways. Obesity is defined as having a body mass index (BMI) at the onset of pregnancy of 30 kg/m^2 or more; however, not all women fulfill these criteria. It has been reported that an estimated 18% of pregnant women are obese or overweight, and pre-pregnancy obesity is the greatest risk factor for obesity during pregnancy[2]. Several maternal and fetal adverse outcomes, such as pregnancy loss, fetal death, gestational diabetes, large for gestational age, gestational hypertension, venous thrombosis, increased rates of cesarean section, and fetal malformations, are associated with obesity during pregnancy and may be a consequence of the excessively inflammatory environment.

Remodeling of the extracellular matrix (ECM) in adipose tissue could alter adipocyte function and adipokine secretion profiles. The matrix metalloproteinase (MMP) family and the fibrinolytic plasminogen/plasmin system mediate the degradation of ECM components and regulate adipose tissue morphology. Alterations of adipose tissue in the ECM and inflammation associated with obesity have attracted considerable attention, highlighting the interaction of these processes in the transcriptome signature of adipose tissue in obese patients[3]. During the chronic inflammatory state associated with obesity, the ECM serves as a scaffold for cell infiltration and a reservoir for adipokines and growth factors[4]. A subset of the so-called damage-associated molecular patterns in the ECM can also directly activate the inflammatory response during tissue damage.

Before its reclassification as a matricellular protein, periostin was initially characterized as a secreted cell adhesion protein and was previously known as osteoblast-specific factor 2[5]. Periostin is produced by various cytokines and may contribute to the maintenance or exacerbation of inflammation. Additionally, periostin is secreted as a direct result of inflammatory reactions instead of acting to regulate the activities of these responses. The pathophysiology of fibrosis, arthritis, osteoarthritis, and atherosclerosis, as well as carcinogenesis and metastasis, including breast, colon, pancreatic, and ovarian cancers, has been linked to periostin, which was originally cloned from a mouse osteoblast cell line. Multiple signaling pathways, such as Wnt/b-catenin and PI3K/AKT, have also been associated with periostin. Periostin has also been linked to metabolic disorders by suppressing hepatic fatty acid oxidation via the JNK pathway[6]. It is not yet known what role periostin plays in lipid metabolism and obesity in pregnancy.

Tenascin-C (TnC) is also an ECM protein that is significantly expressed throughout organogenesis and is involved in cell interactions during proliferation, migration, epithelial-mesenchymal transition, and parenchymal-mesenchymal interactions[7]. In animal models of diet-induced obesity, increased tissue inflammation was associated with increased expression of TnC and Toll-like receptor 4 in stellate liver cells[8]. TnC has also been shown to be overexpressed in human preadipocytes in response to stimuli secreted by macrophages. TnC is usually expressed in association with MMP, and suppression of MMP inhibits the expression of TnC. MMPs, which are endoproteinases that require zinc and calcium to function, can break down the ECM. Because MMPs perform such a wide variety of tasks, there is growing evidence to suggest that they may play a role as either activators or inhibitors in the processes of tissue remodeling, cardiovascular disease, and obesity. Recent research has established a close relationship between adipose tissue inflammation and ECM, in which MMP and TnC expression may play a critical role. However, to our knowledge, there are no data on the level of TnC in the serum of obese pregnant women or in its putative involvement in adipose tissue inflammation.

The pteridine derivative neopterin is generated by dendritic cells, macrophages, activated monocytes, and endothelial cells. It has been found in many bodily fluids, including plasma, urine, saliva, synovial fluid, and cerebrospinal fluid. The level of neopterin in body fluids is used to assess the systemic immunological and inflammatory responses in various diseases[9]. Macrophages are the most abundant type of immune cells in a healthy placenta. The T helper type 2 immune response appears to be represented in the placenta by a predominance of alternatively activated macrophages. Obesity during pregnancy results in an enhanced inflammatory response in the placenta, characterized by accumulating numerous macrophage subsets

and the production of proinflammatory mediators, a hallmark of chronic villous inflammation[10]. Before pregnancy, chronic inflammation in obese women triggers a cascade of events leading to an inflammatory environment in the uterus. However, no studies have been conducted on the association between pregnant obesity, inflammation, and serum neopterin levels[11]. Tissue Inhibitor of Metalloproteinase-1 (TIMP-1) is the major regulatory peptide for extracellular protease enzymes, including MMPs and disintegrin metalloproteinases (ADAMs and ADAMTSs), which are responsible for ECM degradation. It is also secreted by adipocytes, which are increased in obesity, and it supports the formation of adipose tissue. MMPs and TIMPs have recently been linked to obesity-induced adipose tissue enlargement, and TIMP-1 appears to be an interesting candidate for increased fat formation for several reasons. The fat-derived protein TIMP-1 promotes adipose tissue growth when weight gain occurs[12]. However, the exact mechanisms by which obesity-induced TIMP-1 production is triggered in adipocytes remain a mystery. To date, no human studies have been performed to examine blood levels of MMP and TIMP-1 in obese pregnant women, although animal studies have shown alterations in these markers in cardiac and cartilage tissue of offspring of obese mothers[13].

While a few studies have found increased serum levels of neopterin, periostin, TN-C, TIMP-1, and MMP-2 in patients with perinatal complications such as preeclampsia, no studies have examined these ECM protein levels in pregnant women with obesity. Also, the usefulness of these markers in predicting adverse perinatal outcomes has not been extensively studied. Here, we investigated the role of ECM proteins in the molecular mechanism of the inflammatory response in obese pregnant women by comparing serum levels of neopterin, periostin, TnC, tissue inhibitor of metalloproteinase-1, and MMP-2 in obese and normal-weight pregnant women in the third trimester.

