Increase in Endothelin-1 Expression in Umbilical Cord Arteries in Preeclampsia

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ABSTRACT

Objective: Endothelin1 (ET1) is 21- amino acid vasoconstrictor peptide secreted by endotheliumwhich has an important role in the pathoohysiology of preeclampsia (PE). The objective of this study was to evaluate the binding sites and quantitative changes in ET1 in umbilical cord vessels of PE patients.

Methods: This study recruited 40 pregnant women between 20-40 years old at 3rd trimester. All cases selected for this study underwent an elective cesarean section, grouped into 2 groups; PE group of 20 pregnant women (at 3rd trimester) who proved to have pregnancy induced hypertension and proteinuria. The control group was of 20 healthy pregnant females at the same average of gestational age and with the same exclusion criteria and no PE, underwent elective caesarean section. Umbilical cord tissues were taken from the maternal side, fixed with formalin, paraffin, embedded sections of umbilical cord were treated with Endothelin1 antibody. The immunoreactivity of ET1 was assessed using Aperio image scope software. Statistical analysis was done using SPSS program.

Results: The results demonstrated a significant increase (P = 0.001) of ET1 expression in cord vessels of PE group with respect to control group (mean 28.5±1.7, 2.6±0.4 respectively).

Conclusion: It is concluded that ET1 is markedly increase in PE and may be the cause behind promoted vascular smooth muscle cell contraction and blood pressure elevation in PE.

Keywords: Endothelin1; preeclampsia; pregnancy; umbilical cord (Siriraj Med J 2020; 72: 167-173)

INTRODUCTION

The umbilical cord is the fundamental connection between developing fetus and the placenta. It is made of three blood vessels; two small arteries, which carry the deoxygenated blood from the fetus to the placenta and a one large vein which carries nutrition-rich oxygenated blood to the fetus; this vein is unlike the regular veins in that it contains a layer of smooth muscles.[1] These blood vessels lie in an embryonic gelatinous connective tissue known as Wharton's jelly, all are enclosed in a layer of amnion.[2] The blood vessels of human umbilical cord are dissimilar from the main vessels of the same caliber in the body for many reasons; exudation of fluid take place in these vessels and participate to the formation of the

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amniotic fluid.[3] Umbilical vessels lack vasa vasorum, thus rely on their own oxygen supply, making them more vulnerable to modification in hemodynamic status.[4]

Hypertension is a commonly occurs during pregnancy. Preeclampsia (PE) is disorder that occurs in pregnancy that damages both the mother's circulation and the fetal growth. The risk factors for development of PE includes obesity, insulin resistance and hyperlipidemia all these conditions will lead to increase in the release of inflammatory mediators and rise the oxidative stress which will eventually lead to dysfunction of the endothelium.[5] PE occurs at 20 weeks gestation and onward, it is characterized by an elevation in blood pressure higher than "140/90 mm Hg" accompanied by significant proteinuria " ≥300 mg/dl in 24-hour urine collection".[6] PE affects many pregnancies and is still considered as a dangerous risk to mother and fetus.[7,8] PE is much higher in association with intrauterine growth retardation because there might be a chronic hypoxia which leads to contraction of the placental vascular bed which eventually increases the arterial resistance.[4,8]

