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Research Article

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# EVALUATION OF SCUBE-1 LEVEL AND CAROTID INTIMA MEDIA THICKNESS IN RECURRENT PREGNANCY LOSS

# TEKRARLAYAN GEBELİK KAYIPLARINDA SCUBE-1 DÜZEYİNİN VE KAROTİS İNTİMA MEDİA KALINLIĞININ DEĞERLENDİRİLMESİ

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## Öz

**Amaç:** Tekrarlayan gebelik kaybı olan hasta grubu ile tekrarlayan gebelik kaybı öyküsü olmayan hasta grubu arasında serum signal peptid C1r/C1s, Uegf, ve Bmp1 (CUB) epidermal growth factor-like domain-containing protein-1 (SCUBE-1) düzeyinin ve karotis intima media kalınlığının değerlendirilmesi.

**Materyal ve Metot:** Prospektif ve tek merkezli çalışmaya 20-40 yaş arasında 30 tekrarlayan gebelik kaybı öyküsü olan, 30 tekrarlayan gebelik kaybı öyküsü olmayan hasta olgusu alınmıştır. Tekrarlayan gebelik kaybı olanlarda ve kontrol grubunda anatomik, kalıtımsal, endokrin, trombofilik defekti olanlar çalışma kapsamına alınmamıştır. Hasta grubu ile kontrol grubu arasında SCUBE-1 düzeyinde ve ölçülen karotis intima media kalınlığı arasında istatistiksel fark olup olmadığı araştırılmıştır.

**Bulgular:** Demografik özellikler açısından hastalar ve kontrol grupları arasında gravite, parite ve düşükler dışında istatistiksel olarak anlamlı fark bulunmamıştır. TGK kaybı olan hastaların ortalama SCUBE1 düzeyleri (16,44±5,43) kontrol grubunun ortalama SCUBE1 düzeylerinden (10,17±5,19) istatistiksel olarak anlamlı şekilde yüksektir (p=0,001). TGK kaybı olan hastaların ortalama CIMT düzeyleri (0,60±0,09) kontrol grubunun ortalama CIMT düzeylerinden (0,44±0,07) istatistiksel olarak anlamlı şekilde yüksektir (p=0,001).

**Sonuç:** TGK olan grupta yüksek serum SCUBE-1 ve CIMT seviyeleri, iskemi ve endotel disfonksiyonunun TGK etiyolojisinde rol oynayabileceğini düşündürebilir. Gelecekteki çalışmalar TGK'nın tanı, tedavi ve yönetimine ışık tutabilir.


**Anahtar Kelimeler:** Düşük, tekrarlayan, SCUBE1 protein, karotis iç-orta kalınlığı.


## Abstract

**Objectives:** Evaluation of the signal peptide complement C1r/C1s, Uegf, and Bmp1 (CUB), and epidermal growth factor-like domain-containing protein-1 (SCUBE-1) levels and carotid intima media thickness (CIMT) between the group of patients with and without recurrent pregnancy loss (RPL).

**Materials and Methods:** This prospective and single-center study included 20-40-year-old 30 patients with and 30 patients without a history of RPL. No patients with any anatomical, hereditary, or endocrinological thrombophilic defects were included. A comparison of SCUBE-1 levels and CIMT were made between the patient and control groups.

**Results:** There were no statistically significant differences between patients and control groups in terms of demographic characteristics except for gravidity, parity, and miscarriages. The mean SCUBE-1 level in the RPL group was statistically significantly higher compared to the control group (16.44±5.43 vs.10.17±5.19, respectively, p=0.001). The mean CIMT value in RPL patients was statistically significantly higher than that of the control group (0.60±0.09 vs.0.44±0.07, respectively, p=0.001).

**Conclusion:** High levels of serum SCUBE-1 and CIMT in RPL may suggest that ischemia and endothelial dysfunction may be involved in the etiology of RPL. Future studies may shed light on the diagnosis, treatment, and management of RPL.