Materials and Methods

Study Design and Setting

This prospective cross-sectional study was conducted between April 27, 2021 and December 01, 2021 in the Gynaecology Department of Inonu University School of Medicine. A total of 84 pregnant women were enrolled and three groups were formed, each with 28 participants. The groups were divided according to the World Health Organization BMI classification: Control (BMI 18-24.9 kg/m^2), Class I obesity (BMI 30-34.9 kg/m^2), and Class II-III obesity (BMI ≥35 kg/m^2). The following are the criteria by which patients were enrolled in the study: (1) a viable singleton pregnancy; (2) 37^{+0} to 40^{+6} weeks of gestation (confirmed by first trimester ultrasound); (3) normal fetal anatomy; and (4) patients who understood both the oral and written instructions in Turkish and gave their written consent to participate. Exclusion criteria were as (1) coexisting medical conditions (diabetes, hypertension, renal failure, chronic hepatic disease, pulmonary or cardiovascular disease); (2) multiple pregnancy; (3) fetal death; (4) severe anomalies (fatal or those requiring prenatal or postnatal surgery) or chromosomal abnormalities; and (5) tobacco and alcohol use. All participants gave their informed consent before the study was conducted. This research was carried out in accordance with the principles outlined in the Declaration of Helsinki, and it received ethical approval number 2021/138 from the Clinical Research Ethics Committee of Inonu University.

Serum Analysis

Every pregnant woman who participated in the study had a small amount of blood drawn from a peripheral vein into biochemical tubes (2-3 mL) upon admission to the hospital. Serum was separated from blood samples by centrifuging them at 3500 rpm for 15 min at room temperature, and then placed in microcentrifuge tubes for long-term storage at -80 °C. Neopterin, periostin, TnC, TIMP-1, and MMP-2 were quantified in the serum samples using kits for enzymatic immunoassays (E3155Hu, E2063Hu, E3226Hu, E1414Hu, and E0796Hu; Bioassay Technology Laboratory Ltd, Birmingham, UK). The assay ranges for neopterin, periostin, TnC, TIMP-1, and MMP-2 were 0.1-38.0 nmol/L, 1-400 ng/mL, 0.5-150 ng/mL, 20-6.000 ng/L, and 0.3-90.0 ng/mL, respectively. The coefficients of variation for inter- and intra-assay precision were less than 10% and less than 8%, respectively, for all enzyme-linked immunosorbent assay (ELISA) kits. All serum samples were kept in the refrigerator and the ELISA tests were run in the laboratories at the School of Pharmacy. For each patient, age, BMI (weight in kilograms divided by square of height in meters), BMI before pregnancy, gestational week, gravidity, parity, adverse perinatal outcomes, mode of delivery, cord blood pH, cord blood base deficiency, histological results, neonatal outcomes, and serum levels of protein markers including neopterin, periostin, TnC, TIMP-1, and MMP-2 were recorded.

Histological Evaluation

Samples of placental tissue fixed in 10% formalin were examined under a light microscope. For the preparation and paraffin embedding of the placental tissue samples, the usual methods of tissue processing were used. To examine the tissue, 5-mm-thick sections were cut from the paraffin blocks, placed on slides, and hematoxylin and eosin (H-E) staining was performed. A Leica DFC280 microscope with a Leica Q Win and an image analysis system from Leica Micros Imaging Solutions Ltd (Cambridge, U.K.) was used to analyze the tissue sections. Histopathologic examination of damaged tissue was performed for each parameter, such as fibrin deposition, villous stroma disruption, villous basement membrane thickening, syncytial knots, and inflammatory cell infiltration in the placental samples. At least five microscopic regions were examined to evaluate the samples semiquantitatively. A three-point scale was used to score each specimen, indicating 0 none, 1 mild, 2 moderate, and 3 severely. The histopathologic score was calculated based on these parameters.

Power Analysis

Because of the power analysis performed, with a predicted effect size of 0.40 (moderate), the minimum sample size per group required to compare the changes in neopterin levels between the control group, the class I obesity group, and the class II-III obesity group was calculated to be 28 subjects with a power of 90% at a 95% confidence level[1].

Statistical Analysis

Mean ± standard deviation (SD), median (minimum-maximum), and number (percent) were used to summarize the data (percent). The Shapiro-Wilk test was used to verify that the data followed a normal distribution. Statistical analysis was performed using one-way analysis of variance, Kruskal-Wallis tests, and Pearson chi-square tests. Perinatal complication prediction performance and optimal cut-offs for the variables were established using receiver operating characteristic (ROC) analysis. Assuming a p-value less than 0.05 indicates statistical significance. The analysis was performed in IBM SPSS Statistics version 25.0. Histopathologic data were expressed as mean ± SD. Histological results were compared using Kruskal-Wallis analysis of variance.

