Research Article / Araştırma Makalesi

Investigation of Bax and Phospho-Tau Protein Expression in Preeclampsia Placenta

Preeklampsi Plasentada Bax ve Fosfo-Tau Protein Ekspresyonunun Araştırılması

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Abstract

Background: Preeclampsia is a pregnancy complication with a high morbidity and mortality rate. Bax is a proapoptotic protein and in recent years, 14-3-3 tau proteins are considered as key regulators of many processes, such as apoptosis. In this study, we investigated the expression of phospho-tau and Bax in preeclampsia apoptosis immunohistochemically.
Materials and Methods: Placental tissues of 25 healthy and 25 preeclamptic pregnant were included in the study. Placental samples were fixed with 10% neutral buffered formalin. Routine paraffin wax tissue protocol was used.
Results: According to histological micrograf, the trophoblastic cells in the villi were normal in the control group. Mild dilatation in blood vessels was seen. Hyalinized and necrotic areas in chorionic villi and an increase in fibrinoid tissue in root villi were detected in preeclampsia sections. Phospho-tau and Bax primary antibodies were used for immunohistochemical evaluation. The sections from of the preeclampsia group were highly positive for syncytiotrophoblasts and villous connective tissue. It was also statistically different from the control group ($p<0.05$).
Conclusions: When we evaluate the results shows that phospho-tau and Bax may be determinant proteins in the apoptosis pathway of preeclampsia.

Key Words: Apoptosis, Bax, Phospho-tau, Placenta, Preeclampsia

Öz

Amaç: Preeklampsi, morbidite ve mortalite oranı yüksek bir gebelik komplikasyonudur. Bax proapoptotik bir proteindir ve son yıllarda 14-3-3 tau proteinleri, apoptoz gibi birçok sürecin anahtar düzenleyicileri olarak kabul edilmektedir. Bu çalışmada preeklampsi apoptozunda fosfo-tau ve Bax ekspresyonunu immünohistokimyasal olarak araştırdık.
Materyal ve Metod: 25 sağlıklı ve 25 preeklamptik gebe plasenta çalışmaya dahil edildi. Plasental örnekler %10 nötr tamponlu formalinle sabitlendi. Rutin parafin doku protokolü kullanıldı.
Bulgular: Histolojik sonuçlara göre kontrol grubunda villuslardaki trofoblastik hücreler normaldi. Kan damarlarında hafif genişleme görüldü. Preeklampsi kesitlerinde koryon villuslarında hyalinize ve nekrotik alanlar ve kök villuslarında fibrinoid doku artışı saptandı. İmmünohistokimyasal değerlendirme için fosfo-tau ve Bax primer antikorları kullanıldı. Preeklampsi grubundan alınan kesitler sinsityotrofoblastlar ve villöz bağ dokusu açısından pozitifti. Kontrol grubundan istatistiksel olarak da farklıydı ($p<0.05$).
Sonuç: Bulguları incelediğimizde, fosfo-tau ve Bax'ın preeklampsinin apoptoz yolağında belirleyici proteinler olabileceğini düşünmekteyiz.

Anahtar Kelimeler: Apoptoz, Bax, Fosfo-tau, Plasenta, Preeklampsi

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Introduction

Preeclampsia (PE) is a pregnancy complication that increases maternal and fetal mortality (1). It is a syndrome characterized by symptoms such as hypertension, proteinuria, edema and maternal organ dysfunction occurring after the 20th week of pregnancy (2,3). It has been reported that PE, is associated with more than 70,000 maternal and more than 500,000 fetal deaths annual worldwide (4,5).

PE is related with defects in the remodeling of the spiral arteries that impede blood flow to the placenta (6). In normal pregnancy, with the invasion of the endometrium by trophoblasts in the placenta, the spiral arteries remodel and turn into sinusoids with low resistance and high flow. However, spiral arteries cannot be reshaped in PE due to disruption of trophoblast invasion (7). PE has been associated with disruptions in the development of the placenta, dysfunction in the endothelial structure, and imbalances in the angiogenesis process (8). This disruption compromised blood flow and thus restricts blood circulation to the placenta and fetus, causes ischemic hypoxia and oxidative stress. This condition is considered placental disease that affects both the fetus and the mother (9,10).

