# Immunohistochemical Evaluation of Pecam-1 (CD-31) and Icam-3 (CD-50) in Preeclamptic Placenta

## Preeklamptik Plasentada Pecam-1 (CD-31) ve Icam-3 (CD-50) Moleküllerinin İmmunohistokimyasal Olarak Değerlendirilmesi

Neşe LORTLAR, MD,[a]
Sevtap KILIÇ, MD,[b]
Yeşim BARDAKÇI, MD,[b]
Gürer BUDAK, MD,[a]
Suna ÖMEROĞLU, MD[c]

[a]NanoMedicine Research Laboratory,
Gazi University,
[b]Division of IVF, Dr. Zekai Tahir Burak
Women's Health Education and
Research Hospital,
[c]Department of Histology and
Embriyology, Gazi University
Faculty of Medicine, Ankara

Yazışma Adresi/Correspondence:
Sevtap KILIÇ, MD
Dr. Zekai Tahir Burak Women's Health
Education and Research Hospital,
Division of IVF, Ankara,
TÜRKİYE/TURKEY
sevtapkilic@gmail.com

**ABSTRACT Objective:** Preeclampsia has been related to abnormalities in cellular adhesion molecule expression. Preeclampsia complicates 3-5% of all pregnancies in the world. Our primary objective was to compare the expression of two intercellular adhesion molecules, platelet endothelial cell adhesion molecule (PECAM-1/CD-31) and intercellular adhesion molecule-3 (ICAM-3/CD-50) in preeclamptic and healthy placental beds. **Material and Methods:** A prospective controlled clinical trial was conducted involving placentas from 30 women with preeclampsia and 30 normal pregnant women. Formalin fixed and paraffin embedded placental sections were analyzed using peroxidase-antiperoxidase indirect immunohistochemical methods. Two different investigators evaluated the intensity of staining independently. The intensity of the immunreactivity was analyzed using a semi-quantitative score and statistical analyses were performed, during which Kolmogorov-Smirnov, Student's t, and Mann-Whitney U tests were applied to clinical and histopathological data. **Results:** PECAM-1 immunoreactivity was high in placental trophoblasts of preeclamptic women and in their vessel endothelium when compared with the normal placenta. On the other hand, no significant differences were found in ICAM-3 intensity between pre-eclampsia and control group placentas. **Conclusions:** Increased immunoreactivity of PECAM-1 in endothelial cells may cause endothelial injury, but it seems that ICAM-3 (CD-50) may be irrelevant to the etiopathogenesis of preeclampsia.

**Key Words:** Cell adhesion molecules, immunohistochemistry, preeclampsia, placenta


**ÖZET Amaç:** Preeklampsi, hücre adezyon moleküllerinin ekspresyonundaki anormalliklerle ilişkilendirilmiştir. Preeklampsi, dünyada tüm gebeliklerin %3-5'ini komplike etmektedir. Bu çalışmadaki amacımız, iki hücre içi adezyon molekülünün [trombosit endotel adezyon molekülü (PECAM-1/CD-31) ve intraselüler adezyon molekülü-3 (ICAM-3/CD-50)] preeklamptik ve sağlıklı plasentalarda ekspresyonunu karşılaştırmaktır. **Gereç ve Yöntemler:** Otuz sağlıklı ve 30 preeklamptik gebenin plasentaları toplanarak prospektif, kontrollü bir klinik çalışma yapıldı. Formalin ile fikse edilip parafine gömülmüş olan plasental kesitler, peroksidaz-antiperoksidaz dolaylı immünohistokimyasal yöntem ile analiz edildi. Boyanma yoğunluğu iki farklı araştırmacı tarafından, bağımsız olarak değerlendirildi. İmmünohistokimyasal reaksiyon yoğunluğu, yarı niceliksel bir puanlama sistemi ile değerlendirildi ve istatistiksel olarak analiz edildi. Kolmogorov-Smirnov, Student's t ve Mann-Whitney U testleri klinik ve histopatolojik verilerin analizinde kullanıldı. **Bulgular:** PECAM-1 immünreaktivitesi preeklamptik kadınların plasental trofoblastlarında ve onların damar endotellerinde normal gebelerinkinden daha fazlaydı. Diğer yandan, ICAM-3 immünreaktivite yoğunluğu, preeklampsi ve kontrol gruplarının plasentalarında farklı bulunmadı. **Sonuç:** PECAM-1'in, endotel hücrelerinde immünreaktivitesinin artması, endotel hasarına neden olabilirse de, ICAM-3 (CD-50) preeklampsi etyopatogenezi ile ilişkisiz görünmektedir.