Endothelins are family of 21-amino acid peptides encoded by 3 genes and produced in several tissues, mainly by endothelial cells lining blood vessels; they are group of vasoconstrictor peptides covering three isoforms, endothelin-1, endothelin-2, and endothelin-3. "ET1, ET2, and ET3" characterize by the presence of 2 intramolecular disulfide bonds.[9] ET1 is the main isoform made by vascular endothelium. Once formed, ET1 acts as a paracrine and autocrine mediator rather than an endocrine hormone, it has strong vasoactive performance and has been involved in the pathogenesis of a lot of vascular diseases like hypertension,[10] which can cause fibrosis of blood vessels and a state of inflammation due to increase in cytokine production.[11] When ET1 is released by the endothelium, it performs its action on ETA and ETB receptors of the neighboring endothelium or smooth muscle cells by paracrine or autocrine manner. The genes for ETA and ETB receptors had been cloned. ETA and ETB receptors on smooth muscle induce many cellular activities such as contraction, proliferation cell hypertrophy and apoptosis,[12] studies in animal models representative of PE, have shown that endothelin receptor blockers prevent the development of this disease.[13] Large quantity of Endothelin-1 was demonstrated in human umbilical vessels, amniotic membrane ,amniotic fluid and placenta.[14] This research aims to evaluate the binding sites for Endothelin-1 in the vessels of the umbilical cord and to quantify the differences in expression of Endothelin-1 in these vessels of women with in PE.

MATERIALS AND METHODS

Sample collection

The sample size was determined using Raosoft tool with the confidence level set at 95% and the margin of error is 10% with the resultant sample size required is 20 patients with preeclampsia.

The present study enrolled 40 pregnant women ageing between 20-40 years old at the third trimester who attended AL- Imamayn Al-Khademyiayn medical city hospital in Baghdad who underwent elective cesarean section. The choice of patients undergoing cesarean section was because we wanted to standardize the method of delivery and it is easier to attend to the patient and harvest the fresh placenta immediately upon delivery.

The exclusion criteria are patients with hypertension before pregnancy, diabetes mellitus, vascular diseases and smoking.

The Patients were grouped into two groups of 20. Group I "PE group" consisted of pregnant women (at third trimester) who had systolic blood pressure (BP) ≥140 mmHg, diastolic BP ≥90 mmHg and proteinuria of at least 1+ (≥300 mg/dl). Group II "control" consisted of normotensive pregnant women at the same average age (systolic BP <140 mmHg and diastolic BP <90 mmHg). Written consent was obtained from the patients after explaining the procedure to them. The present study was approved by the Head of the Postgraduate Committee, Department of Applied Embryology, High Institute of Infertility Diagnosis and ART, Al-Nahrain University.

Immunohistochemical staining with Endothelin-1 antibody (ET-1)

The placenta and umbilical cord was collected during the caesarean section procedure. Transvers pieces of the umbilical cords "one cm in thickness" was taken from the maternal side (area close to the placenta) of each patient, fixed in 10% buffered formal saline, dehydrated by ascending concentrations of ethanol, cleared in xylene, impregnated and embedded in paraffin wax. Paraffin sections of 4 µm thickness were placed on positively charged slides then the sections incubated overnight at room temperature. The tissue sections were de-paraffinized and rehydrated, blocked with peroxidase and serum blocking reagents, treated with ET-1was purchased from Abcam (a30536) Primary antibody was diluted in a serum block-to (1/100) µg/ml as determined by titration and was added in sufficient volume to cover the tissue.and incubated overnight with biotinylated secondary antibody and HRP-streptavidin complex respectively, then treated with DAB chromogen, stained by haematoxylin as counter stain, dehydrated

by series of ethanol, cleared in xylene and covered with cover slips. The immunoreactivity of ET1 was assessed quantitatively by Aperio Image Scope software. The computerized analysis of the immunohistochemical reactivity of ET1 using Aperio image scope program was done by choosing a determined area from each sample which had no spaces and then enter the picture to the program which read the negative, weak positive, positive and strong positive reaction in the cells. The positive reading included positive and strong positive cells we excluded the weak positive. The accuracy of the reading was ensured by repeating the reading three times and taking the mean.

Statistical analysis

The IBM Corp. SPSS Statistics for Windows, Version 23 Armonk, NY: IBM Corp. was used to analyze the data. All data in this study are presented as mean ± SEM. Data were analyzed by Mann-Whitney U test, the value of $p < 0.05$ was considered as statistically significant.