**Keywords:** Abortion, habitual, SCUBE1 protein, carotid intima-media thickness.

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## Introduction

Recurrent pregnancy loss (RPL) is defined as two or more consecutive losses of pregnancy confirmed via an ultrasonographic or histopathological examination.[1] RPL occurs only in 2% of the pregnant women. No exact etiology is identified to explain RPL in 50% of the patients. RPL is one of the complex and challenging conditions in reproductive medicine and it can be a difficult process for patients, families, and physicians. When RPL presents with an unidentifiable etiology, it may cause anxiety and concerns in patients and also clinicians.[2]

Changes in coagulation factors and hemostatic disorders in the pregnancy process cause an obstruction in placental bed vessels, resulting in RPL.[3] Additionally, a physiologically ischemic environment develops in the placental bed in early pregnancy. This ischemia is a mechanism; which protects trophoblasts from potential harms of oxidative stress. An exaggerated such ischemic environment in susceptible persons is another factor involved in the etiology of RPL.[4]

The cell surface protein Signal peptide complement C1r/C1s, Uegf, and Bmp1 (CUB), and Epidermal growth factor-like domain-containing protein (SCUBE) are considered important vascular biological markers.[5] SCUBE family includes different members. SCUBE-1 and SCUBE-2 are cell-surface proteins and they are expressed by thrombocytes and endothelial cells.[6,7] Previous studies have revealed the critical role played by SCUBE-1 in the detection of hypoxia, endothelial dysfunction, and vascular damage.[8] Because of rising blood levels in ischemic processes, SCUBE-1 is a protein that is likely to be involved in the relationship between RPL and ischemia.

Vascular structures need an intact endothelium in order to perform their functions adequately and accurately.[9] Carotid intima media thickness (CIMT) is an important predictor and a well-known early atherosclerosis marker for future cardiovascular diseases such as myocardial infarction and stroke. The measurement of CIMT is a repeatable, inexpensive, fast, and risk-free imaging method used for the early recognition of vascular pathologies.[10] It is known that this proinflammatory marker used in the early diagnosis of atherosclerosis; which is a vascular endothelial disorder, may develop due to an impaired endothelial bed.[11,12]

It may be accepted the ischemic conditions may be involved in the pathophysiology of recurrent abortion. There have been no well-known ischemic markers for the diagnosis or early prediction of RLP. Ischemic markers may be used as an early predictive marker of RPL. An exhaustive literature review was failed to reveal any studies investigating the role of SCUBE-1 and CIMT in the pathophysiology of RPL.

In this study, we aimed to investigate the role of changing plasma SCUBE-1 levels and CIMT as indicators of endothelial dysfunction and vascular damage in the etiology of RPL.

## Materials and Methods

This was a prospective case-control study conducted at the clinic of University hospital.

*Patient selection*

A total of 60 women were included in this study and they were divided into two groups. Group 1 included 30 women admitted to our clinic due to the absence of fetal cardiac activity or of a fetal pole in the ultrasonographic examination. Group 2 included 30 consecutive healthy pregnant women who had no history of a pregnancy loss previously and their pregnancy continued as uncomplicated to term after the blood samples were collected and CIMT was measured in the first trimester. Patients with a history of diabetes and vascular disease were excluded from the study.

Assessments for RPL included tests for diabetes, thyroid problems, autoimmune antibodies, clotting factors, parental karyotypes, and previous hysterosalpingography for identifying uterine abnormalities. Patients diagnosed with chromosomal, anatomical, endocrinological, and autoimmune disorders or with the antiphospholipid syndrome; explaining the etiology of RPL, were excluded from Group 1. Women with a history of ischemic disease, diabetes, heart disease, and hypertension were not included in either group. Also; women with multiple pregnancies, fetal anomalies, or smokers and drug users were excluded.

*Blood sampling*

Maternal blood samples of 5 mL were drawn from the antecubital vein; placed in a non-heparinized tube; left for coagulation, and centrifuged. The samples were centrifuged within one hour at 1000g for 15 minutes. Separated plasma was collected and stored at -80°C until the time of the analysis. Serum levels of human SCUBE-1 were quantified by enzyme-linked immunosorbent assay (ELISA) using commercially available matched antibodies (Eastbiopharm, Hangzhou, China). The intra-assay and inter-assay coefficients of variation were <8% and 10%, respectively. The sensitivity of the cut-off value was calculated to be 0.156 ng/mL.

