Available online on 15.12.2024 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

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Research Article

Placental Adrenomedullin and Soluble Endoglin Expression in Preeclamptic Placentas

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Article Info:

Article History:

Received 18 Sep 2024
Reviewed 11 Oct 2024
Accepted 28 Nov 2024
Published 15 Dec 2024

Cite this article as:

Ünal BK, Akkuş M, Türe Z, Acar GEA, Ölmez M, Yıldırım E, Aşır F, Ağaçayak E, Yıldızhan E, Placental Adrenomedullin and Soluble Endoglin Expression in Preeclamptic Placentas, Journal of Drug Delivery and Therapeutics. 2024; 14(12):20-26 *DOI: http://dx.doi.org/10.22270/jddt.v14i12.6905*

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Abstract

Aim: This study aims to evaluate the placental expression levels of two recently discovered proteins, adrenomedullin (ADM) and soluble endoglin (sEng), in preeclamptic pregnancies.

Materials and Methods: Placental tissue sections were obtained from 20 preeclamptic and 20 normotensive patients and processed for paraffin embedding. Hematoxylin-eosin and immunostaining methods were applied to the sections, which were then examined under a light microscope and photographed.

Results: In the preeclamptic group, more intense pathological changes were observed in placental tissues compared to the control group, including an increase in Hofbauer cells, degeneration, congestion, and hemorrhage in villous capillaries. Immunohistochemical analyses revealed sEng and ADM expression in both groups, with a concentration of these proteins in cytotrophoblast and syncytiotrophoblast cells. sEng expression was 34.83% in the control group and 49.22% in the preeclamptic group, showing a significant increase (p=0.000). ADM expression was 51.72% in the control group and 22.08% in the preeclamptic group, with a significant decrease observed (p=0.000).

Conclusion: We suggest that trophoblast cells play an indirect functional role in the development of preeclampsia or in response to the homeostatic disturbances it causes. We believe that further research is needed to obtain new findings that could support this hypothesis.

Keywords: Placenta, Umbilical cord, Adrenomedullin, Soluble Endoglin

INTRODUCTION

The placenta is a temporary organ that facilitates circulation between the fetus and mother, playing a critical role in fetal development and nutrition [1]. Formed from both fetal and maternal tissues, the placenta provides the fetus with oxygen, nutrients, and antibodies, while also aiding in the removal of waste products. As pregnancy progresses, structural changes occur within the placenta, with the development of placental villi creating a selective barrier between fetal and maternal blood circulation. This barrier, together with the placenta's endocrine functions, is crucial for healthy fetal development [2, 3].

Preeclampsia, a serious health complication that can arise during pregnancy, is characterized by high blood pressure and proteinuria, posing significant risks for both mother and fetus [4]. Known to be exclusive to humans, preeclampsia can present in two clinical forms—mild and severe—according to the American College of Obstetrics and Gynecology (ACOG) [5, 6]. The poorly understood pathophysiology of this condition contributes to complications such as preterm birth and mortality. It is thought that genetic predisposition may play a role in preeclampsia, with studies showing a 2-4 times higher risk in families with consanguineous marriages. Women with a maternal or sibling history of preeclampsia also face an increased risk of developing the condition [7, 8].

Endoglin is a surface membrane protein primarily found in vascular endothelial cells and syncytiotrophoblasts, playing a crucial role in angiogenesis and vascular regulation. Soluble Endoglin (sEng), part of the TGF-β receptor family, functions as a co-receptor for TGF-β, exhibiting anti-angiogenic effects [9]. Also known as CD105, Endoglin is thought to be involved in hematopoiesis, cardiovascular development, and angiogenesis. Structurally, it contains an extracellular domain with disulfide bonds and a short phosphorylated cytoplasmic tail [10]

Endoglin is highly expressed in various cells such as vascular endothelial cells, chondrocytes, and syncytiotrophoblasts in term placentas. It is also present in monocytes, erythroid precursors, and hematopoietic stem cells [11, 12]. Elevated levels of circulating sEng have

been observed in conditions like atherosclerosis and various cancer types (including breast, colorectal, and myeloid cancers) [13]. Stepan and colleagues reported increased sEng levels in pregnancies with intrauterine growth restriction but without preeclampsia-specific maternal symptoms, suggesting that sEng may play a role in other placental pathologies beyond preeclampsia [14].