RESULTS

The umbilical arteries of the control group have constricted, folded shaped endothelium and their wall consist of pale staining layer of variable thickening in the center called 'tunica intima' which is surrounded by muscular layer called 'tunica media'. The medial layer is closely attached to the surrounding Wharton's jelly which is merged with tunica adventitia in umbilical vessels (Fig 1) while in PE group this layer is clearly separated from the jelly due to the presence of strong vasoconstriction which may lead to narrowing the lumen Fig 1.

In the control group, SMCs of arterial wall are fusiform shaped concentrically closely arranged with each other with elongated, large nuclei having wavy like appearance (Fig 2A). While in PE group, the muscle cells are irregularly arranged and the nuclei become small sized losing their longitudinal appearance. Irregular spaces appear between SMCs lead to accumulation of these cells in groups due to increase inter cellular fluid which is associated with edema. The presence of these spaces in the PE cords made it easier to distinguish between the muscle cells than it is in the control group (Fig 2B).

The immunoreactivity for ET1 appeared as small dark brown granules or deposits concomitant with the structural arrangement of the cord when visualized by DAB using the haematoxylin as a counter stain. The reaction mainly occurred in the cytoplasm of smooth muscle fibers and to a lesser extent in the endothelium lining blood vessels (Fig 3A). The strongest staining reactivity pattern was observed in the smooth muscle fibers seen as high intense brownish granules especially in the arteries of PE samples (Fig 3B).

The positivity of the vascular smooth muscle cells varied between strong positive and positive. In spite of the difference in the thickness of vascular smooth muscle layer in between PE group and control group, the number of cells that showed strong positive reaction to ET1 was significantly high (Fig 4A). In contrast, the control samples showed large number of weakly stained smooth muscle cells when treated with ET1 antibody, tunica adventitia showed no reactivity at all (Fig 4B). The expression percentage of ET- 1 was significantly increased in PE patients compared to the control group Fig 5.

DISCUSSION

Several theories have been suggested about the eventual cause of preeclampsia, it is clear that in PE there is an abnormal vascular remodeling.[15] It has been demonstrated that the remodeling of spiral arteries is not complete in these patients.[16] Reduction of utero-placental perfusion as a consequence of anomalous cytotrophoblast invasion of the spiral arterioles is a prompting episode leading to preeclampsia.[17] Ischemia/hypoxia in the placenta is believed to induce abnormal endothelial function leading to the release of vasoactive substances such as "nitric oxide, endothelin, and angiotensin II" that have intense effects on blood flow and arterial pressure regulation.[18,19] Due to the fact that human umbilical cord vessels have a special feature in being deficient of innervation, the action of the vasoactive substances seemed to be decisive in monitoring the tone of the umbilical vessels; several studies have demonstrated that the production of vasoactive substances, such as nitric oxide and ET1 are changed in PE in comparison with normotensive pregnancies.[20,21]

The choice of patients undergoing cesarean section was because we wanted to standardize the method of delivery and it is easier to attend to the patient and harvest the fresh placenta immediately upon delivery. We don't think that the mode of delivery would affect the rate of expression of ET1.

ET-1 is a strong vasoactive peptide, its concentration is increased in PE and plays a serious role in the pathophysiology of PE; there are many investigation on the role of ET1 in the stimulation of hypertension in PE. Indeed, ET1 was observed to prompt vasoconstriction via the ETA receptor, which had been shown to induce hypertension in PE.[22,23] The concentration of ET1 was found to be 3 times higher in the plasma umbilical cord than in