Hypoxia can lead to increased oxidative stress in the placenta and apoptosis in trophoblast cells. All these processes can cause preeclampsia (11). Even though it is thought that there are many underlying causes of PE with a complex pathogenesis, its specific mechanism of action still remains uncertain (12). Histologically in preeclamptic placentas; fibrin deposition, syncytiotrophoblast apoptosis, villous necrosis and shallow invasion of trophoblasts are seen (13). It has been declared that apoptosis has a critical role in the physiological and pathophysiological mechanism of the placenta (14).

Apoptosis (programmed cell death) is a critical process for maintaining tissue homeostasis throughout life and plays an important role in placental development (11). Bax is a proapoptotic protein that plays a role in the apoptosis process. Increased Bax protein expression is indicative of the level of advanced apoptosis (15). Studies have shown that 14-3-3 proteins are an inhibitor that regulates Bax activity in apoptosis. It has been stated that 14-3-3 proteins are key regulators of numerous processes such as mitosis and apoptosis in animals and exhibit antiapoptotic properties. It is include in various biological processes such as the control of apoptosis (16,17). It has also been found that 14-3-3 tau proteins exhibit antiapoptotic properties. Seven isiforms have been identified in the 14-3-3 gene, 14-3-3 ß, g, e, h, s, t/q, and z (17). However, phosphorylation of the 14-3-3 protein (phospho-tau) causes Bax to dissociate from 14-3-3, which leads to apoptosis and translocation of Bax to mitochondria (16–18).

In this study, we investigated the relationship between of phospho-tau and Bax proteins with preeclampsia.

Materials and Methods

Collecting the placental samples

The study was carried out with the permission of Dicle University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee, numbered 2023/147. Placental tissues of 25 pregnant women with preeclampsia diagnosis and 25 control (healthy) pregnant women from Dicle University Medical Faculty Hospital Gynecology and Obstetrics Clinic were included.

The 2013 guidelines of the American College of Obstetricians and Gynecologists were used to define preeclampsia. Preeclampsia was defined as a systolic blood pressure ≥140 and a diastolic blood pressure ≥90 and the presence of protein ≥300 mg in a 24-hour urine test. (17). The values of the control and preeclampsia groups are shown in Table 1.

Table. 1. Systolic BP (mmHg) and Diastolic BP (mmHg) values of the study participants

Parameters	Control (n=10) (mean±SD)	Preeclampsia (n=10) (mean±SD)
Systolic BP (mmHg)	118.16±8.42	147.20±16.27
Diastolic BP (mmHg)	67.64±6.54	94.63±10.44

The tissues fixed in 10% formaldehyde was applied a paraffin wax embedding procedure. Sections of 4 µm were cut from the obtained blocks and stained with H&E, phospho-tau and Bax primary antibodies for histopathological and immunohistochemical evaluation, respectively.

Hematoxylin-Eosin (H&E) staining

Sections were deparaffinized in xylene for 2x15 minutes. Then, sections passed through decreasing alcohol series were kept in hematoxylin staining solution for 6 minutes and washed in running water for 5 minutes. Sections taken in eosin staining solution were kept for 4 minutes and passed through a rapidly rising alcohol series. Sections cleaned in xylene for 2x15 minutes were closed with entellan. Micrographs of the preparations evaluated under the light microscope were taken (Zeiss Imager A2 Zen 3.0 software (Germany)).

Immunohistochemical staining

Placental sections obtained from paraffin blocks were deparaffinized in xylene for 2x15 minutes. Sections passed through decreasing series of ethyl alcohol were brought to distilled water and washed with phosphate buffer solution (PBS). Sections were then microwaved in EDTA buffer solution (pH: 8.0, catalog no: ab93680, Abcam, Cambridge, USA) for antigen retrieval. Sections cooled to room temperature were preserved in PBS solution. Sections were kept in hydrogen peroxide solution (Catalog No: TA-015-HP, Thermo Fischer, USA) to block endogenous peroxidase activity.