Results

Class II-III obese patients were significantly older than class I obese and normal-weight patients (p<0.001). As expected, there was a significant difference in BMI between the normal-weight, class I obese, and class II-III obese groups (p<0.001). The groups were comparable in terms of gestational age at hospital admission (p=0.583). Additionally, there were significant differences in pre-pregnancy BMI between the groups (p<0.001). In the prenatal period, the rate of preeclampsia was significantly higher in class II-III obese pregnant women compared to the other groups (p=0.008). The rates of gestational diabetes mellitus, fetal growth restriction, and preterm birth were similar among the groups (p=0.117, p=0.913, and p=0.367, respectively). There were no significant differences in gestational age at birth or neonatal birth weight in all three groups (p=0.994 and p=0.237, respectively). Neonatal morbidity rates were comparable between groups (p=0.747), and there was no perinatal mortality in the entire study group. Maternal characteristics and perinatal and neonatal outcomes of the study population are summarized in Table 1.

The serum levels of neopterin, periostin, TnC, tissue inhibitor of metalloproteinase-1, and MMP-2 in the third trimester are summarized in Table 2. Neopterin, periostin, and TIMP-1 were significantly higher in obese class II-III patients than in class I obese and normal-weight patients (p=0.002, p<0.001, and p<0.001, respectively). There was no significant difference in MMP-2 levels between the groups (p=0.769). TnC levels were lowest in the normal-weight group and highest in class II-III obese pregnant women (p<0.001). The comparison of serum levels of neopterin, periostin, TIMP-1, TnC, and MMP-2 is shown in Figure 1.

ROC curve analysis was performed to evaluate the ability of serum biomarkers to predict pre-eclampsia. TnC showed the best performance in predicting pre-eclampsia [area under the curve (AUC)=0.82, 95% confidence interval (CI), 0.72-0.90, p<0.001]; however, periostin also proved effective (AUC=0.71, 95% CI, 0.60-0.80, p=0.007) (Table 3) (Figure 2).

In the control group, placental tissue showed a normal histological appearance. Patients in the control group had normal appearance of villi with normal vasculosyncytial membranes and syncytiotrophoblastic layer (Figure 3). Fibrin-containing fibrinoid deposits, and villous stroma disruption were observed more frequently in the placental villi of the class II-III obesity group and class I obesity group compared with the control group. Also, the fragmentation of the vasculosyncytial membranes and irregularities in the syncytiotrophoblastic layer was observed in the obese groups compared with the control group. Additionally, syncytial knots were more frequent in obese patients in class II-III obese patients than in class I obesity and control groups (Figures 4 and 5). Table 4 shows the histopathological scores of the control, class I obesity, and class II-III obesity groups.

Discussion

This study showed that serum levels of neopterin, periostin, TnC, and TIMP-1 were significantly higher in class II-III obese pregnant women than in class I obese and normal-weight women. Particularly in patients with extreme obesity, insights into the molecular factors associated with increased perinatal complications and obesity and elucidation of the pathophysiological mechanisms are needed to develop effective diagnostic and therapeutic strategies. Adequate vascular and ECM remodeling is strongly associated with the progressive and balanced increase in adipocytes in response to total calorie intake. Increasing adiposity, on the other hand, is probably linked to a condition of inflammatory processes in adipose tissue that promote ectopic lipid accumulation. The results of this study showed significantly increased serum levels of ECM proteins, which regulate inflammatory responses, in class II-III obese pregnant women. Histopathological analyses of the placentas of the obese pregnant women also showed higher histopathological scores than those of the control group. Histopathological findings of placental damage, such as fibrin deposition, villous stroma disruption, villous basement membrane thickening, syncytial knots increment, and inflammatory cell infiltration, were observed more frequently in placental samples from obese pregnant women. In line with this, recent studies have revealed that macrophages play a significant role in the inflammatory state in adipose tissue and ECM remodeling that is considered a pathological status rather than adaptive feedback[14]. Evidence also suggests

that as chronic inflammation progresses, the adipose tissue undergoes adipocyte hypertrophy, proinflammatory invasion, regulation of angiogenesis, and enhanced ECM synthesis. Adipose tissue macrophages are dominant contributors of proinflammatory mediators that sustain inflammation throughout the obesity process. Therefore, a paracrine circuit between adipocytes and macrophages may impact the progression of metabolic disorders in various organs due to obesity-related dysregulation of adipocytokine production.

Many biological processes involve pteridines like neopterin and biopterin, which are produced by the oxidation of tetrahydrobiopterin, and both tetrahydrobiopterin and its derivative products can be detected in physiological fluids and tissues. Neopterin is an effective biomarker for inflammatory diseases because it sensitively detects T helper type 1 immune response in humans[15]. In this study, we found significantly higher serum neopterin levels in women with class II-III obesity compared with the other groups. Consequently, higher neopterin levels are frequently observed in disorders associated with oxidative stress, and it has been hypothesized that neopterin may serve as a signal for oxidative stress indirectly resulting from immune system dysfunction. Sugulle et al.[16] found elevated neopterin levels in pregnant women with late-onset preeclampsia. Similarly,

