Original Article

Histological Study of Human Placenta in Preeclampsia and the Role of Decidual Natural Killer Cells and Macrophages in its Pathogenesis

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ABSTRACT

Background: Preeclampsia (PE) is a hazardous pregnancy condition, if untreated can result in serious complications to the mother and the fetus.

Aim: Investigating the histological changes of human placenta in PE and the involvement of natural killer (NK) cells and macrophages in its pathogenesis.

Materials and Methods: Forty pregnant women were included; 20 were normal clinically and considered the control (group I) and the other 20 were included in PE (group II). Blood samples were taken just before delivery to assess serum interleukins (IL)-10 and -12 and transforming growth factor (TGF)-β1. Placental sections were stained with H&E, Mallory's trichrome and immunohistochemically with Bcl-2, vascular endothelial growth factor (VEGF), CD56 and CD68. Then morphometric and statistical studies were done.

Results: Group II (PE) showed significantly decreased IL-10 and significantly increased IL-12 and TGF-β1 compared to group I. It also presented different histological alterations in placental sections mainly in the chorionic plates and villi. There was significant increase in area percent of collagen fibers and VEGF, and in the number of syncytial knots, CD56 positive decidual NK cells and CD68 positive decidual macrophages. But there was significant decrease in the area percent of Bcl-2 immunoexpression.

Conclusion: Preeclampsia is associated with marked inflammatory and immune responses, and decidual NK cells and macrophages are highly imperative cells in its pathogenesis.

Key Words: Decidual macrophages, decidual natural killer cells, placenta; preeclampsia.

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INTRODUCTION

Supporting the fetal life, its development and wellbeing are provided by the materno-fetal unit; umbilical cord and placenta[1]. Altered placental morphology and histology in pregnancies complicated by preeclampsia (PE) or eclampsia results in placental impaired function, which accounts for different fetal and neonatal complications[2]. Preeclampsia is a serious clinical disorder affecting about 3-8% of pregnant females after 20 weeks of gestation and is a principal reason of fetal and maternal morbidity worldwide; accounting for about 15% of premature births and 14% of pregnancy-linked maternal deaths[3].

The major pathological feature of PE is defective placentation. Trophoblasts in early placentation invade the maternal uterine spiral arteries and convert them into vessels with large diameters and low resistance to blood flow. In PE, this is impaired and the exact cause is still not clear, however, genetic variations, defective trophoblast differentiation or immunological factors are most likely involved[4,5]. Defective placentation is accompanied with augmented vascular resistance, increased platelet aggregation, coagulative system activation, in addition to endothelial malfunction and altered angiogenesis[6,7]. Abnormal uterine spiral arteries reduce the uteroplacental perfusion leading to local placental hypoxia. Hypoxia causes oxidative stress of the syncytiotrophoblasts (STB), inflammation, necrosis, apoptosis and structural damage. Also it endorses the release of vasoactive and pro-inflammatory mediators from the placenta into the maternal circulation, causing the clinical syndrome[3,4].

Natural killer (NK) cells are innate immune cells known by their great cytolytic capability against tumor-transformed and virus-infected cells[8]. They constitute the main immune cell in the decidua throughout the first trimester of pregnancy accounting for about 70% of local lymphocytes. Decidual NK (dNK) cells contribute directly to the beginning of spiral arteries remodeling through the secretion of several cytokines, angiogenic factors and enzymes, also they indirectly affect uterine vessels remodeling through altering the growth and differentiation of extra villous trophoblasts (EVT), plus their migration and

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invasion[3]. CD56 is a well-known marker to characterize and distinguish NK cells from other immune cells like monocytes or T cells[9].

Two types of macrophages are present within the human placenta; Von Hoffbauer cells in the fetal villi, plus decidual macrophages in maternal decidua basalis. Decidual macrophages are the second plentiful leucocytes in human decidua constituting about 20% to 30% of the decidual cells. They are imperative participants in feto-maternal immune fine-tuning by granting a sufficient microenvironment to support cellular growth and hinder risky inflammatory reaction. Abnormal behavior of these macrophages influences the placental development and trophoblast function, leading to a diverse of unfavorable pregnancy outcomes including PE[10]. CD68 marker is the most frequently used one for the identification of macrophages[11].