**Anahtar Kelimeler:** Adezyon molekülleri, immünohistokimya, preeklampsi, plasenta

The placenta plays a critical role for the developing fetus, ensuring both its continuity and protection.[1] Fetal blood is carried to the human placenta via two umbilical arteries which are functionally terminal arteries.[2]

Preeclampsia complicates 3-5% of all pregnancies in the world. It is associated with hypertension and proteinuria occurring after week 20 of pregnancy. Oxidative stress induces endothelial dysfunction, which plays an important role in the pathophysiology of vascular-related disease, including the ischemia-reperfusion injury associated with hypertension. Accumulated evidence has supported a major role for endothelial oxidative stress in the pathology of preeclampsia.[3] The main consequence of placental ischemia is generalized endothelial dysfunction, which largely is responsible for the clinical symptoms and complications of this condition.[4]

In the examination of the placental vasculature, from the mean stem to the smallest segment, the villous tree is important, in terms of assessing placental pathology associated with intrauterine growth retardation secondary to preeclampsia.[5]

Preeclampsia has been related to abnormalities in cellular adhesion molecule expression. Cellular adhesion molecules include integrins, selectins, cadherins and the immunoglobulin super family.[6] They are effective in carrying out the cell functions in addition to their roles in cellular proliferation, migration, differentiation and maturation at prenatal and postnatal developmental stages.[6] The integrins bind to fibronectin, laminin, collagen and other molecules, and to members of the immunoglobulin super family, like intercellular adhesion molecule (ICAM)-I, -2 and -3(CD-50), vascular cell adhesion molecule (VCAM-I), and platelet endothelial cell adhesion molecule (PECAM-I= CD-31). Selectins are cell adhesion molecules that bind to carbohydrates expressed on cells.[7] PECAM-I, with a molecular weight of 130 kd, is an adhesion molecule that is structurally expressed by circulating blood cells, including thrombocytes, monocytes and T-lymphocytes, as well as at the intercellular junction of endothelial cells. It plays an important role in angiogenesis and leukocyte extravasation.[8-11] PECAM-1 is localized on cell-to-cell borders of adjacent endothelial cells, suggesting a possible role in angiogenesis.[12] Several studies have demonstrated that PECAM-1 supports leukocyte migration and plays a role in spiral artery transformation.[13]

The interaction of maternal leukocytes and decidual cells with invading trophoblasts is to be established through cell adhesion molecules. The expression of cell adhesion molecules in fetal and maternal placental bed biopsies recently has been studied. Studies on the role of adhesion molecules in preeclamptic women have produced conflicting results In addition; conflicting reports exist regarding the expression of cell adhesion molecules in preeclamptic placenta.[14-17]

Herein, our aim was to compare the expression of two of the intercellular adhesion molecules, PECAM-1 (CD-31) and ICAM-3 (CD-50), in preeclamptic and healthy placental beds, using an immunohistochemical staining method.

## MATERIAL AND METHODS

### 2.1. POPULATION CHARACTERISTICS

In this study, 30 pregnant patients with a predetermined diagnosis of preeclampsia were accepted to the Dr. Zekai Tahir Burak Women's Health Education and Research Hospital between September 2006 and May 2007. The Educational Planning and Coordination Board of the hospital approved this study and all patients were informed about the voluntary nature of their involvement in the study.

A group of 30 healthy pregnant women in labor was designated as the control group with voluntary participation. All preeclampsia patients had hypertension (>140/90 mm-Hg), 300 mg/dL daily proteinuria and mildly impaired liver function tests. In the present study, all patients were examined by ultrasonography for umbilical artery Doppler flow and birth weight calculations.

None of the patients involved in the study reported any history of systemic diseases, like hypertension, renal disease or collagen vascular disease. Placental tissues were removed from preeclamptic

and normal patients during the postpartum peri-od.