Adrenomedullin (ADM) is a peptide derived from the adrenal medulla, first referenced in 1993 in a study examining its effects on blood pressure. Initially isolated by Japanese scientists from pheochromocytoma cells, subsequent clinical studies have measured ADM protein levels and identified relevant receptors for this peptide [15]. In humans, ADM consists of 52 amino acids, containing disulfide bridges and amidated tyrosine [16]. It is understood that the ADM gene is expressed not only in the adrenal medulla but also in endothelial cells, where it is secreted from vascular endothelial cells along with proteins like nitric oxide (NO) and endothelin [17].

Recent studies have shown that ADM levels decrease following ultrafiltration during hemodialysis, indicating a regulatory role of the body in maintaining ADM levels. Additionally, ADM has been reported to contribute to hypotension in hemodialysis patients [18]. Other studies on hemodialysis suggest ADM's autocrine and paracrine effects on vascular wall stimulation, hypoxia, complement pathways, platelet activation, leukocyte protease release, and during heparin treatment [19].

This study aims to investigate and compare the expression levels of Adrenomedullin and soluble Endoglin (sEng) in placental tissue sections from preeclamptic pregnancies using histological and immunohistochemical methods.

MATERIAL-METHODS

Study design

Our study was initiated with ethical approval received from Dicle University Faculty of Medicine Ethics Committee (Date: 15/08/2018, Approval No: 59). A total of 40 pregnant participants, 20 preeclamptic and 20 normotensive pregnant patients, regardless of age, who applied to Dicle University Faculty of Medicine Hospital Gynecology and Obstetrics Clinic were included in the study. Placentas were collected after obtaining informed consent form from the participants. After the collected placentas were washed with physiological saline, they were protected with 10% buffered neutral formalin in the operating room under appropriate conditions for tissue follow-up and sent to the Laboratory of Histology and Embryology Department of Dicle University Faculty of Medicine.

Histological tissue tracking

After the placentas were taken to the Dicle University Faculty of Medicine, Department of Histology and Embryology Laboratory, 1x1x1 cm sections were taken from both the central maternal and fetal sides. These sections were kept in 10% neutral formalin for 16 hours, then cleaned with water and dehydrated in alcoholic solutions. Then, it was made transparent with xylol and infiltrated with paraffin. 5µm thick sections were taken from the tissues turned into paraffin blocks. Sections were stained with Hematoxylin-Eosin (H-E), adrenomedullin and soluble endoglin immunostains [20].

Hematoxylin and Eosin Staining

Paraffin sections were first kept in xylene for 2x30 minutes. Then, they were kept in decreasing alcohol solutions for 10 minutes each and then washed with distilled water. Sections were placed in Harris hematoxylin solution for 8 minutes and then rinsed under tap water for 5 minutes. After tap water, the sections were left to rest in distilled water for a few minutes. For counterstaining, sections were immersed in eosin solution for 5 min. For the dehydration process, the sections were soaked in increasing alcohol solutions and finally kept in xylene for 2x45 minutes for polishing and sealed with entellan [21].

Immunohistochemical Staining

Tissue sections with a thickness of 5 µm were kept in xylol for 2x30 minutes and then kept in increasing alcohol series for 10 minutes respectively. After waiting for a while in distilled water, the sections were microwaved in EDTA solution at 90°C for 5 minutes for antigen retrieval and then cooled at room temperature for 30 minutes. After the tissue sections were dried in distilled water, they were taken into the immunohistochemistry box and the tissue locations were drawn with a hydrophobic pen. Phosphate Buffer Saline (PBS) was added to the sections for 3x5 minutes to ensure that the box remained moist. Then, PBS and hydrogen peroxide solution were added and left for 20 minutes. Soluble Endoglin primary antibody (cat# ab107595, Abcam, Cambridge, UK) and Adrenomedullin primary antibody (cat# ab69117, Abcam, Cambridge, UK) were pipetted onto the demarcated tissues and the sections were kept at +4°C overnight. The next day, the sections were washed in PBS for 3x5 minutes. After waiting for 14 minutes with biotinylated secondary antibody, it was washed again with PBS for 3x5 minutes. Streptavidin-prexoidase was dropped and left for 15 minutes, and then washed with PBS for 3x5 minutes. Staining was carried out for 10-15 minutes by dropping DAB on the sections, and then they were washed with PBS for 3x5 minutes. For counterstaining, the sections were stained with Mayer hematoxylin for 45 seconds and then washed in tap water for 5 minutes. Finally, the sections were passed through an increasing alcohol series, kept in xylol for 2x30 minutes and covered with entellan. The prepared preparations were examined using a Zeiss Imager A2 light microscope and Zen 3.00 software program [22].