Ultra V Blocking solution (Catalog No: TA-015-UB, Thermo Fischer, USA) was applied to the sections that were taken back to PBS to prevent non-specific binding. The sections were then incubated in the refrigerator (+4 °C) overnight with phospho-tau (p-Tau S396, Thermo Fischer, USA) and Bax (sc- 20067 Santa Cruz,Italy) primary antibodies. The sections were then kept at room temperature, the sections were washed in PBS solution and biotinylated secondary antibody was applied. Sections washed with PBS solution were then kept in streptavidin peroxidase solution (catalog no: TS-015-HR, Thermo Fisher, USA). Sections washed in PBS were treated with diaminobenzidine (DAB) (catalog no: TA-001 HCX, Thermo Fischer, USA). Sections washed with PBS solution were treated with hematoxylin solution for 40 seconds and then rinsing to tap water for 5 minutes. Sections passed through increasing series of ethyl alcohol were covered with entellan. Micrographs of the preparations evaluated under the light microscope were taken. (Zeiss Imager A2 Zen 3.0 software (Germany)).

Statistical analysis

Shapiro-Wilk test was used to check the suitability of the data with normal distribution. Comparisons between control and patient groups were performed using the independent samples t-test. The $p<0.05$ level was chosen for statistical significance during the analyses. Statistical analyzes were performed using Analyze-it for Microsoft Excel Method Comparison Edition (v30.2, Analyze-it Software Ltd., Leeds, UK).

Results

Histological results

When the sections of the control placentas were examined; Decidual cells were polygonal and small fibrinoid structures were detected in places. In the villi, the trophoblastic cells were round and normal. Mild dilatation and congestion were seen in blood vessels. Few syncytial knots were seen. In preeclampsia sections, fibrinoid tissue increase in root villi and hyalinized and necrotic areas in chorionic villi were detected. Intense inflammation and bleeding foci were detected in the intervillous area. Dilatation and intense congestion were observed in the blood vessels (Figure 1).

Phospho-Tau and Bax immunohistochemical results

In the control group sections, Bax immunoreactivity was generally negative in vascular structures, syncytiotrophoblasts, roots and floating villi, villous connective tissue and vascular endothelium. It was observed that Bax immunoreactivity was intense in the preeclampsia group compared with the control group. Bax expression was increased in floating villi, syncytiotrophoblasts, syncytial knots, and villous connective tissue (Figure 2,3).

Negative phospho-tau immunoexpression was observed in control group sections, syncytiotrophoblasts, villous connective tissue cells, and endothelial cells. In the sections of the preeclampsia group, particularly intense phospho-tau expression was observed in villous connective tissue and syncytiotrophoblasts. phospho-tau immunoreactivity was negative in syncytial nodes (Figure 4,5).