*Ultrasonography measurement*

CIMT was measured by one of the authors (T.E.) using a high-resolution 7.5 MHz linear array ultrasound transducer (Hitachi EUB 6500, Osaka, Japan). Using two-dimensional ultrasonography; the measurement was performed at the far end of the right carotid artery wall, 10 mm proximally to the bifurcation. Measurements were performed at three points at a scanning session and synchronization with R-wave peaks in ECG was performed to prevent potential errors, which might result from variable arterial compliance. The mean CIMT

value was calculated out of 6 measurements obtained in two scanning sessions. The intra-observer intraclass correlation coefficient was 0.951 for CIMT.

*Statistical analysis*

For statistical analysis, the NCSS (Number Cruncher Statistical System) 2007&PASS (Power Analysis and Sample Size) 2008 Statistical Software (Utah, USA) program was used. When the quantitative study data were analyzed; besides the use of descriptive statistical methods, Student's t-test and Mann-Whitney U test were used for the two-group comparisons of normally and non-normally distributed data, respectively. Fisher's exact test was used for the comparison of the qualitative data. Pearson's correlation analysis was used for the assessment of parameter correlations. Significance was evaluated at levels of $p<0.01$ and $p<0.05$.

*Ethical considerations*

The Local Research Ethics Committee approved the study protocol (approval number 2012/143) and informed consent was obtained from all participants.

## Results

A total of 60 participants with a mean age of 32.10 ± 4.28 years were included in our study. The comparison of demographic factors in groups 1 and 2 was given in Table 1.

SCUBE-1 levels in group 1 were 16.44 ± 5.43 ng/mL, while it was 10.17 ± 5.19 in healthy controls (Student t-test, $p<0.001$, figure 1). The recurrent pregnancy loss group had also a significantly high CIMT value than that in group 2 (0.60 ± 0.09 vs. 0.44 ± 0.07 mm, respectively, $p<0.001$, Student t-test, figure 2). A statistically significant correlation was detected between the SCUBE-1 levels and CIMT in the whole group (Pearson correlation, $r=0.28$; $p=0.03$).

SCUBE-1 and CIMT had a more important diagnostic value for recurrent pregnancy loss. Within the scope of developing recurrent pregnancy in the whole group, ROC analysis showing the relationship between serum SCUBE-1 levels and habitual abortion is given in figure 3. The serum level of SCUBE-1 was used as a marker for the development of recurrent pregnancy loss, and the sensitivity and specificity were calculated as 87.7% and 66.7% for the limit value 11.14 ng/ml (AUC 77.4%, $p <0.001$, 95% CI 0.655-0.894), respectively. Furthermore, ROC analysis showing the relationship between CIMT and habitual abortion is given in figure 4. The measured CIMT was used as a marker for the development of recurrent pregnancy loss, and the sensitivity and specificity were calculated as 96.7% and 56.7% for the limit value 0.45 mm (AUC 99.1%, $p <0.001$, 95% CI 0.838-0.982), respectively.

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**Table 1.** Comparison of demographic factors in groups 1 and 2.

| | **Group 1 (Recurrent pregnancy loss, n=30)** | **Group 2 (Healthy pregnant controls n=30)** | ***p*** |
|---|---|---|---|
| Age (years) | 30.47±4.83 | 30.73±2.90 | *0.120* |
| BMI (kg/cm$^2$) | 25.25±2.05 | 24.47±1.66 | *0.108* |
| Gravidity | 3.30±1.02 (3.0) | 2.77±0.68 (3.0) | ***0.038***[a] |
| Parity | 0.30±0.60 (0.0) | 2.73±0.64 (3.0) | ***0.001***[a] |
| Miscarriages | 3.00±0.64 (3.0) | 0.03±0.18 (0.0) | ***0.001***[a] |

Student t-test or [a]Mann Whitney U test was used for comparison.