Immunohistochemical and Statistical Analysis

After the tissue sections taken from paraffin blocks and stained with sEng and ADM were micrographed, the images were analyzed in Image J (University of Wisconsin, USA) software. A total of 10 randomly selected areas from the sections were selected for threshold analysis in the software, taking into account color contrast differences. The proportion of DAB positive areas on the total tissue stained with hematoxylin was determined and percentage values

were calculated. The obtained percentage data were evaluated with the normality test in SPSS 24 (IBM, USA) software and then statistically analyzed using the independent t test. Statistical significance was accepted as $p<0.05$.

RESULTS

Histopathological findings

Figure 1 shows Hematoxylin Eosin staining transverse sections of placentas belonging to the groups. In the Hematoxylin Eosin stained sections of the control group, it was observed that the placenta had a normal structure and the villi were organized in an orderly manner. In addition, vascularization, trophoblast cell organization and villus stromal area were found to be normal. In the placenta sections of the control group, structurally normal Hofbauer cells were observed in the regions near the villous capillaries (Figure 1A).

In the sections in the preeclampsia group, prominent hemorrhagic foci in the intervillous region, congestion and dilatation in the villus capillaries, and structural disorders of the villous stroma were observed. Hofbauer cells did not appear morphologically normal in preeclampsia sections. Compared to the control group, it was determined that the number of syncytial bridges and nodes increased, villous capillary lumens expanded, and there was dense cell infiltration in the connective tissue in preeclampsia sections. Additionally, in preeclampsia sections, it was observed that trophoblastic cells completely disappeared in some areas and there was a slight increase in the number of Hofbauer cells as well as morphological deteriorations (Figure 1B).

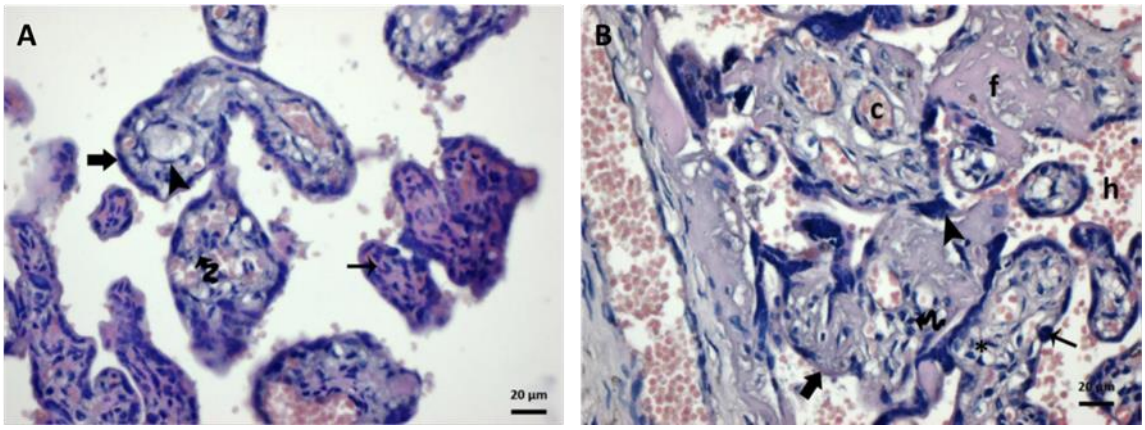

Figure 1: Hematoxylin Eosin staining of placental tissues. A) Control group; thick arrow: villi, arrowhead: capillary organization, thin arrow: stromal space, curved arrow: Hoffbauer cells. B) Preeclampsia group; arrow: syncytial nodes, arrowhead: syncytial bridges, thick arrow: loss of trophoblastic cells, curved arrow: increase in Hofbauer cells, c: congestion and dilatation in villous capillaries, h: dense hemorrhagic foci in the intervillous area, star: deterioration in stromal organization, f: hyalinizing fibrous areas, Bar: 20µm, Magnification: 40X