Consequently, this study aimed at detecting the histological changes in human placentas from pregnancies complicated by preeclampsia and revealing the possible pathology of ischemic damage to placental tissue, in addition to investigating the pivotal role of NK cells and macrophages in the pathogenesis of PE.

MATERIALS AND METHODS

Selection of cases

Forty pregnant women were included from the Obstetrics and Gynaecology Department, Cairo University Hospitals; 20 were normal clinically and considered the control group, whereas the other 20 were included in PE group. Written informed consents were obtained from all participants and the following exclusion criteria were fulfilled[7,12]

- Mother age less than 20 or more than 35 years.
- Multiple gestations.
- Duration of pregnancy less than 30 weeks.
- History of smoking or alcohol intake.
- Intake of any medications.
- Diabetes mellitus or gestational diabetes.
- Intrahepatic cholestasis of pregnancy.
- Major fetal anomalies.
- Co-existing or preconceptional maternal conditions like polycystic ovary syndrome, thrombophilic conditions, autoimmune disease, renal, thyroid, cardiovascular or liver diseases.

Grouping

- Group I (control group): 20 healthy primigravidae, normoglycemic, normotensive with no detected abnormality on routine clinical assessment. In the third trimester, they were admitted to Cairo University Hospital delivery unit for vaginal delivery or caesarian section.

- Group II: (PE group): 20 patients selected in accordance with the "International Society for the Study of Hypertension in Pregnancy". PE was marked out as de novo hypertension after week 20 of gestation (diastolic blood pressure ≥90 mmHg and/or systolic ≥140 mmHg) along with the presence of one or more of these new-onset conditions: proteinuria (300 mg or more protein in 24h urine sample), fetal growth restriction (uteroplacental dysfunction) or other maternal condition including liver involvement, renal insufficiency, hematological or neurological complications[7].

Biochemical study

When women arrived to the delivery unit, venous blood samples were taken and centrifuged to obtain sera that were stored at -20 0C until analysis to detect inflammatory cytokines. Interleukins (IL)-10 and -12 (Mabtech, Stockholm, Sweden) and transforming growth factor (TGF)-β1 (R&D Systems, MN, USA) were measured using sandwich ELISA as described in the user manual[12, 13, 14].

Histological Study

Placental biopsies were taken instantly after delivery nearly 3 cm from the cord insertion; halfway between the basal and chorionic plates to be away from peripheral areas of tears or calcification. Specimens were fixed in formalin then embedded in paraffin. Serial sections of 5μm thickness were subjected to:

- Hematoxylin & eosin (H&E)[15].
- Mallory's trichrome stain[15]
- Immunohistochemical staining[16] using:

1) Bcl-2 rabbit monoclonal antibody (ab182858; Abcam, USA). It has cytoplasmic localization.

2) Vascular endothelial growth factor (VEGF) mouse monoclonal antibody (ab1316; Abcam, USA). It shows cytoplasmic localization.

3) CD56 recombinant human antibody (NCL-L-CD56-504; Leica Biosystems Newcastle Ltd, UK). It is detected on the membranes of NK cells.

4) CD68 mouse monoclonal CD68 antibody (NCL-L-CD68; Leica Biosystems Newcastle Ltd, UK). It is localized in the cytoplasm and cell membranes of macrophages.

Then sections were counterstained with Mayer's hematoxylin.

Morphometric studies

This was done by means of image analyzer computer system (Leica Qwin 500, UK). Using an objective lens x10, the following parameters were measured in 10 non-overlapping fields in 10 randomly chosen sections:

- Percentage of syncytial knots (SN); their number relative to the number of villi.
- Area percent of collagen fibers.
- Area percent of Bcl-2 immunoreaction.
- Area percent of VEGF immunoreaction.
- Number of CD56 positive cells.
- Number of CD68 positive cells.

Statistical Analysis:

This was accomplished for biochemical and morphometric results via SPSS package version 22 (SPSS Inc., Chicago, USA). Comparisons were done using ANOVA (analysis of variance) followed by post hoc Tukey test. Results were noted as mean and standard deviation and regarded as statistically significant when $p<0.05$[17].