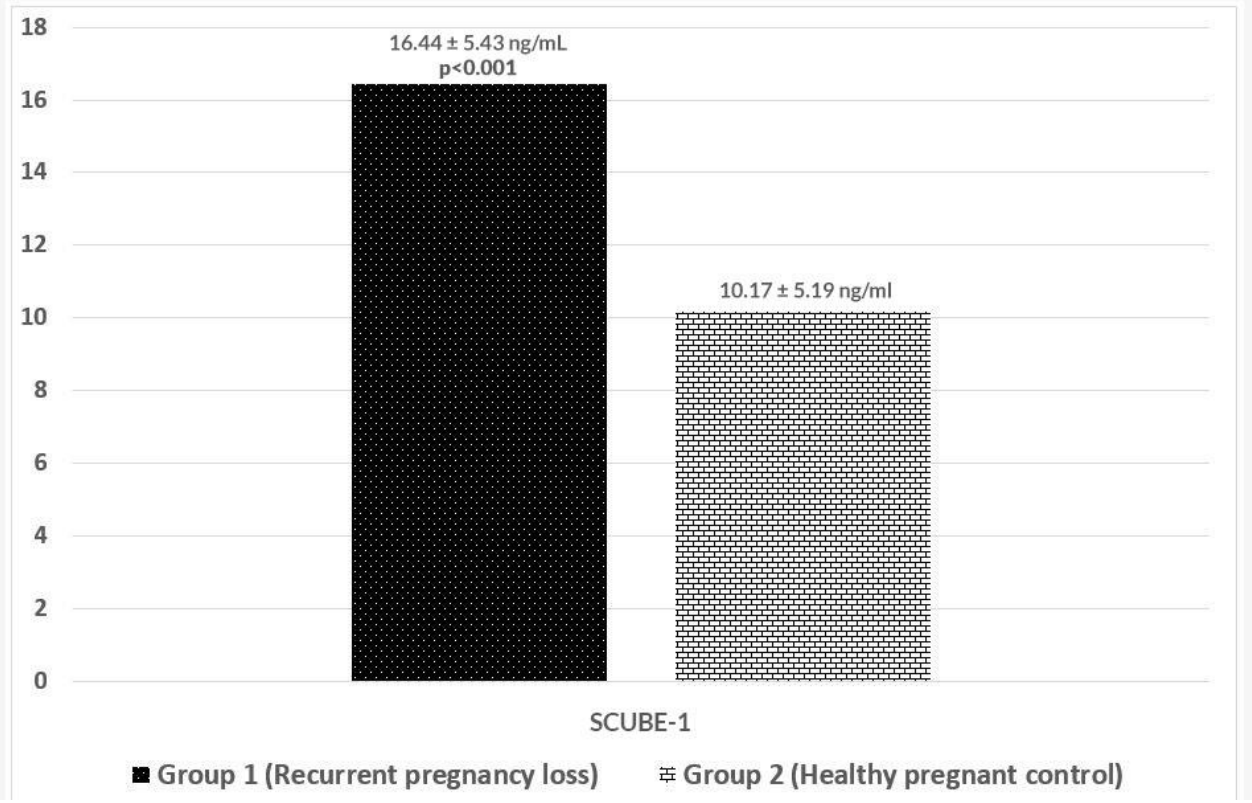


**Figure 1.** Comparison of mean serum scube-1 levels in group 1 (Recurrent pregnancy loss and 2 (Healthy pregnant controls).