Figure 2 shows sEng immunoexpression of placental tissues. In the control group, sEng expression was observed in placental sections, concentrated throughout the stroma, syncytial bridges and trophoblastic cells. sEng positivity was also detected in villus capillary endothelium and Hofbauer cells (Figure 2A). In the preeclampsia group, sEng expression was again seen to be intense in stromal areas, syncytial bridges, cytotrophoblastic and syncytiotrophoblastic cells; A more intense staining was noted compared to the control group. Similar sEng expression to the control group was observed in villous capillary endothelium and Hofbauer cells (Figure 2B).

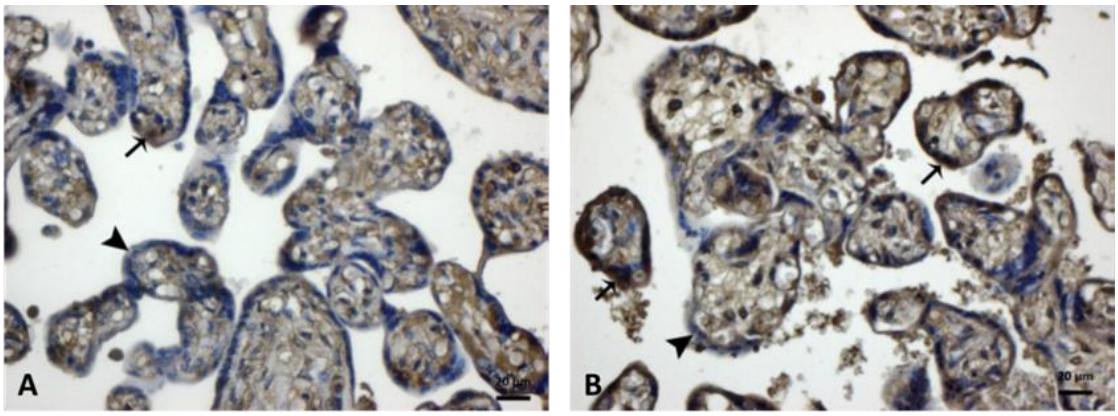

Figure 2: sEng immunoexpression of placental tissues. A) Control group; arrow: positive areas, arrowhead: negative areas. B) Preeclampsia group; arrow: areas where expression is intensely positive, arrowhead: negative areas. Bar: 20µm.

Figure 3 shows ADM immunoexpression of placental tissues. In control group placenta sections, ADM expression was observed in villous capillary endothelium and trophoblastic areas (Figure 3A). In the preeclampsia group, ADM expression was observed in villous capillary and trophoblastic areas, while weak expression was observed in Hofbauer cells and capillary endothelium. Although intense ADM expression was observed in cyto and syncytiotrophoblast cells in the preeclampsia group, it was noted that the expression in these cells was generally low (Figure 3B).