RESULTS

Biochemical Results

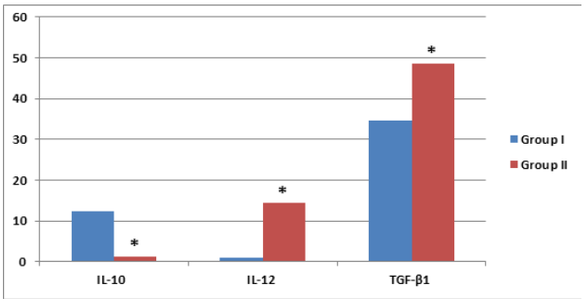
The results are demonstrated in (Table 1) and Histogram 1.

Table 1: Serum levels of inflammatory markers (mean ± SD) for the control and preeclampsia groups in pg/ml

Groups	IL-10	IL-12	TGF-β1
Group I	12.41±3.63	0.94 ±0.46	34.52 ±2.61
Group II	1.13 ±0.77*	14.43 ±1.97*	48.50±1.54*

* Significant compared to group I ($P < 0.05$)

Histogram 1: Serum levels of inflammatory markers in pg/ml for the control and PE groups.



* Significant compared to group I ($P < 0.05$)

Histological results

H & E results

Placental sections from group I (the control) showed the chorionic plate (fetal side); the chorionic mesoderm containing fibroblasts and blood vessels and lined by STB at the surface facing the maternal blood. The amniotic mesoderm containing mesenchymal cells and covered by amniotic epithelium was separated from the chorion by chorionic cavity (Fig. 1a). The basal plate (maternal side) in contact with uterine wall was made up of anchoring villi and decidua basalis. The decidua basalis displayed enlarged glycogen-containing decidual cells. Rohr's fibrinoid could be detected on the side of the basal plate facing the intervillous space (Fig. 1b). Different types of tertiary villi were present; stem villi the largest with condensed fibrous stroma having central artery and vein, mature intermediate villi had loose stroma with numerous capillaries, terminal villi had the smallest diameter (Fig. 1c). Each villous was lined by STB and few cytotrophoblasts and had a core of mesenchymal connective tissue containing fetal capillaries lined by flat endothelium. The villi were separated from each other by intervillous space filled with maternal blood. Syncytial knots (SN), a feature of mature placenta, were seen as clusters of STB nuclei. Thin vasculosyncytial membrane; where fetal capillaries in terminal villi face a very thin layer of STB, was seen (Fig. 1d).

Group II (PE group) revealed diverse placental affection. Changes mainly appeared in the chorionic plates and villi, whilst, the decidua did not show significant change. The chorionic plate was covered by thickened Langhan's layer of fibrinoid with loss of STB layer (Fig. 2a). Crowded villi with decreased intervillous spaces and increased fibrinoid deposition (homogeneous acidophilic deposits) were seen (Fig. 2b), in addition to hemorrhage in the intervillous space and decreased fetal blood capillaries within the villi (Fig. 2c). Numerous SN and distorted villi were also detected, with increased fibrinoid deposits within and around the villi. Hyalinization of fetal blood capillaries was evident; appearing as homogeneous acidophilic material

(Fig. 2; d and e). Increased thickness of vasculosyncytial membrane and walls of fetal blood vessels due to fibrinoid deposition was seen (Fig. 2; e and f).

Mallory's trichrome results

Group I presented numerous collagen fibers within the stroma of stem villi around fetal blood vessels (Fig. 3a) and very minimal collagen fibers within terminal villi (Fig. 3b). In group II, dense excessive collagen fibers were detected within stem mature intermediate villi and terminal villi (Fig. 3; c and d).

Bcl-2 Immunostained results

Group I showed widespread immunoreaction for Bcl-2 which appeared as brown cytoplasmic reaction in most of STB (Fig. 4a). On the other hand, placentas from group II exhibited Bcl-2 immunoreactivity in some STB cells (Fig. 4b).