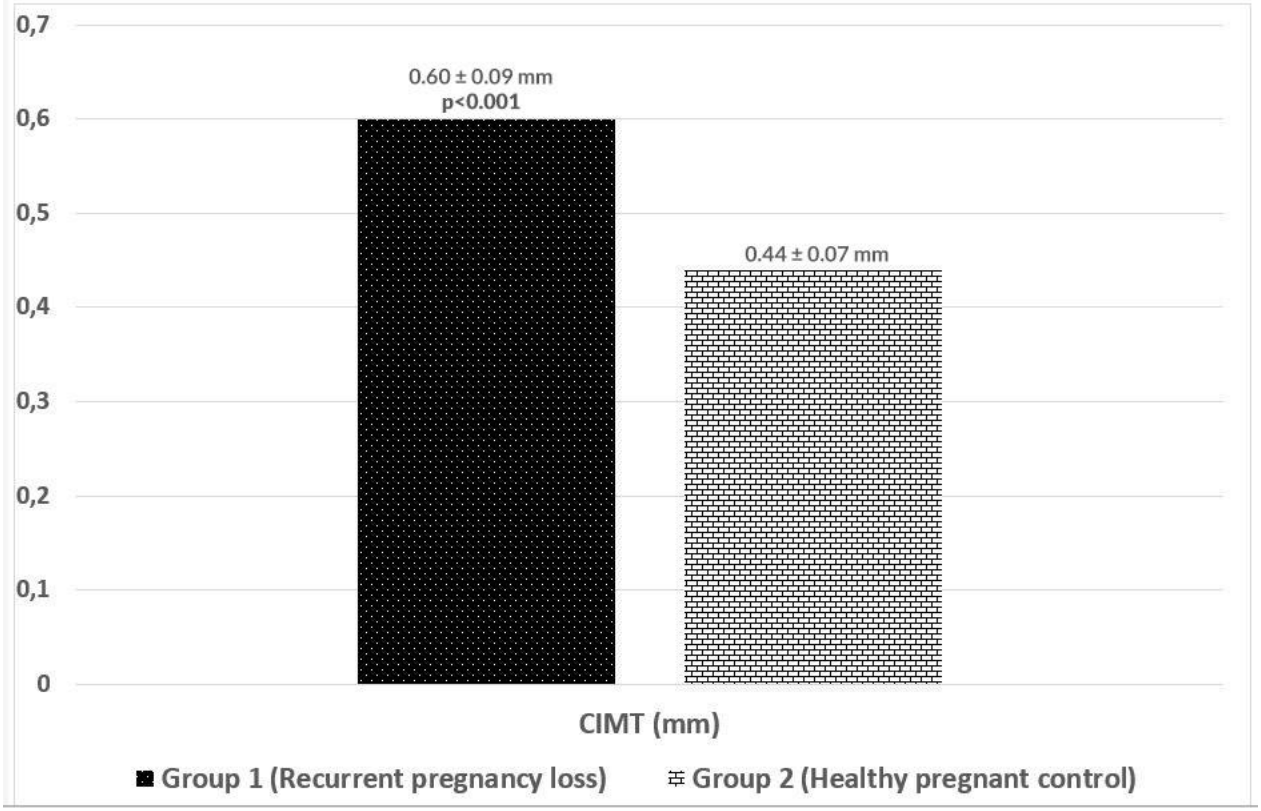


**Figure 2.** Comparison of mean CIMT in group 1 (Recurrent pregnancy loss and 2 (Healthy pregnant controls).

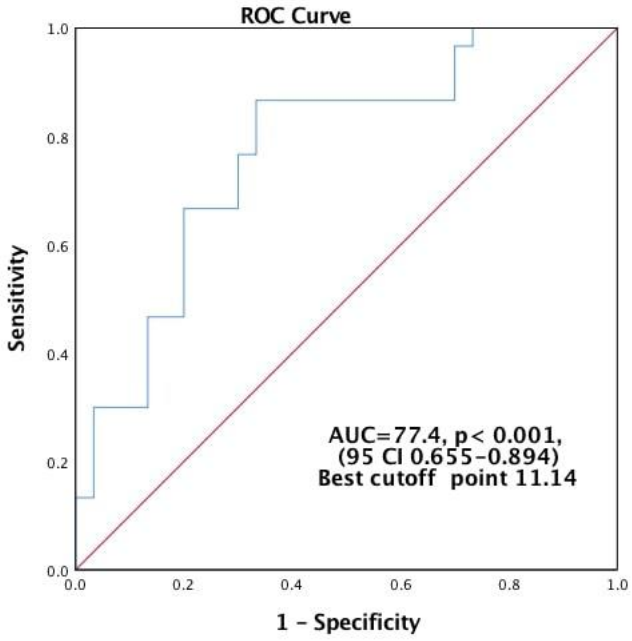


**Figure 3.** ROC analysis showing the relationship between serum SCUBE-1 levels and recurrent pregnancy loss.

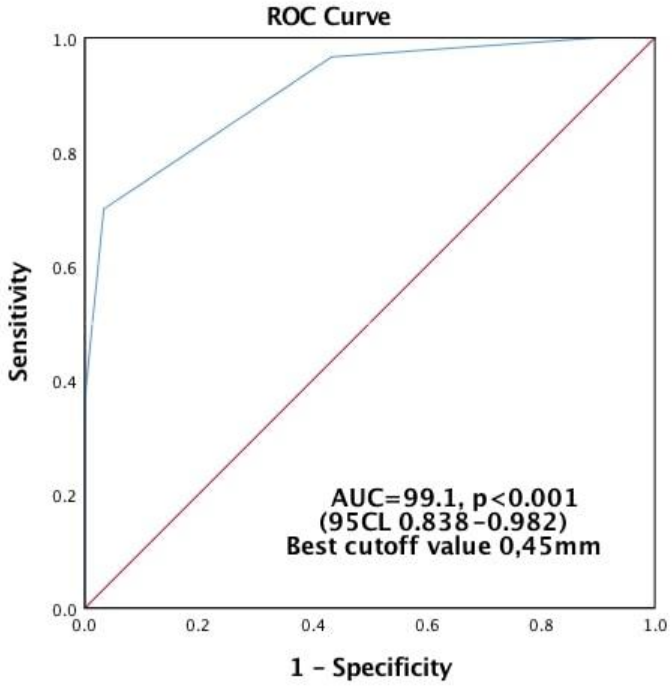


**Figure 4.** ROC analysis showing the relationship between CIMT and recurrent pregnancy loss.

## Discussion

In our study, we found statistically significantly higher SCUBE-1 levels and CIMT in patients with a history of RPL compared to the women in the health pregnant control group. Our study is the first to demonstrate increased SCUBE-1 levels and CIMT in pregnancy loss as a marker of placental failure and ischemia.

According to the generally accepted opinion, maternal intraplacental circulation starts immediately after implantation.[13] In the early stages of pregnancy, the obstruction of the decidual vessels by the endovascular trophoblast cells creates a relatively hypoxic uterine environment. This environment is thought to protect trophoblasts from the oxidative stress-induced damage.[14] Reactive oxygen radicals act as second messengers in many intracellular signaling cascades targeting cellular hemostasis and microenvironmental regulation. At high levels, they damage biological molecules that will cause cell death or loss of function. Exposure of syncytiotrophoblasts to excessive oxidative damage in early pregnancy is considered to be a major facilitating factor for miscarriages.[14] Anatomical evidence is available, demonstrating the presence of a thinned trophoblast layer characterized by defective placentation, spiral arteries, and decreased cytotrophoblast invasion in approximately 2 out of 3 early pregnancy losses.[15,16] There is clear evidence to show that miscarriages result from placentation disorders and the presence of changes in villi is considered a result rather than a cause as described previously.[17]

The vast majority of miscarriages in the first-trimester result from defective placentation. Compared to the control group, the higher maternal serum SCUBE-1 levels found in the RPL patients might have resulted from the presence of a more ischemic environment facilitating defective placentation. SCUBE-1 levels increase in ischemic conditions such as acute coronary syndrome or acute ischemic stroke.[8] In light of studies showing that RPL can occur after ischemia following endothelial damage, it has been suggested that SCUBE-1 protein levels can be elevated in women with a history of RPL.