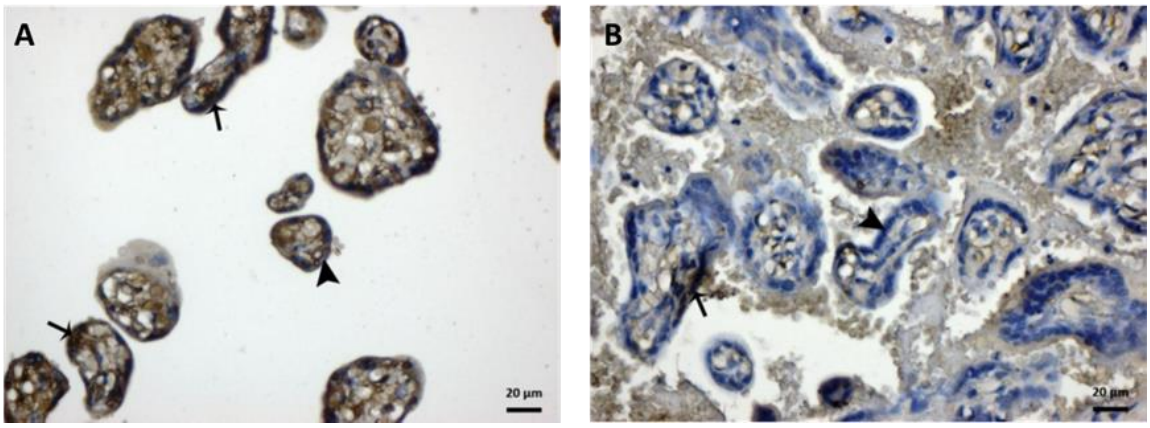

Figure 3: ADM immunoexpression of placental tissues. A) Control group; arrow: areas where expression is intensely positive, arrowhead: negative areas. B) Preeclampsia group; arrow: expression positive areas, arrowhead: negative areas. Bar: 20µm

Statistical findings

In our statistical analysis, it was determined that sEng expression was expressed in 34.83 ± 0.52% of the control group and 49.22 ± 0.69% of the preeclampsia group, and this difference was statistically significant as a result of the independent t-test (p = 0.000). In immunohistochemical sections, more intense ADM expression was observed in the stromal and capillary endothelial areas of placentas in the control group compared to the preeclampsia group. These observational findings were subjected to Image J analysis and the results were confirmed to be compatible with software analyses. In the quantitative evaluation, ADM expression was expressed in 51.72 ± 1.83% in the control group and in 22.08 ± 1.10% in the preeclampsia group, and it was determined that this difference showed a statistically significant decrease (p = 0.000) (Table 1).

Table 1. Expression percentages of sEng and ADM in control and preeclamptic placentas

sEng Expression Percentage (%)					
Groups	**Average**	**Minimum**	**Maximum**	**SE**	**p value**
Control	34,83	32,18	37,45	0,52	<0,01
Preeclampsia	49,22	45,60	52,13	0,69	
ADM Expression Percentage (%)					
Groups	**Average**	**Minimum**	**Maximum**	**SE**	**p value**
Control	51,72	45,47	63,43	1,83	<0,01
Preeclampsia	22,08	17,14	27,91	1,10	

DISCUSSION

Preeclampsia is a condition that occurs in 5-8% of pregnancies and carries a risk of serious morbidity and mortality for the mother and fetus. It is characterized by symptoms such as increased blood pressure, proteinuria and edema. The pathophysiology of preeclampsia involves multisystem defects such as abnormalities in the coagulation system, endothelial dysfunction, and vascular response disorders. Especially during pregnancy, hypertension stands out as one of the important causes of maternal and fetal mortality [23]. As stated by Gant et al. [24], many hemodynamic adaptations occur in the body during normal pregnancy. However, disruption of these adaptations can lead to problems such as preeclampsia and intrauterine growth restriction. It is suggested that vasoactive agents, especially factors such as adrenomedullin (ADM), have an important role in regulating fetoplacental circulation during pregnancy.

ADM is a growth-promoting, angiogenic and natriuretic peptide secreted by decidua and trophoblast cells. During pregnancy, ADM levels increase with placentation, but return to pre-pregnancy levels after birth. Changes in placental tissues play a role in the pathogenesis of conditions such as spontaneous abortion, gestational diabetes and preeclampsia. Yotsumoto and colleagues observed early death of fetuses due to placental insufficiency in mice with ADM gene defect [25]. In the study conducted by Di Lorio et al., it was observed that ADM levels in the blood of pregnant women were higher than in non-pregnant women [26]. ADM plays an important role during pregnancy thanks to its vasodilator properties, and changes in plasma levels are associated with pregnancy complications such as fetal growth restriction and preeclampsia. Li and his team showed that fetal growth and placental development were severely impaired in female mice with reduced ADM expression. In the same study, it was emphasized that ADM expression increased in fetal trophoblast cells and maternal uterine wall at the time of implantation and that this decrease had negative effects on reproduction [27].

Eng (CD105), the cell surface coreceptor of TGF-β1 and TGF-β3 isoforms, modulates the effects of TGF-β1 and TGF-β3 by showing high expression in endothelial cells and syncytiotrophoblasts. Shivalingappa and colleagues showed that sEng caused severe preeclampsia in pregnant rats when administered together with sFlt1 [28]. In their study, Levine et al. showed that sEng levels increased in the last two months of normal pregnancy, but in women who developed preeclampsia, these levels increased earlier and more significantly and reached their peak at the onset of clinical disease [29].