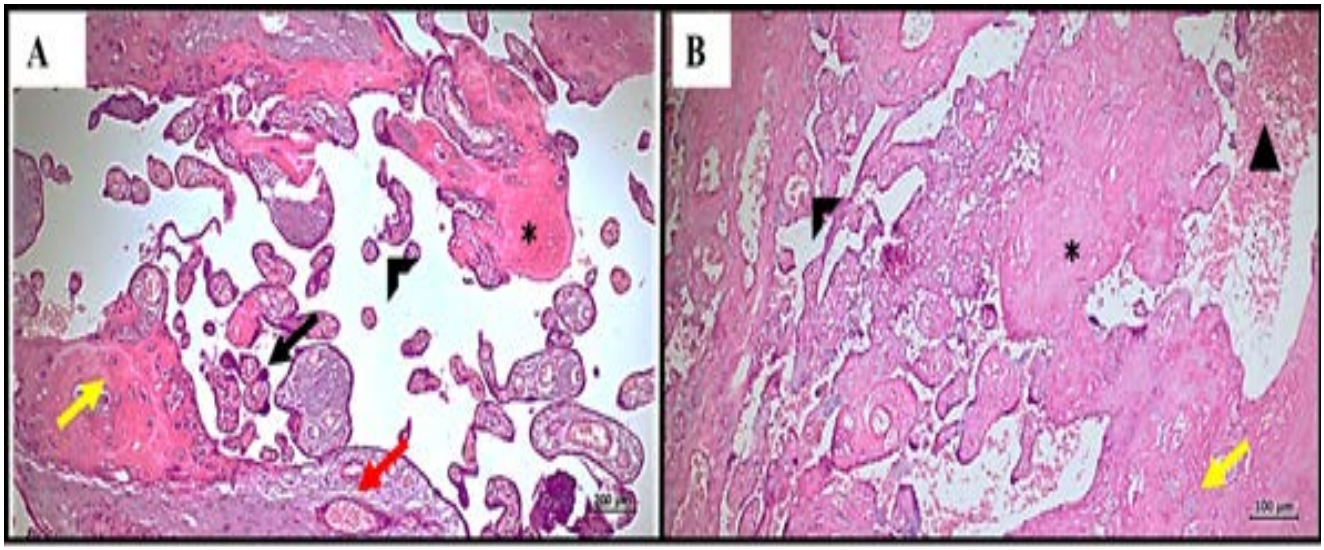

Figure 1. Control placenta (A), decidual cells are polygonal (black arrow), small fibrinoid (asterisk) structures, mild dilation and congestion in blood vessels (red arrow), few syncytial knots (yellow arrow), round-shaped trophoblastic cells (arrowhead) in normal villi (H&E Bar: 200 µm). Preeclampsia (B), fibrinoid tissue increase in root villi (asterisk), hyalinized and necrotic areas in chorionic villi (yellow arrow), intense inflammation and bleeding foci in the intervillous area (triangle), intense congestion (arrowhead) with dilatation of blood vessels (H&E Bar: 100 µm).