## 2.2. PLACENTAL SAMPLES

One of the indications for routine sampling of placenta for histological examinations in our study was preeclamptic pregnancy. The protocol for sampling involved taking a 1 cm full thickness placental block adjacent to terminal villi and immediately fixing it in 10% formalin solution for 72 hours. After fixation, the tissues were washed under running tap water for 24 h and dehydrated with 50, 60, 70, 80, 90, 96 and 100% concentrated ethanol. The specimens were then laid in a 1:1 ratio of immersion oil and absolute alcohol for 1 h, followed by immersion oil overnight, for transparency. After the application of xylol, the specimens were made into paraffin blocks using a 1:1 xylol and paraffin mixture for 1h and paraffin for 6 h in an incubator.

## 2.3. IMMUNOHISTOCHEMISTRY METHODS FOR PECAM-1 AND ICAM-3 IN PLACENTA

We used *Avidin-Biotin Peroxidase Complex* techniques for immunohistochemical staining.[18] After incubating overnight at 37°C for 1 h at 60°C, slides were de-waxed in two changes of xylene (15 min each) and rehydrated with descendent ethyl alcohol gradient for 10 min each, followed by two 5 min changes of distilled water. Sections were boiled in a microwave oven in citrate buffer (Catalog # AP-9003-500, Lot CT14070, LabVision Corporation, Fremont, CA, USA) for 5 min at 650 W and for 3 x 5 min at 550 W. After 20 min at room temperature, the tissue was rolled with a Pap-pen (Super Pap pen, PN IM3580, Beckman Coulter Company, France). After washing with distilled water and then with phosphate-buffered saline (PBS, Lot 0446B002, Lab-Vision Corporation, Fremont, CA,USA), endogenous peroxidase activity was blocked in 3% hydrogen peroxide (Catalog #TA-125-HP, Thermo Scientific, Fremont, CA, USA) for 20 minutes. After washing with PBS, ultra V block (Catalog #TA-125-UB, Thermo Scientific, Fremont, CA, USA) was applied for 5 minutes. After a 1-hour application of primary antibodies (CD31/PECAM-1 Ab-6 (Clone 1A10) Cat#MS-1873-R7: ready to use, CD50/ICAM-3 Ab-

3 (Clone 186-2G9) Cat.#MS-624-P0,-P1, or –P, Fremont, CA 94539 USA) diluted (1:100 concentration) with antibody diluents (Catalog # TA-125-UD Thermo Scientific, Fremont, CA, USA), the samples were washed with PBS. Secondary antibody, biotinylated goat antipolyvalent (Catalog #TP-125-BN, Thermo Scientific, Fremont, CA, USA) was applied for 20 min. After washing with PBS, slides were then exposed to streptavidin peroxidase (TP-125-HR, Thermo Scientific, Fremont, CA, USA) for 20 min. After rewashing with PBS, the specimens were placed in AEC (3-Amino-9-Ethyl Carbazole) (Catalog #TA-125-HA, Thermo Scientific, Fremont, CA, USA) chromogen for 10 minutes. Finally, counterstaining with Mayer's hematoxylin (Catalog # TA-125-MH, Lot MH13533, LabVision Corporation, Fremont, CA, USA) was performed for 2 minutes.

## 2.4. ANALYSIS OF SPECIMENS

All slides were evaluated with a Leica DMI 4000 B light microscope *(Wetzlar, Germany)* and photographed with Leica QWin ProV 3.4.0 (Calidris and SoftHard Technology Ltd. Switzerland)

Two different researchers, who were blinded to clinical diagnosis, evaluated the intensity of staining independently. The following staining intensity designation was used: 0; no involvement; 1(+); slight involvement; 2(++); mild involvement; and 3(+++); strong involvement of primary antibody. PECAM-1 staining was determined in vascular endothelial cells in both groups. ICAM-3 was determined in circulating blood cells and in the terminal villi of controls. The entire slide was examined for each specimen and final scoring was based on the predominant areas with the highest grade of staining intensity. Labeling intensity was graded semiquantitatively and the HSCORE was calculated using the following equation: HSCORE $= \sum Pi(i+1)$, where $i$ is the intensity of labeling with a value of 1, 2 or 3 and $Pi$ is the percentage of labeled epithelial and stromal cells for each intensity, varying from 0% to 100%.[19]

## 2.5. Statistical Analysis:

Kolmogorov-Smirnov, Student's t test, and Mann Whitney U test were run, using SPSS version 11.0

statistical software. A statistically significant difference was defined as p < 0.05 and all tests were 2-tailed.