Eng (CD105) plays an important role in controlling vascular tone by regulating nitric oxide-dependent vasodilation and controls placental implantation and spiral artery remodeling. In the pathophysiology of preeclampsia, the role of Eng is based on systemic endothelial dysfunction and placental implantation disorders, as well as abnormalities in spiral artery remodeling. Many studies have been done on this subject. Nikuei and colleagues examined the dysregulation of Eng expression in preeclampsia patients and investigated the diagnostic accuracy of sEng. In their study, they found that Eng mRNA levels increased significantly in patients with severe and early-onset preeclampsia, and sEng levels increased significantly in all preeclampsia patients compared to the control group, reaching the highest levels in patients with early-onset and severe preeclampsia [30].

The imbalance between pro-angiogenic and anti-angiogenic factors plays an important role in the pathophysiology of preeclampsia. This imbalance may exist even before clinical symptoms appear. Complications such as placental ischemia and endothelial dysfunction, similar to preeclampsia, can also be associated with low birth weight in normotensive pregnancies [31, 32].

In our study, we evaluated the microscopic morphological differences between control and preeclamptic placenta samples and the expressions of anti-angiogenic sEng and pro-angiogenic ADM. In our histopathological examinations, we detected fibrous hyalinized structures, an increase in the number of syncytial nodes and bridges, capillary dilatation, intense congestion and degeneration in the preeclampsia group. Additionally, extensive hemorrhage was observed in the intervillous area. These findings are compatible with placental pathologies caused by preeclampsia by Huppertz et al. [33]. The increase in cytotrophoblast proliferation and syncytial node/bridge in preeclampsia can be explained by factors such as hypoxia and increased apoptosis [34].

In immunohistochemical analyses, we observed that sEng expression increased and ADM expression decreased in preeclamptic placentas. Yang Gu and colleagues reported that sEng increased in preeclamptic placentas [35]. In our study, we thought that sEng expression was especially concentrated in trophoblast cells in preeclamptic term placentas, and that these cells created an anti-angiogenic response by affecting vascular endothelium through paracrine signaling pathways. Additionally, a mechanism that potentiates the paracrine effect of trophoblasts has been demonstrated, consistent with findings that sEng inhibits TGF-β1 and inhibits nitric oxide synthase activity [28].

It is known that ADM is released into the system at increased levels in normotensive pregnancies and controls the fluid flow and homeostasis of the kidney and peripheral vascular structures. Studies have shown that ADM levels decrease in preeclampsia [36, 37]. In our study, we observed that ADM expression decreased in preeclamptic placentas. This decrease may lead to disruption of homeostasis due to disruption of a mechanism in preeclampsia and trigger the development of the disease.

We interpreted the relationship between ADM and sEng in two ways: Our first hypothesis is that a mechanism in the placenta shapes preeclampsia by influencing ADM expression. Our second hypothesis is that ADM and/or sEng try to balance excessive vascularization and maintain homeostasis through a negative feedback mechanism.

Creating hypotension by intravenous infusion of ADM is a mechanism related to nitric oxide production [17]. In preeclamptic placentas, sEng's blocking of nitric oxide synthase and the decrease in ADM expression also creates an anti-angiogenic response by reducing nitric oxide production. These findings suggest that anti-angiogenic and pro-angiogenic factors cooperate in preeclampsia, creating a mechanism to maintain homeostasis.

CONCLUSION

In conclusion, we found that sEng and ADM showed intense expression, especially in trophoblast cells, and that the effects of both factors on angiogenesis were important in preeclamptic placentas. We found findings that these two factors cooperate in the vascular changes associated with preeclampsia.

Acknowledgement

This study is a part of doctoral thesis of Beyza Karadede, from Dicle University, Department of Histology and Embryology.

Conflict of Interest: The authors declare no potential conflict of interest with respect to the contents, authorship, and/or publication of this article.

Author Contributions: All authors have equal contribution in the preparation of manuscript and compilation.

Source of Support: Nil

Funding: This study was funded by Dicle University Scientific Research Platform (DÜBAP) with project number: TIP.18.006

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Ethics approval: The ethical approval was taken from Dicle University Faculty of Medicine Ethics Committee (Date: 15/08/2018, Approval No: 59).

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