Table 1. Maternal characteristics and perinatal and neonatal outcomes of groups

		Group			p-value
		Control (n=28)	Class I Obesity (n=28)	Class II-III Obesity (n=28)	
		Mean ± SD	Mean ± SD	Mean ± SD	
Age (years)		29.86a±5.32	29.11a±5.04	34.93b±4.22	**<0.001***
Prepregnancy BMI (kg/m²)		21.11a±2.05	26.48b±2.8	32.42c±3.91	**<0.001***
Weight (kg)		64.07a±5.25	82.14b±7.12	97.11c±10.39	**<0.001***
Height (cm)		163.29a±5.46	161.46a±7.14	159.75a±6.51	0.125*
BMI (kg/m²)		24.47a (18.87-24.98)	31.15b (30.08-34.14)	36.76c (35.16-46.09)	**<0.001****
		Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	
Gestational age at admission		38a (37-40)	37.5a (36-40)	37.5a (36-41)	0.583**
Gravidity		2.0a (1.0-8.0)	3.0a (1.0-9.0)	3.0a (1.0-6.0)	0.309**
Parity		1.0a (0.0-4.0)	1.0a (0.0-4.0)	1.0a (0.0-4.0)	0.242**
Gestational age at birth (weeks)		38a (37-40)	38a (37-41)	38a (37-41)	0.994**
Birthweight (g)*		2945a (2545-4100)	3057.5a (2540-3700)	3180a (2530-4800)	0.237**
Cord blood pH		7.37a (7.27-7.47)	7.37a (7.17-7.6)	7.36a (7.18-7.43)	0.589**
Cord blood base excess		-3.95a (-18-6.2)	-4.4a (-10.5-0.8)	-3.7a (-8.1-1.3)	0.423**
		Count (Percent)	Count (Percent)	Count (Percent)	
Mode of delivery	Cesarean section	28a (100.00%)	27a (96.43%)	27a (96.43%)	0.599***
	Vaginal delivery	0a (0.00%)	1a (3.57%)	1a (3.57%)	
Gender	Female	17a (60.71%)	11a (39.29%)	15a (53.57%)	0.263***
	Male	11a (39.29%)	17a (60.71%)	13a (46.43%)	
Gestational DM		3a (10.7%)	3a (10.7%)	8a (28.6%)	0.117***
Preeclampsia		0a (0.00%)	2a, b (7.14%)	7b (25.00%)	**0.008*****
FGR		4a (14.29%)	5a (17.86%)	4a (14.29%)	0.913***
Preterm birth		3a (10.71%)	7a (25.00%)	6a (21.43%)	0.367***
Fetal distress		3a (10.71%)	2a (7.14%)	2a (7.14%)	0.856***
Admission to NICU		4a (14.29%)	8a (28.57%)	11a (39.29%)	0.109***
Neonatal morbidity		3a (10.71%)	4a (14.29%)	5a (17.86%)	0.747***

*: One-way ANOVA test; **: Kruskal-Wallis test; ***: Pearson chi-square; There is a statistically significant difference in the group categories that do not contain the same letter on a row basis, BMI: Body mass index, DM: Diabetes mellitus, HT: Hypertension, FGR: Fetal growth restriction, NICU: Neonatal intensive care unit, Min-Max: Minimum-Maximum, SD: Standard deviation, Bold values show p<0.05

pregnant women with gestational diabetes had greater serum neopterin levels compared with healthy controls and the inflammatory response in GDM was closely related to obesity[17]. Our current clinical finding is well supported by the literature and suggests the potential importance of neopterin in the development of pregnancy complications associated with obesity.

Table 2. Serum neopterin, periostin, Tenascin-C, Tissue inhibitor of metalloproteinase-1 and matrix metalloproteinase-2 levels of control and study groups

	Group			p-value
	Control (n=28)	Class I Obesity (n=28)	Class II-III Obesity (n=28)	
	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	
Neopterin (nmol/L)	4.07a (1.76-9.44)	4.33a (2.13-18.37)	12.39b (2.42-19.58)	**0.002****
Periostin (ng/mL)	44.57a (10.33-97.48)	63.68a (10.59-93.49)	79.39b (10.64-120.33)	**<0.001****
Tenascin C (ng/mL)	932a (557-8757)	2637b (1137-5857)	5672c (1147-12587)	**<0.001****
MMP-2 (ng/mL)	18.67a (12.03-45.56)	21.15a (13.55-80.94)	18.25a (7.5-85.14)	0.769**
TIMP-1 (ng/L)	127a (99.75-597.22)	198.48a (98.1-822.82)	795.63b (104.91-998.1)	**<0.001****

*: One-way ANOVA test; **: Kruskal-Wallis test; ***: Pearson chi-square; There is a statistically significant difference in the group categories that do not contain the same letter on a row basis, MMP: Matrix metalloproteinase, TIMP: Tissue inhibitor of metalloproteinase, Min-Max: Minimum-Maximum, bold values show p<0.05