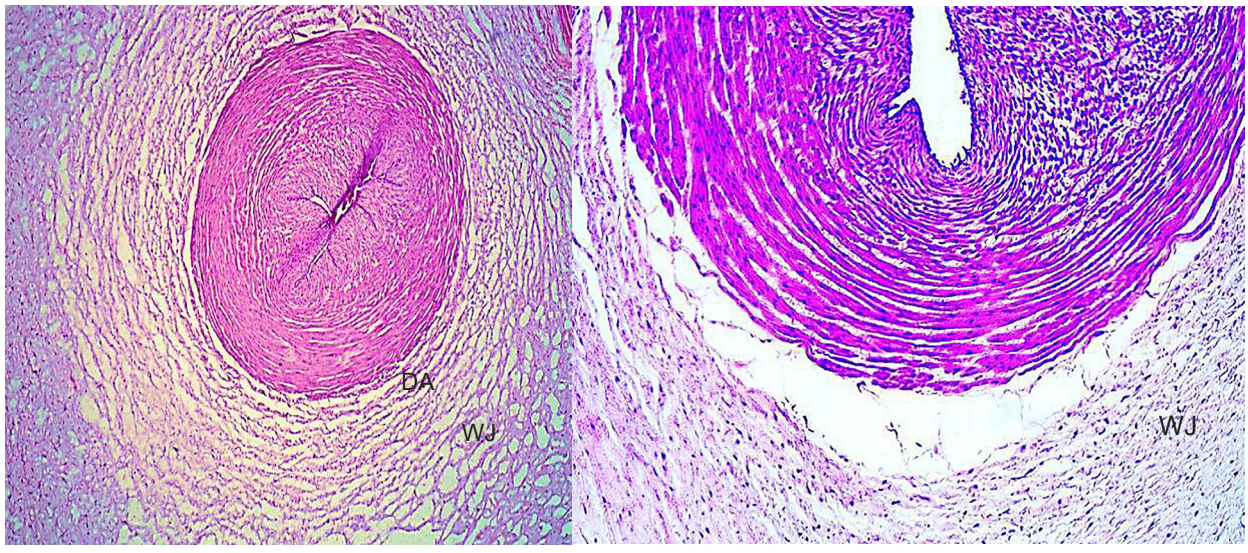

Fig 1. **(A)** Cross section of umbilical cord of control group shows the general appearance of the umbilical artery where its tunica adventitia (AD) merged with Wharton's jelly (WJ) control group, H&E stain, X40. **(B)** Cord in PE group show the separation of the medial layer of umbilical artery from the surrounding Wharton's jelly, H&E, X100.

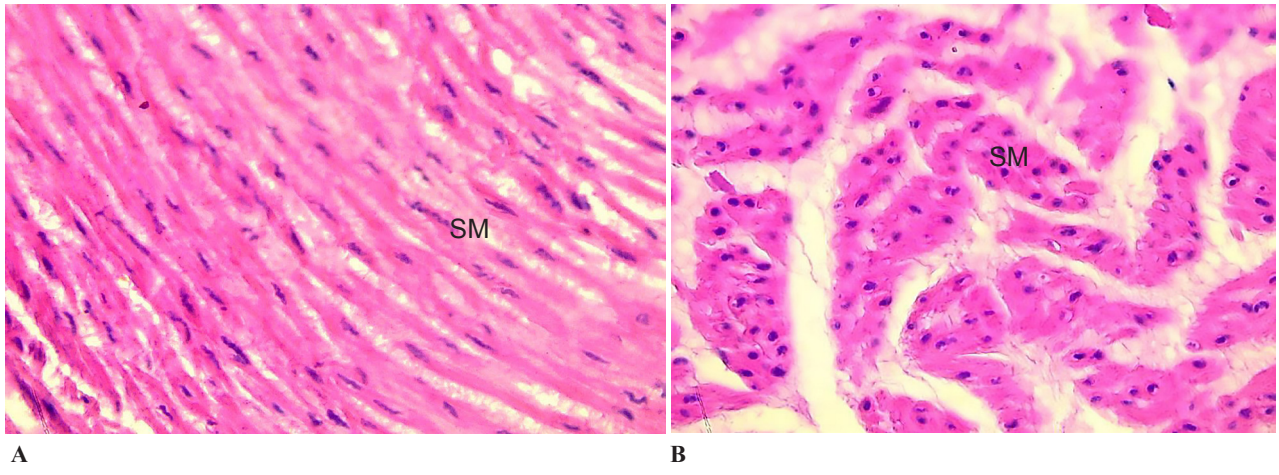

Fig 2. **(A)** Cross section of the umbilical artery shows the normal concentrically arranged smooth muscle (SM) in arterial wall of control group. **(B)** Irregularity of SM with an increase in the inter-cellular spaces between these cells in PE group. H&E stain, X400