# RESULTS

## CLINICAL CHARACTERISTICS

As seen in Table I, no statistically significant difference was observed between the two groups in terms of mean maternal age (p= 0.310). Infants of preeclamptic mothers had a significantly lower (p= 0.001) birth weight and gestational age (p= 0.001) than infants born to controls. Moreover, a statistically significant inter-group difference was observed in the systolic/diastolic (S/D) blood pressure index of the umbilical artery, with the ratio on Doppler blood flows higher in those with preeclampsia (p= 0.001).

## PLACENTAL IMMUNOHISTOCHEMISTRY

In tissues from the preeclampsia group, PECAM-1 mild to strong immunoreactivity of the placenta was observed in vascular formations. While this positive immune reactivity was observed in endothelial cells, there was no involvement of trophoblastic cells in the preeclamptic placenta. When evaluating the preeclamptic tissues mild to strong PECAM-1 (CD31) immunoreactivity was identified in vascular endothelial epithelia.

| **TABLE 1:** Clinical characteristics of preeclamptic women and the control group. | | | |
|---|---|---|---|
| | **Preeclamptic** | **Control Group** | |
| | **Group (n= 30)** | **(n= 30)** | **p value** |
| Age (year) | 29.26 ± 5.63 | 27.66 ± 3.58 | 0.310 |
| Gestational age (week) | 36.22 ± 1.43 | 38.84 ± 0.84 | **0.001** |
| S/D index of umbilical artery | 3.53 ± 0.70 | 2.53 ± 0.42 | **0.001** |
| Birth weight (g) | 2576.90 ± 377.89 | 3433.10 ± 378.47 | **0.001** |

S/D: Systolic/diastolic; p <0.05 is significant

PECAM-1 positive immune reactivity had an HSCORE of 193.0% ± 55.2 in placental villous endothelial cells from the preeclampsia group (Figure 1: A, B). The HSCORE value was 114.0% ± 43.9 in the control group (Figure 1: C, D). Staining was localized primarily across the entire vascular epithelia cytoplasm. Positive immune reactivity was lower in the control group (Figure 1: C, D). Moreover, a significant difference was observed between the two groups (p= 0.001) (Figure 1: A, B, C, D)

There was no ICAM-3 (CD-50) immunoreactivity in the preeclampsia group, for which the HSCORE was 89.0% ± 23.8 (Figure 2: A, B). The HSCORE value was 82.0% ± 17.4 for the control group. On the other hand, slight involvement of ICAM-3 in circulating blood cells was observed in only two patients (5.9%) in the control group (Fi-
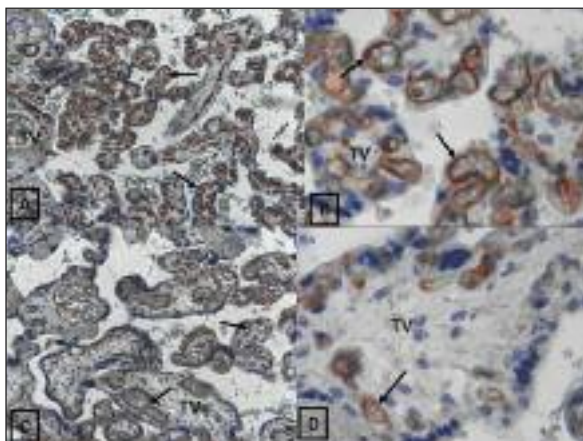


**FIGURE 1:** In terminal villi (TV), arrows indicate mild PECAM-1 immunopositivity in placental villous endothelial cells in preeclamptic placental sections (A, B) and slight to mild PECAM-1 immunopositivity in the control group is indicated with arrow (C, D). (Bar: 30 μm)
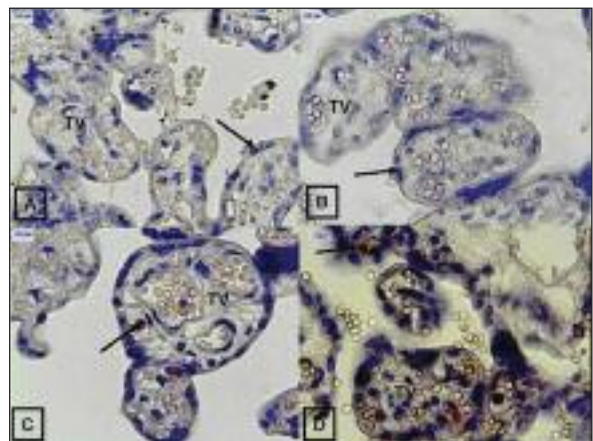