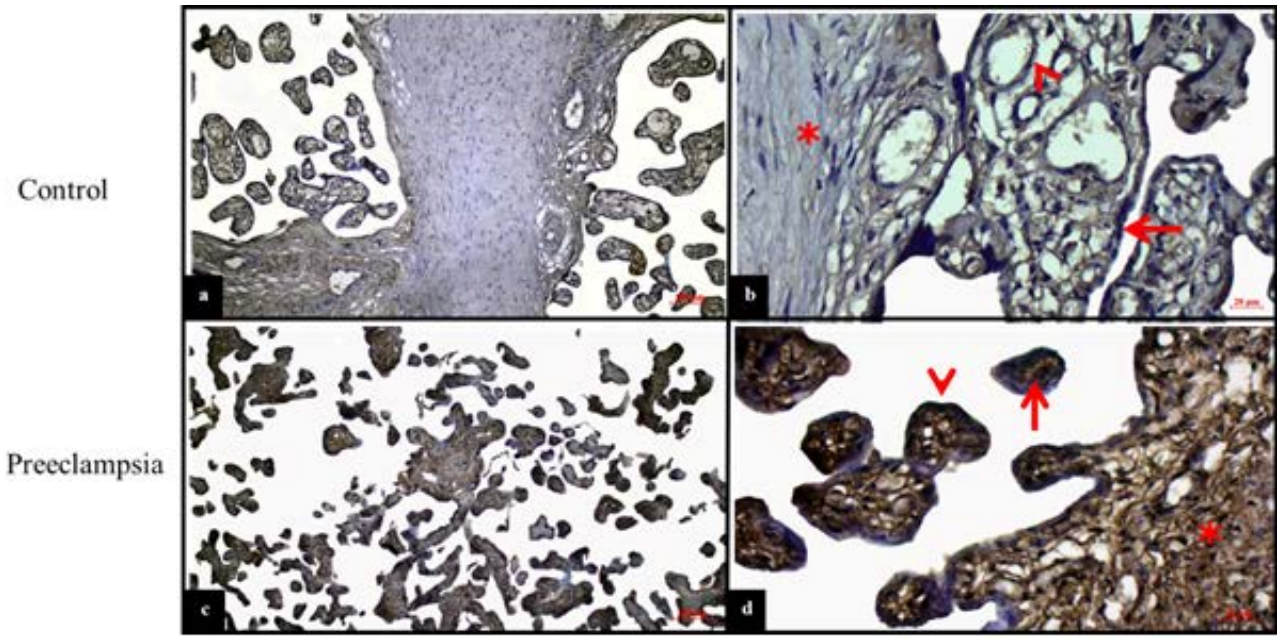

Figure 2. a; Control group, negative Bax expression (bar: 100 µm), b; Control group, negative Bax expression, villous connective tissue (∗), vascular endothelium (>), villous trophoblasts (→) (bar: 20 µm), c; Preeclampsia group, intense Bax expression (bar: 100 µm), d; Preeclampsia group, intense Bax expression syncytial knots (→), syncytiotrophoblast (>), villous connective tissue (∗) (bar: 20 µm).

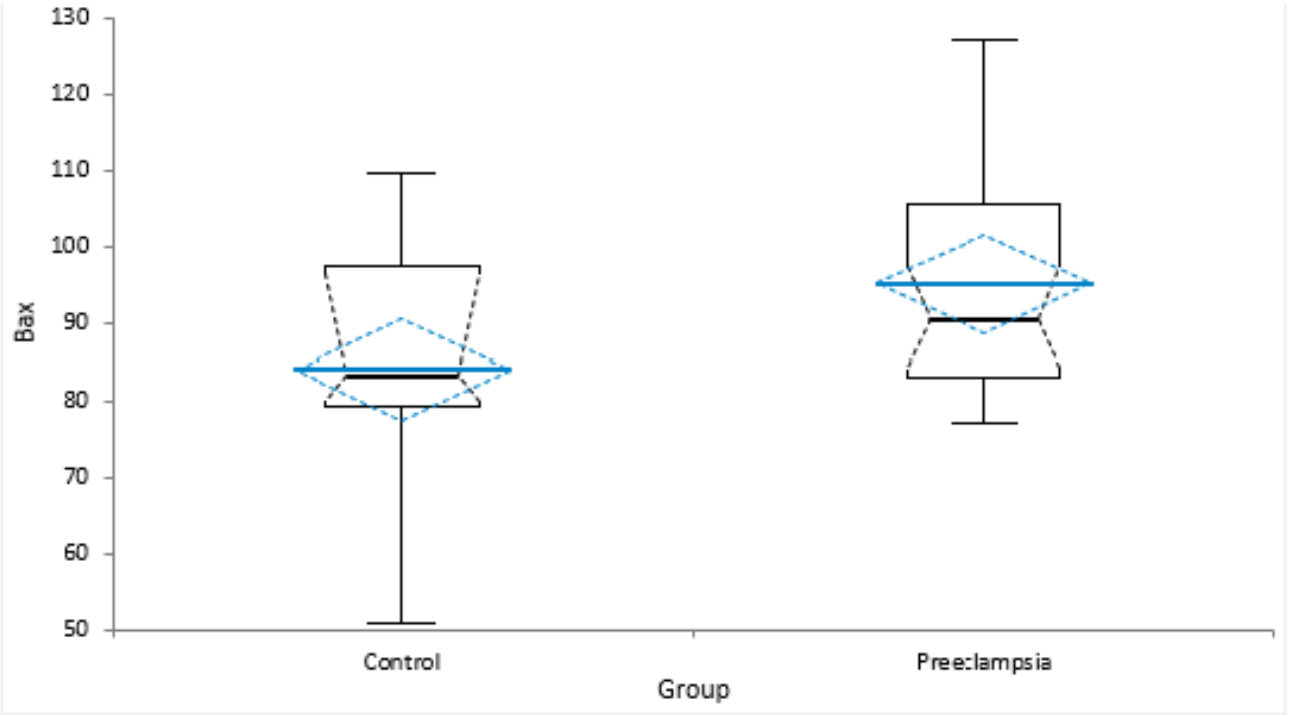

Figure 3. Bax expression in placentas of control and preeclamptic patients: The difference between control and preeclampsia placentas was statistically significant ($p<0.05$).