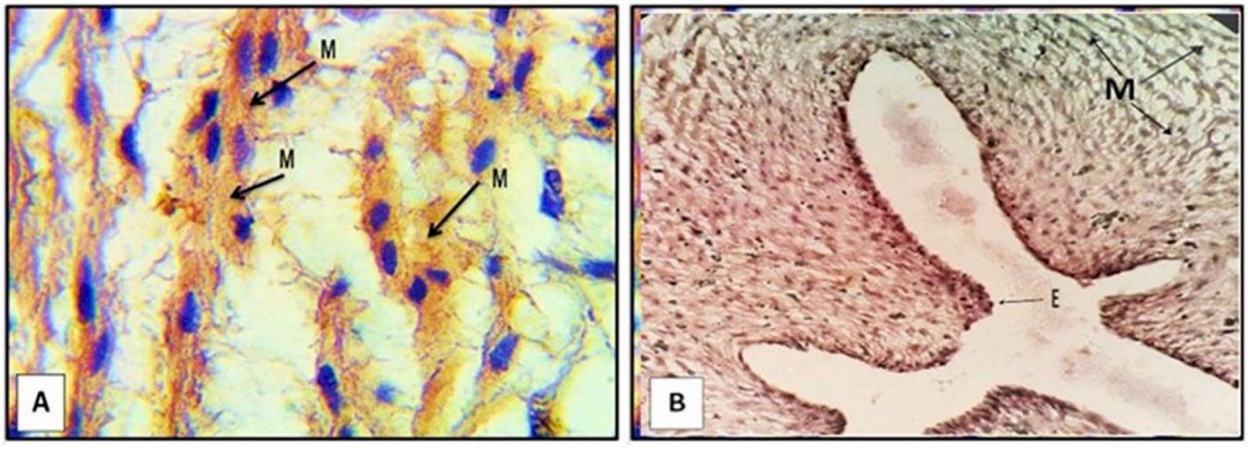

Fig 3. Cross section in umbilical artery with immunohistochemical staining with endothelin 1 antibody showing the positive expression of endothelin 1 in the cytoplasm of smooth muscle (M) and in endothelial cells (E). Endothelin1 Ab, PE group, magnification **(A)** x100, **(B)** x 40.