**FIGURE 2:** No immunreactivity for ICAM-3 in the placental terminal villous (TV) endothelial cells from preeclamptic (A, B) and control groups (C, D). Arrows indicate slight immunopositivity of ICAM-3 in circulating blood cells in the control group (Bar: 10 μm).)

gure 2: C, D). Between the two groups, no significant difference was identified (p= 0.15).

## DISCUSSION

Preeclampsia is associated with impaired trophoblastic invasion and consequent failed spiral artery modification, which affects multiple organs and systems.[20] The pathological adrenergic innervation of spiral arteries observed in preeclampsia causes placental tree dysfunction, and this in turn is associated with fetal growth retardation.[7,21]

Preeclampsia is the most common disease-related cause of uteroplacental dysfunction. Therefore, the impaired umbilical artery blood flow occurs by vascular endothelial pathology. This pathologic endothelial injury stimulates endothelial cell activation, which results in the expression of cell adhesion molecules.[22,23] Early enhanced activation of endothelial cells, platelets and leukocytes seem to be present in preeclamptic patients, especially in those that develop severe preeclampsia.[24]

PECAM-1 (CD-31) is a member of the cell adhesion molecule (CAM) family, which is expressed by platelets and vascular endothelium. Platelets and neutrophils are involved in maternal placental vascular damage in preeclampsia. ICAM-1 expressed on trophoblasts are involved in preeclampsia pathogenesis and is regulated by cytokines. Cell adhesion molecules expressed in the placental tree probably mediate recruitment of these cells. It remains controversial whether platelets and neutrophils mediate damage to trophoblasts or to the villous vasculature.[25,26]

Failure of trophoblastic invasion and spiral artery transformation in preeclampsia and fetal growth retardation are not well understood. Recent studies have suggested that cytotrophoblasts that invade spiral arteries mimic the endothelial cells they replace and express PECAM-1. Pathology in fetal uteroplacental circulation is related to increases in PECAM-1 and ICAM-1 mRNA expression in endothelial cells.[23,27]

Despite the importance of trophoblastic invasion and vascular remodeling, these processes remain controversial. However, they are thought to include changes in the expression of cell adhesion molecules, matrix metalloproteinases and their tissue inhibitors, and growth factors and their receptors.[28,29]

Gonzales et al demonstrated that the levels of CD-31 (PECAM-1) and CD-42 endothelial microparticles increased and claimed this as evidence of endothelial injury in preeclamptic women.[8]

Chaiworapongsa et al identified decreased levels of P-selectin, E-selectin and VCAM-1, whereas ICAM-1 and PECAM levels remained unchanged in preeclamptic women. ICAM-1, VCAM-1, PECAM-1 and P-selectin plasma levels varied according to the severity of hypertension. ICAM-1, VCAM-1 and PECAM-1 levels also appeared to be significantly increased in patients with pregnancy-induced hypertension (PIH) relative to healthy controls. [9-11]

In a study by Coukos et al, PECAM-1 expression appeared to be constitutive in this subpopulation of trophoblastic cells. The molecule only migrated to the cell surface in the presence of endothelial cells. Moreover, PECAM-1 localization became polarized in sites of trophoblastic cell contact. As a result, our findings were similar to the placental involvement identified by Coukos et al.[30]

PECAM-1 is expressed in the placental bed of normal and preeclamptic pregnancies, but there are no differences between normal and preeclamptic pregnant women. Consequently, it seems that PECAM-1 should not be implicated in the etiology of preeclampsia.[17]

## CONCLUSION

Preeclampsia is an angiogenic pathologic condition that originates at the beginning of the implantation process. For this reason, PECAM-1 molecules may be associated with preeclampsia. PECAM-1 expession levels, one piece of evidence of leukocyte activation, are higher in pre-eclamptic women's placental villous endothelium than normal pregnant women's as relatively. In conclusion, PECAM-1 over-expression from endothelial cells may cause endothelial injury, but ICAM-3 (CD-50) may be irrelevant to the etiopathogenesis of preeclampsia.

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