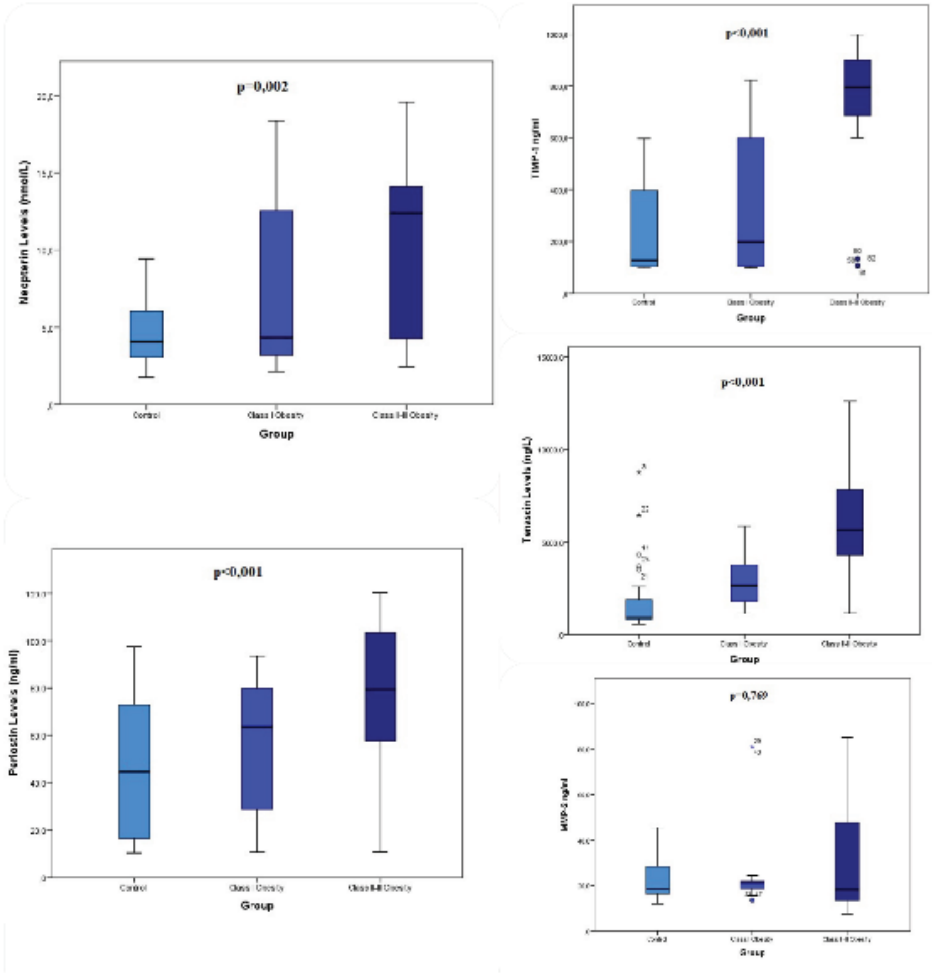

Figure 1. Comparison of neopterin, periostin, tissue inhibitor of metalloproteinase-1, Tenascin C, and matrix metalloproteinase-2 levels of the study groups compared to the control group

Maternal obesity is a major problem in today's world as more and more women of childbearing age are overweight. It has both short- and long term harmful effects on the mother and baby. The placenta of obese women exhibits altered function related to increased inflammation and oxidative stress[18]. Challier et al.[10] also observed that the chronic inflammatory state of pre-pregnancy obesity, which continues during pregnancy, increases macrophages and proinflammatory mediators in the placenta. They suggested that fetal metabolic programming of obesity and insulin resistance syndrome may occur because of this inflammatory environment. In this study, although no significant differences were found in the early neonatal outcomes of lean and obese pregnant women, the rate of preeclampsia was significantly higher in class II-III obese patients. Besides, the predictive value of ECM proteins for preeclampsia was also evaluated as new potential biomarkers, and TnC and periostin were found to be effective in predicting pre-eclampsia. Accordingly, Ribatti et al.[19] demonstrated tenascin expression in preeclamptic decidua in association with angiogenesis and showed that both angiogenesis and tenascin expression is induced by implants from preeclamptic decidua. The placenta and skin contain the greatest levels of TnC among human tissues. The placental mesenchymal villi, which are

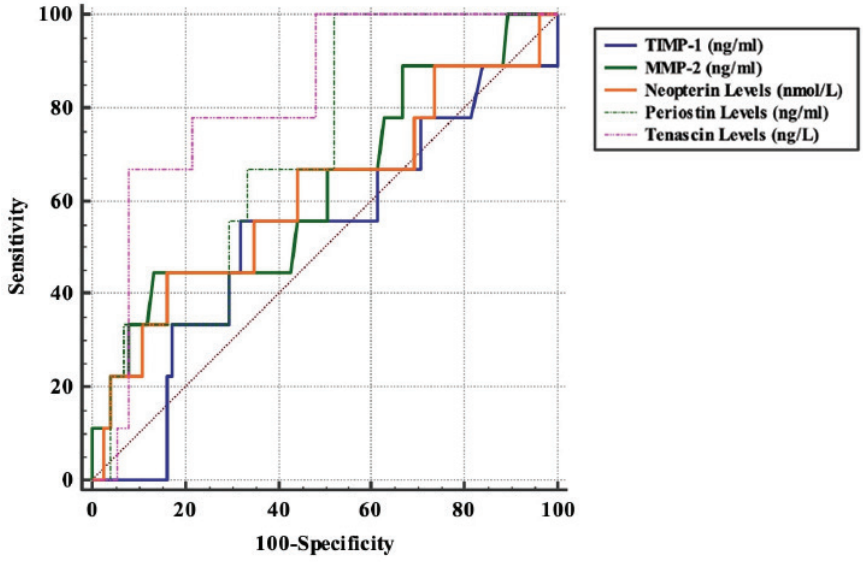

Figure 2. Receiver operating characteristic analysis showing the utility of neopterin, periostin, tissue inhibitor of metalloproteinase-1, Tenascin C, and matrix metalloproteinase-2 levels in the study cohort for predicting preeclampsia

Table 3. ROC analysis showing the predictive value of biomarkers for preeclampsia