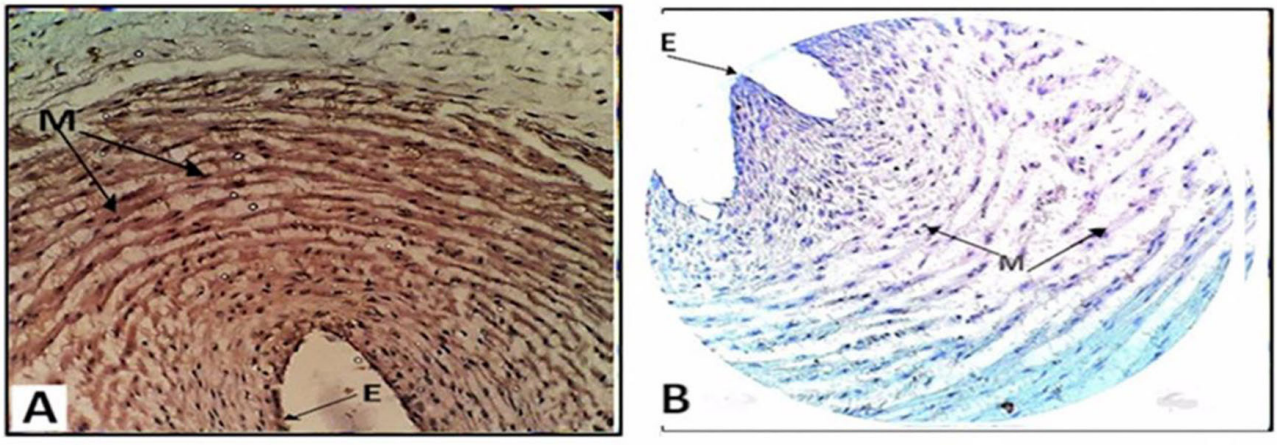

Fig 4. Cross section of umbilical artery showing intense brown pigmentation of ET1 immune-localization in the endothelial cells (E) and the smooth muscle (M) of the PE group **(A)** and the very weak reaction to the anti endothelin1 of the control group **(B)** endothelin1 Ab, magnification X400.

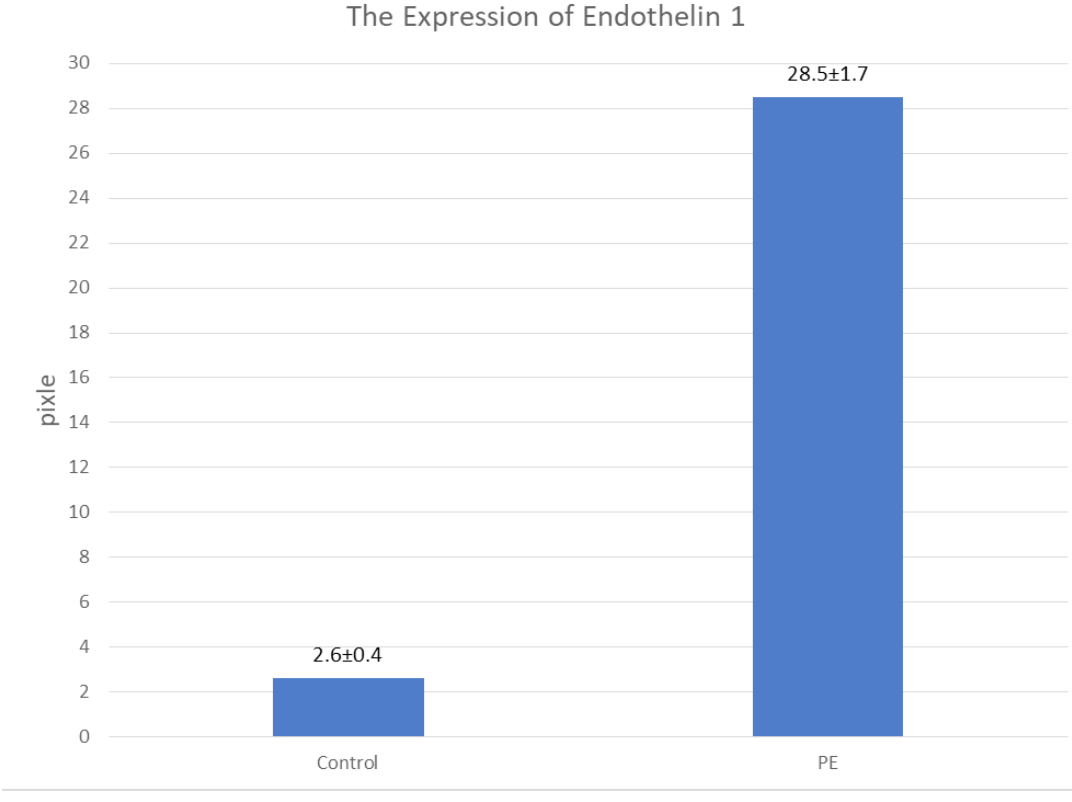

Fig 5. The expression of endothelin 1 in preeclampsia group with respect to control group presented as mean±SEM, p-value <0.05.