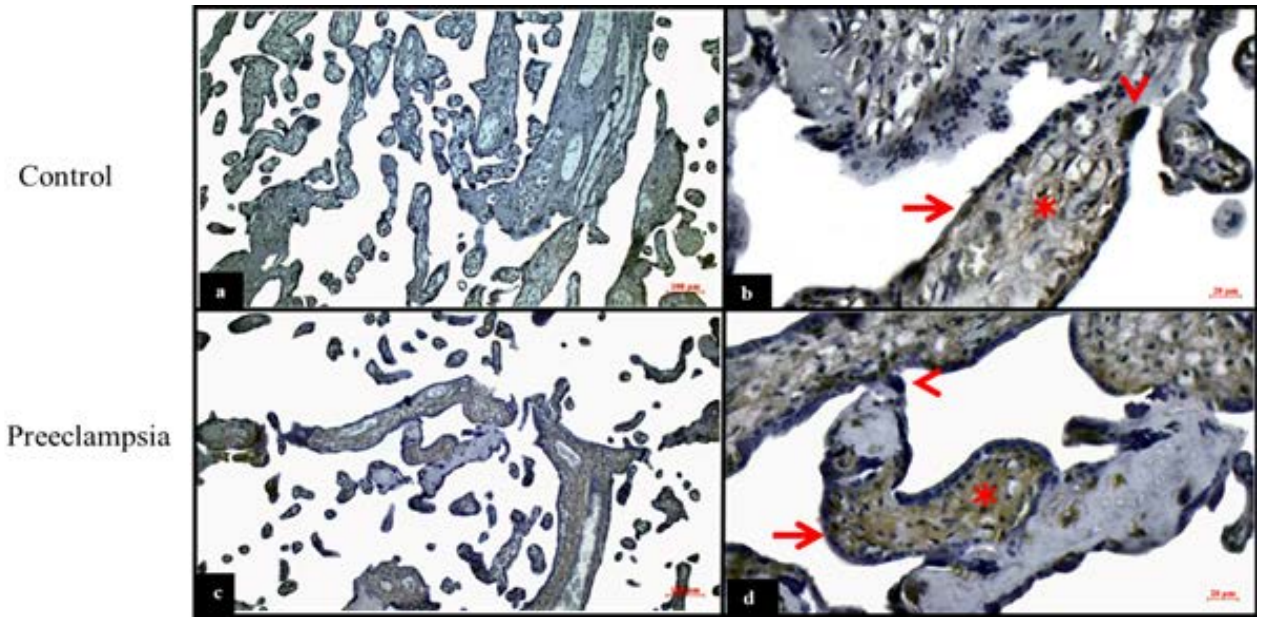

Figure 4. a; Control group, negative phospho-Tau expression (bar:100 µm), b; Control group, negative phospho-Tau expression (bar: 20 µm), villous connective tissue (∗), syncytial knot (>), syncytiotrophoblast (→), c; Preeclampsia group intense phospho-Tau expression (bar: 100 µm), d; Preeclampsia group, intense phospho-Tau expression villous connective tissue (∗), syncytial knot (>), syncytiotrophoblast (→) (bar: 20 µm).