Variables	Cut-off	Sensitivity	Specificity	LR+	LR-	PPV	NPV	AUC (95% CI)	p-value[a]
Neopterin	3.00	44.44 (13.7-78.8)	84.00 (73.7-91.4)	2.78	0.66	25.0	92.6	0.61 (0.50-0.71)	0.102
Periostin	53.4	100.0 (66.4-100.0)	48.00 (36.3-59.8)	1.92	0.0	18.8	100.0	0.71 (0.60-0.80)	**0.007**
Tenascin C	6627	66.67 (29.9-92.5)	92.00 (83.4-97.0)	8.33	0.36	50.0	95.8	0.82 (0.72-0.90)	**<0.001**
MMP-2	29.17	44.4 (13.7-78.8)	86.67 (76.8-93.4)	3.33	0.64	28.6	92.9	0.63 (0.51-0.73)	0.254
TIMP-1	599.75	55.56 (21.2-86.3)	68.00 (56.2-78.3)	1.74	0.65	17.2	92.7	0.53 (0.42-0.64)	0.806

MMP: Matrix metalloproteinase, TIMP: Tissue inhibitor of metalloproteinase, AUC: Area under the curve, LR: Likelihood ratio, PPV: Positive predictive value, NPV: Negative predictive value, ROC: Receiver operating characteristic, bold values show p<0.05

Table 4. The histopathological score of groups

Groups	Placenta histopathology score (mean ± SD)
Control group	0.51±0.07[a]
Class I obesity	1.76±0.08[b]
Class II-III obesity	2.33±0.09[c]

SD: Standard deviation, The mean differences in the values bearing different superscript letters within the same column are statistically significant (p<0.001)

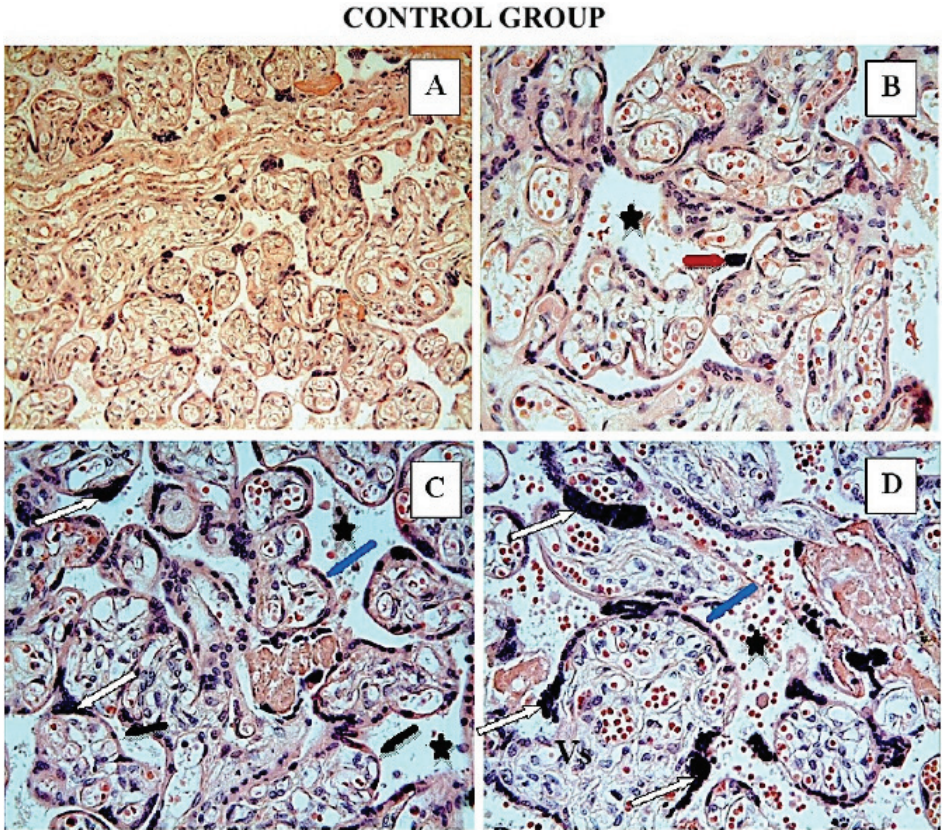

Figure 3. (Control group). Normal villi were observed in patients with body mass index between 18-24.9 kg/m^2. (A). The villi are characterized by the formation of vasculosyncytial membranes (black arrows) (C), which result from the accumulation of syncytiotrophoblasts in syncytial knots (white arrows) (C, D). The syncytiotrophoblastic layer (blue arrows) (C, D) was observed in normal appearance in this group. Maternal red blood cells (black asterisks) are present in the intervillous space (B, C, D). The villous stroma (D) contains fetal blood capillaries. The fetal blood capillaries are filled with fetal red blood cells. A: H-E; X20, B, C, D: H-E; X40

assumed to constitute the framework for the promotion and differentiation of the villous tree, exhibit high levels of tenascin. Villous expansion, cell growth, and formation of fibrinoid deposits have all been linked to TnC expression in the human placenta[20]. In agreement with our results, serum periostin concentrations were observed to be considerably higher in women diagnosed with pre-eclampsia compared with normotensive controls by Sasaki et al.[21] This study's *in situ* hybridization results showing periostin expression in placental stromal cells suggests that this molecule plays a role in adhesion. Adhesion molecules like periostin and TnC are secreted by the placenta and may regulate inflammation or disrupt cell adhesion. Therefore, inflammation, which is the most important feature of preeclampsia, may be thought to be regulated in part by adhesion molecules that modulate leukocyte and endothelial cell activation.