the plasma of maternal side, and was related to the influence of low pO2 in fetal blood.[24] In spontaneous labor, the concentration of ET1 in the umbilical cord and retroplacental blood plasma was ten times higher than those in the maternal peripheral blood suggesting that an elevation of the intrauterine secretion of endothelin-1 at delivery may stimulate the constriction of the blood vessels in the umbilical cord and placental bed.[25]

Endothelin 1 can also have a prolonged effect on blood pressure regulation The plasma level of ET1 can have substantial long-term effects on circulation and arterial pressure regulation. Thus, oversecretion of ET1 might have a significant role in mediating renal failure and hypertension observed in women with PE.[26] The present investigation might be the first study that demonstrated ET1 in umbilical vessels revealing that the strongest

staining pattern was detected in muscle & endothelial cells particularly in the arteries of PE samples. In PE the dysfunction of endothelial cells is responsible for the different presentations of PE like hypertension and proteinuria which results in a disturbance in the balance between substances that cause vascular dilatation and constriction.[27] Locally, ET1 a potent vasoconstrictor is produced by endothelial cells, increases smooth muscle contractility.[28] It is known that endothelin plays a significant role in the development of PE during pregnancy.[29] therefor, ET1 may stimulated a contractile response in arteries with damaged endothelium, and the severity of the damage in PE might potentiate the effect of ET1.[30] A previous study on pregnancies with intra uterine growth retardation revealed that ET-1 was localized diffusely in placental specimens from normal and IUGR pregnancies. The localization of ET-1 immunoreactivity was much higher in the endothelium of capillaries of villi as well as in the cells of the basal plate in the placenta of normal pregnancy than pregnancies with IUGR.[31]

ET-1 plays a significant role in regulating blood vessel function in all organ systems, ET1 elicited a presser response in vascular smooth muscle cells chiefly mediated by ETA receptors, and a depressor response chiefly mediated by nitric oxide released from endothelial cells through ETB receptors.[32] The present study has demonstrated a strong positivity of the vascular smooth muscle cells in the arterial wall which can lead to the dysregulation of vascular function leading to vascular constriction. Clinically, ET-1 has been implicated for the deterioration of renal function through loss of nephrin as Studies with an endothelin-1 (ET-1) receptor antagonist indicated that ET-1 was the main factor affecting loss of nephrin.glomerular endothelium was found to produce ET-1 when incubated with serum from PE patient, and recombinant ET-1 triggered nephrin shedding from podocytes.[33] which lead to the renal manifestation of preeclampsia which are characterized by proteinurea and hypertension.

ET-1 inhibits cell proliferation and vitality and triggers oxidative stress in the human placenta by altering the balance between oxidants and antioxidants forces in favor of oxidation[34] that's why we can clearly see changes in histological appearance of the umbilical cord of PE patients where the smooth muscle layer looses its uniform shape and shows cellular swelling which is a sign of cell injury. Therefore, according to this study, further research can be conducted on the benefit of blocking the ET-1 receptor in preventing the progression of preeclampsia to eclampsiawhich can save many mothers at risk from developing this disorder that carries high mortality rate.

The main limitation of this study is to get standardized immunohistochemical staining of the samples obtained so we need to observe the correct staining site in the tissue and ignore false positive random staining.and also to be able to preserve the tissue well to allow good preservation of the antigens as it is well known that a good immunohistochemical stain requires fresh tissue samples and the older the tissue the more loss of antigens.

CONCLUSION

It is concluded that ET1 is markedly increase in PE and may be the cause behind promoted vascular smooth muscle cell contraction and blood pressure elevation in PE.

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Conflict of interest statement

All the authors have contributed in this research, we have no conflict of interest to declare.

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