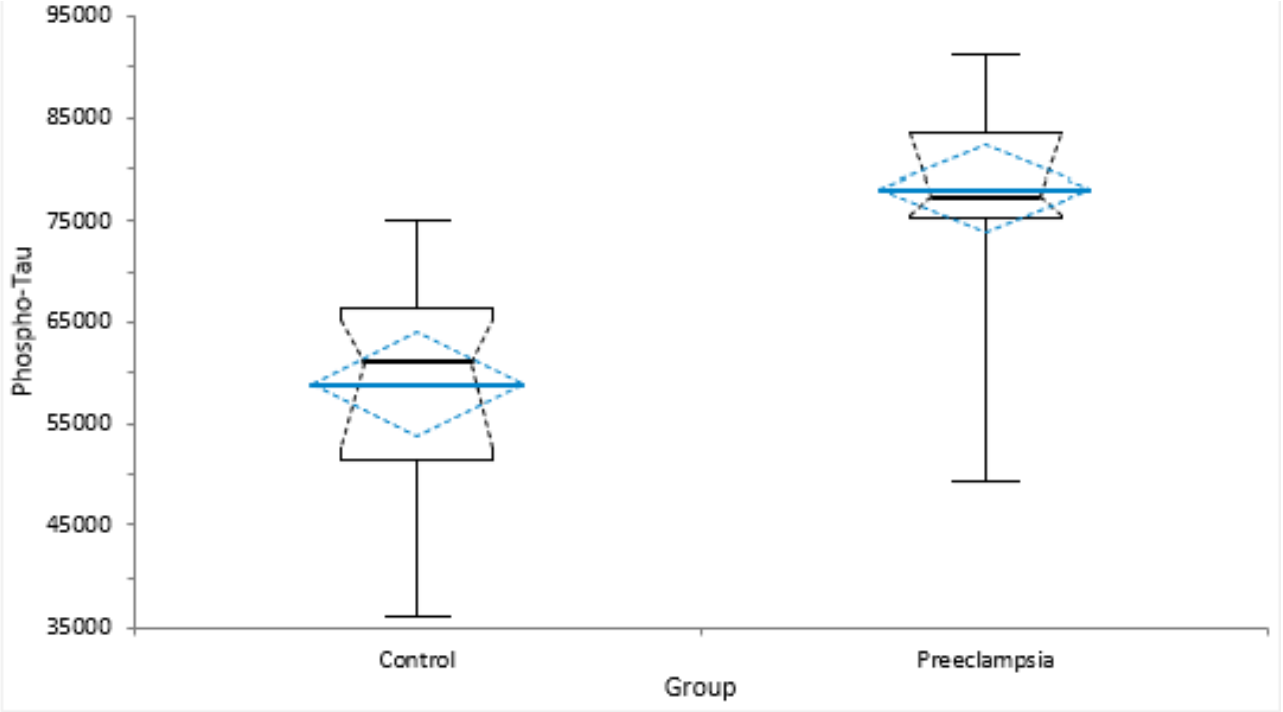

Figure 5. Phospho-Tau expression in placentas of control and preeclamptic patients: The difference between control and preeclampsia placentas was statistically significant ($p<0.05$).

Discussion

PE is known that abnormal development and function of the placenta associated with abnormal invasion and remodeling of maternal uterine arteries by extravillous trophoblasts (19). In addition, angiogenic, apoptotic and epithelial mechanisms are thought to be effective in placental inadequacy (20). However, the pathological mechanism of PE is still not fully determined (21). Apoptosis plays a critical role in the physiology of the human placenta. Extreme placental apoptosis is a typical feature in PE (22).

In the study by L. Hecht et al., hypertrophic decidual vasculopathy, fullness of endothelial cells and damage to the epithelial integrity were detected in the placentas of pregnant women with preeclampsia. Large dilated arteries with mural fibrinoid necrosis and small arteries with medial hypertrophy have been described. In addition, all lesions were associated with loose clusters of small lymphocytes (23). In our study, in parallel with the literature, fibrinoid tissue increase in root villi, hyalinized and necrotic areas in chorionic villi, and bleeding foci with intense inflammation in the intervillous area were observed. Moreover dilatation and intense congestion were observed in the blood vessels (Figure 1).

A few studies have demonstrated defects in the vascular remodeling process in serious complications such as preeclampsia (24). It causes apoptosis due to complications of preeclampsia (13). Cell cycle dysregulation in preeclampsia leads to increased apoptosis. The role of increased apoptosis in placental pathology in PE is not clear but may inhibit the regeneration of syncytiotrophoblasts and may lead to syncytial degeneration. It may lead to the release of vasoactive or inflammatory factors into the maternal circulation (25). Bax is expressed in the villous trophoblast and the cytoplasm of cytotrophoblast in the first trimester and in the syncytiotrophoblast and villous trophoblast in the third trimester (26).

In the study by Sharp et al, increased Bax expression was observed in preeclamptic placentas compared with normal term placentas. It has been stated that Bax is expressed in syncytiotrophoblast, cytotrophoblast and occasionally stromal cytoplasm in normal and PE placental tissue, however apoptosis is reportedly increased particularly in locations where trophoblast damage has occurred (25).