The ECM is critical for adipocyte development and function and therefore is central to weight regulation, obesity, and lipid metabolism[22]. Recent studies have found close associations between adipose tissue inflammation and ECM proteins, and the expression of TIMP-1 and MMPs may play a key role[23]. To the our knowledge, there are no data on serum ECM protein levels in obese pregnant women or their possible involvement in adipose tissue inflammation during pregnancy. In this study, although MMP-2 levels were similar between groups, we observed statistically significantly higher serum TIMP-1 and neopterin levels in class II-III obese pregnant women. In parallel, recent clinical studies have described changes, particularly in the circulating levels of MMP-2 and MMP-9 and endogenous mediators of these MMPs (TIMPs) in obesity and obesity-related metabolic diseases[24]. This is important because the measurement of serum levels of MMPs and TIMPs may help elucidate important mechanisms potentially involved in the pathogenesis of obesity-related pregnancy complications. One of the major roles of MMP-2 is the degradation of ECM components, particularly type IV collagen, the major component of basal membranes. It has also been suggested that MMP-2 plays a crucial role in adipose tissue development and plays a central role in inflammation and vasoconstriction. However, in this study, no statistically

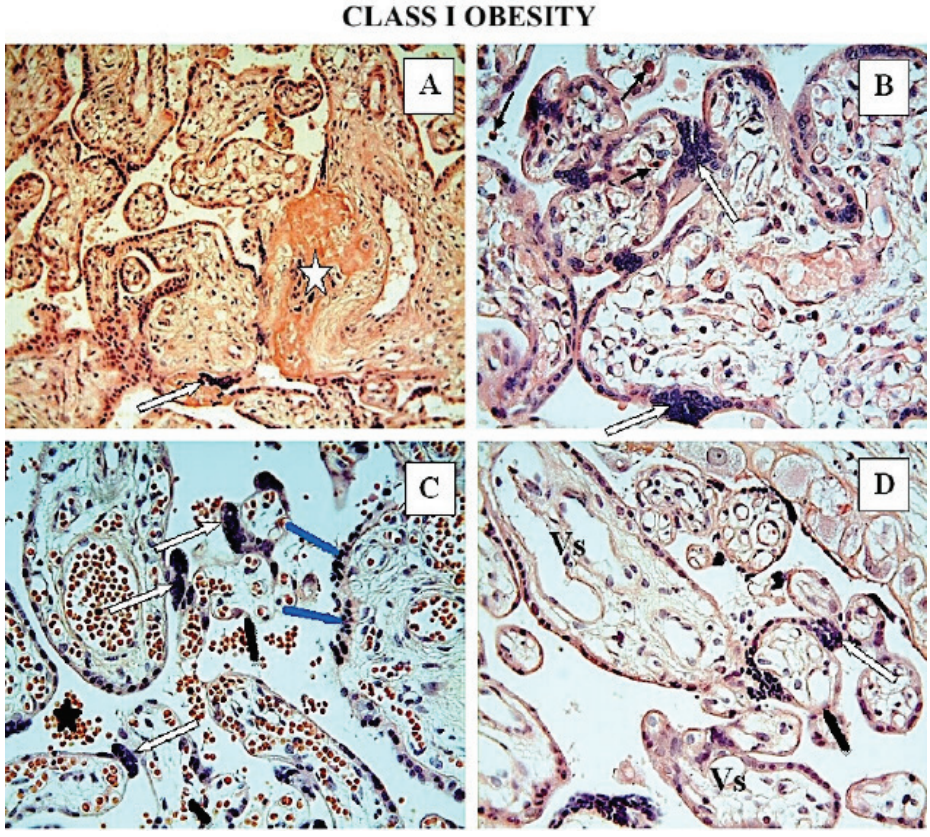

Figure 4. In the class I obesity group, white asterisks indicate some of the fibrin-containing fibrinoid deposits on the surface of the villi (A). In the class I obesity group, syncytial knots were increased. White arrows show that the syncytial knots (A, B, C) bulging into the intervillous space. Rarely fragmentation of the vasculosyncytial membranes was observed (black arrows) (C, D). Irregularities in the syncytiotrophoblastic layer (blue arrows) (C) were detected in the class I obesity group. A small amount of red blood cells were present in the intervillous space (black asterisks) (C). Disruptions of the villous stroma were also noticed. Apoptotic cells (thin black arrows) (B) were observed in the intervillous space and villi. A: H-E; X20, B, C, D: H-E; X40

significant difference was found between serum MMP-2 levels of overweight and normal-weight pregnant women. One possible explanation for these discrepant results is that the measurement of serum MMP levels rather than plasma in this study does not accurately reflect the circulating concentration of the enzyme of interest. The exact function of MMPs in the inflammatory cascade is not yet fully understood. It is assumed that they can be mediated and upregulated by inflammatory cytokines and that they also have an intrinsic influence on the inflammatory process.

Study Limitations

The small size of our sample and the fact that it was collected from a single center are two of our study's weaknesses. However, there were enough participants included in the study to reliably evaluate these protein markers. Additionally, serial serum measurements were not performed during pregnancy, even during the first and second trimesters. Moreover, we were unable to evaluate the expression of these biological markers through pathological examination of placental tissue. One of the study's strongest points is that, to the best of our knowledge, it is the first to analyze these protein biomarkers in obese pregnant women. The prospective cohort design was another strength.