Studies are available in the literature, demonstrating that the levels of ischemia-modified albumin (IMA), which is another ischemia marker, also reach supraphysiological values in abnormal pregnancies. A study has shown that elevated IMA levels are associated with a hypoxic intrauterine environment.[18] Another study has suggested that defective endovascular trophoblastic invasion is associated with elevated maternal serum IMA levels and can be used as a potential biomarker in the prospective evaluation of abortion and preeclampsia due to abnormal placentation.[19,20] Since pregnancy alone may create a predisposition to coagulation, patients with existing diseases that predispose them to coagulation face a high risk for thrombosis and thromboembolism. The ischemic environment resulting from the microthrombi formation in the placental vascular bed may be involved in the occurrence of miscarriages.

In our study, we found statistically significantly higher SCUBE-1 levels in pregnant women with a history of RPL compared to the women in the control group. In the early stages of pregnancy, the uterine environment is physiologically ischemic and this environment is necessary for the normal development of pregnancy. However, the increased ischemic environment may be the cause of pregnancy loss. SCUBE-1 levels, as a marker of ischemia, were found to be higher in the patient group compared to the control group; suggesting that ischemia levels increase further in these individuals during pregnancy.

Studies investigating the relationship between RPL and cardiovascular diseases have shown that patients with RPL are at risk for the development of cardiovascular diseases later in life.[21] Risk factors leading to the development of cardiovascular diseases create a predisposition to both preeclampsia and spontaneous abortions. It has been found out that different degrees of placentation defects occur in both of these conditions.[22,23] It has been demonstrated that impaired trophoblast invasion into the uteroplacental arteries is associated with late pregnancy complications, including preeclampsia and developmental retardation. One of the possible underlying mechanisms in patients experiencing RPL with unknown etiology may be the presence of endothelial dysfunction preceding the occurrence of obstetric complications. Maternal endothelial dysfunction disrupts extravillous trophoblast invasion into the spiral arteries. This invasion reduces the resistance in the uteroplacental vascular system and increases the blood flow, providing an adequate blood supply for fetal development.[24] Various studies show that endothelial dysfunction takes part in the development of hypertension and metabolic syndrome.[25] Endothelial dysfunction is considered an early

marker of atherosclerosis, which occurs before angiographic or ultrasonographic evidence becomes manifest. A noninvasive method used for determining the risk of endothelial dysfunction and cardiovascular disease is the measurement of CIMT with high-resolution ultrasound.[26]

We found in our study that CIMT was statistically significantly higher in patients with RPL compared to the control group. This finding indicates that patients with RPL may have endothelial dysfunction. Future studies; which will investigate CIMT measurements and other markers for endothelial dysfunction, can allow for predicting the prognosis of pregnancy. Treatment to be provided for patients with RPL of unknown etiology remains to be an important problem awaiting solution. The results of our study show that endothelial dysfunction and ischemia may be involved in the etiology of RPL. Although prophylactic antithrombotic and anti-platelet therapy is still controversial in these patients, our findings may suggest that patients will RPL may benefit from such therapy that will reduce ischemia and endothelial dysfunction.

Increasing SCUBE-1 level and CIMT, which are important markers in ischemic processes, may explain the placental ischemic defect that may cause recurrent pregnancy loss. Ischemia in the early placentation period may be a marker predicting SCUBE-1 level and CIMT elevation for RPL. If the SCUBE-1 level is 11 ng / mL or CIMT is above 0.3 mm, it may help to predict RPL early and follow the clinical process. It can also provide information to the clinician and the patient for prognosis early in the placentation.

However, in our study, the limitations of the number of patients, the inability to evaluate the vascular wall thickness in the placenta, and the inability to measure the level of SCUBE-1 in the placental vascular area can be considered as the limitations of the study. We believe that this study will contribute to the literature and be a resource for further studies and conducted further studies will confirm our data on the subject.

In summary, our results demonstrate that ischemia and endothelial dysfunction are involved in the etiology of RPL. We believe that future studies about the place of SCUBE-1 and other systemic ischemia markers, as well as of CIMT and other endothelial dysfunction markers of atherosclerosis, in the etiology of RPL would allow for understanding the pathophysiology of RPL and its proper management. The development of molecules that will correct endothelial dysfunction and act on cellular ischemia may enable us to achieve considerable progress in the treatment of RPL.

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**Disclosure statement**

The authors declare no conflicts of interest.

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