In a study evaluating Bax expression in pathological placentas during pregnancy, it was found that Bax immunopositivity increased in the extravillous trophoblasts of the preeclamptic placenta in the third trimester (27). In a prior study, the preeclampsia group's decidua cells, root villi, syncytial nodes, and vascular endothelium had higher levels of Bax protein expression than the control group's (28). Similar to this, in our current investigation, the Bax immune reactivity was higher in the preeclampsia group than in the control group in floating villi, syncytiotrophoblast, syncytial nodes, and villous connective tissue.

Result of a study on relation of tau and preeclampsia showed that tau expression was significantly decreased in the preeclampsia group (29). In a study conducted by Liu et al. on the disruption of the 14-3-3 tau gene in preeclamptic placentas, it was reported that the 14-3-3 tau expression in the PE group was significantly decreased than in the control group (30). In a study investigated Bax and tau proteins in preeclamptic placentas, they stated that the separation of tau protein and Bax protein is via 14-3-3 zeta phosphorylation. In addition, in their analysis, they stated that tau protein decreased as a result of this separation and the 14-3-3 zeta phosphorylation expression level increased in preeclamptic placentas (31). In this case, we would like to point out that the decrease in the tau protein level and the increase in the phospho-tau level are associated with the increase in apoptosis in PE. The immunoreactivity of the 14-3-3 zeta phosphorylation protein increased in the preeclamptic group and was especially evident in the syncytiotrophoblasts and perivascular cells in the villous nucleus (31). Our results showed that phospho-tau expression in preeclampsia group was importantly increase than control group. A significant increase in phospho-tau expression was observed in the connective tissue cells and syncytiotrophoblasts.

It has been noted that the expression level of p-Tau in the blood is increased especially in some diseases (17). It is known that the most important reason in the pathogenesis of preeclampsia is endothelial dysfunction. In addition, placentation disorder in preeclampsia is another important factor affecting the development of preeclampsia (17). Considering these two reasons together, it is consistent with the literature that p-tau is more highly expressed in villous connective tissue and syncytiotrophoblasts that form during placentation. In addition, phospho-tau expression was intensely observed in connective tissue cells located in intervillous areas (Figure 4). According to the results of a study, it was stated that there is a decrease in 14-3-3 tau expression in preeclamptic placentas and an increase in phospho-tau, and this may increase apoptosis in cells and lead to an increase in the level of placental cellular debris spilled into the maternal blood circulation (30). Preeclamptic pregnant women have a higher concentration of syncytiotrophoblast microvillous membrane (STBM) fragments in their blood circulation compared with pregnant women without complications. It has been reported that STBMs lead to vascular endothelial dysfunction of preeclamptic pregnant women. Placental factors that seek to increase shedding of STBMs into the maternal blood circulation may caused to this, including placental secreted proteins and perhaps 14-3-3 proteins (32). PE placentas are likely to have increased apoptotic expression, which has been confirmed in many studies (33,34).

Conclusion

In conclusion, preeclampsia is a critical disease for both maternal and fetal health. Intense apoptosis of the placenta is observed in preeclampsia. We have investigated the presence of these proteins in preeclampsia apoptosis because

phospho-tau and Bax proteins affects many apoptosis mechanisms. The results of our current experiment indicated that phospho-tau and Bax protein is effective in in the apoptosis pathway of the preeclamptic placenta. However, to completely comprehend the apoptotic process in preeclampsia, we think that further molecular research and pathway of apoptosis investigate are required.

Ethical Approval: This study was carried out in accordance with the rules of research and publication ethics. The study was approved by the Dicle University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee, numbered 2023/147.

Author Contributions:
Concept: S.K., F.A., H.A., E.D.
Literature Review: S.K., H.A.
Design : S.K., F.A., H.A., E.A., E.D.
Data acquisition: S.K., F.A., H.A.
Analysis and interpretation: S.K., F.A.
Writing manuscript: S.K.
Critical revision of manuscript: E.A., E.D.
Conflict of Interest: *The authors have no conflicts of interest to declare.*
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