Conclusion

In conclusion, this study shows that class II-III obese pregnant women had significantly higher serum levels of neopterin, periostin, TnC, and tissue inhibitor of metalloproteinase-1 in the third trimester. Thus, the fetuses of obese women are exposed to higher levels of these protein markers in utero than those of lean women. These higher serum levels may be associated with the adverse perinatal effects of obesity during pregnancy. Additionally, periostin and TnC are considered biomarkers of preeclampsia, and serum levels of these protein biomarkers could be used to support clinical decisions for predicting pre-eclampsia.

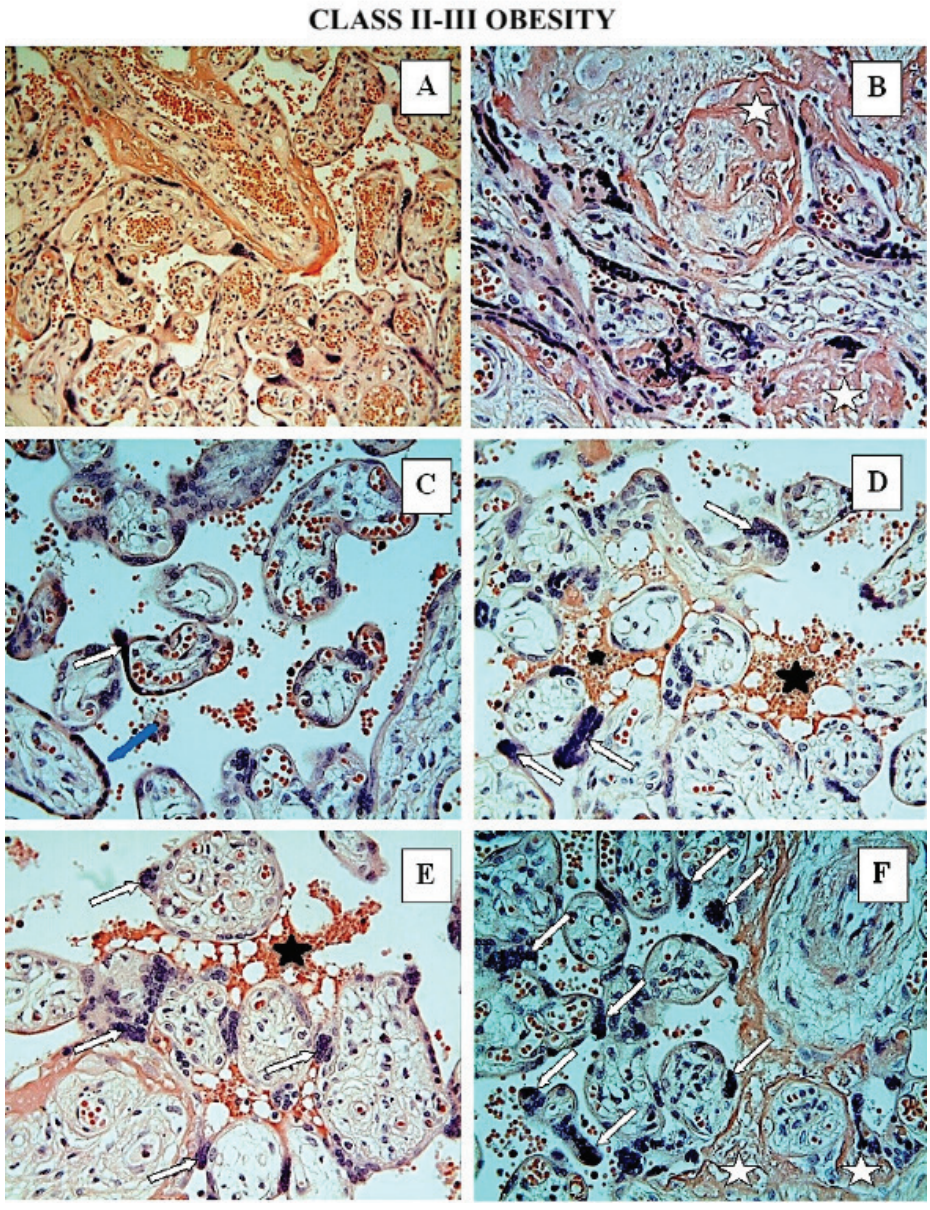

Figure 5. In the class II-III obesity group, deterioration and irregularity of villous structures were noted. White asterisks indicate some of the fibrin-containing fibrinoid deposits (B) on the surface of the villi. Syncytial knots were significantly increased in this group. White arrows indicate the syncytial knots (C, D, E, F) bulging into the intervillous space. The syncytiotrophoblatic layer (blue arrow) (C) was rarely observed in the villi. There is a large number of red blood cells (D, E) in the intervillous space (black asterisks). Disruption of villous stroma and vacuolization (thin black arrows) were observed in the class II-III obesity group. A: H-E; X20, B, C, D, E, F: H-E; X40

Ethics

Ethics Committee Approval: This research was carried out in accordance with the principles outlined in the Declaration of Helsinki, and it received ethical approval number 2021/138 from the Clinical Research Ethics Committee of Inonu University.

Informed Consent: All participants gave their informed consent before the study was conducted.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: R.M., Concept: R.M., Design: R.M., S.Ü., Data Collection or Processing: S.Ü., N.B.T., N.Z.Ç., H.Y., Analysis or Interpretation: R.M., N.B.T., A.Ç., N.Z.Ç., Ş.Y., Literature Search: R.M., Writing: R.M., S.Ü.

Conflict of Interest: No conflict of interest was declared by the authors.

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