VEGF immunostained results

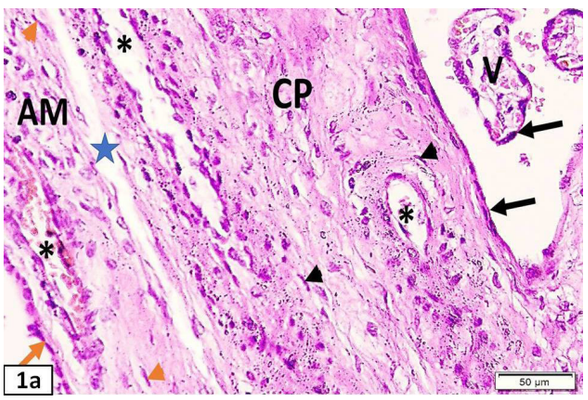
Sections from group I revealed brown cytoplasmic immunoreactivity for VEGF in the endothelial cells lining fetal blood vessels, plus some STB cells (Fig. 5a). Group II showed little or even absent reaction in endothelial cells of fetal vessels especially the hyalinized capillaries, but there was widespread VEGF immunoreaction in most of STB cells and some villous stromal cells (Fig. 5b).

CD56 immunostained results

Very few number of CD56 positive dNK were detected in group I. Positive cells with different sizes were identified by the brownish precipitation that was clearly membranous and to lesser extent cytoplasmic (Fig. 6a). Whereas sections from group II showed increased number of CD56 positive dNK (Fig. 6b).

CD68 immunostained results

Placentas from group I disclosed brown cytoplasmic CD68 positive reaction in the fetal (Von Hoffbauer cells) and decidual macrophages (Fig. 7; a and b). Immunostained sections from group II displayed CD68 positive fetal macrophages as in group I (Fig. 7c), however, the number of CD68 positive decidual macrophages was apparently increased (Fig. 7d).



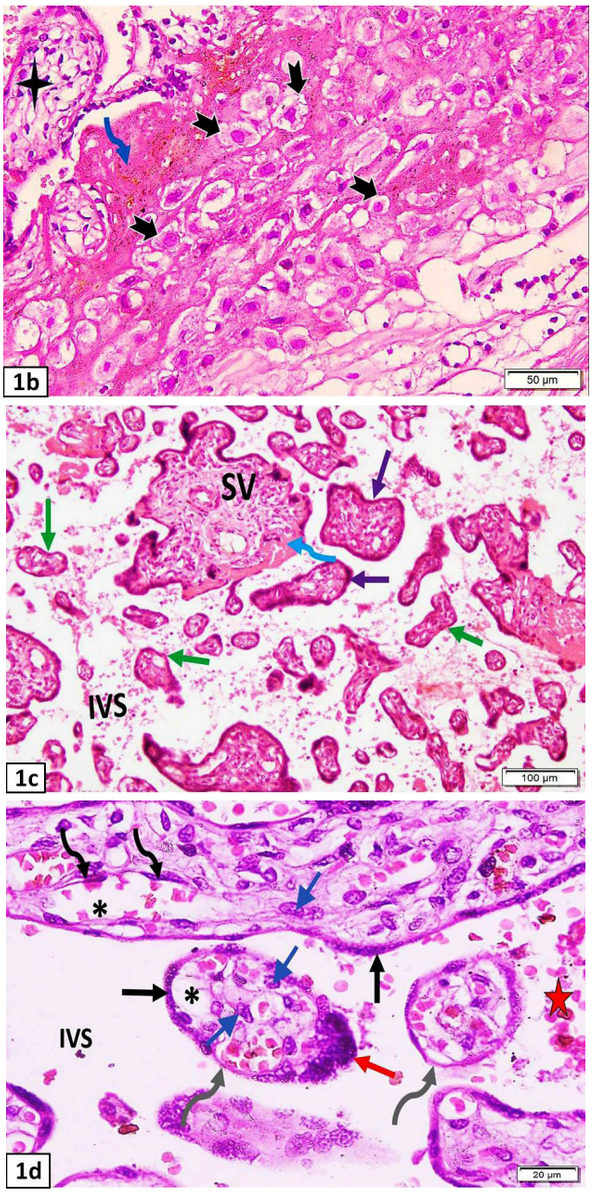
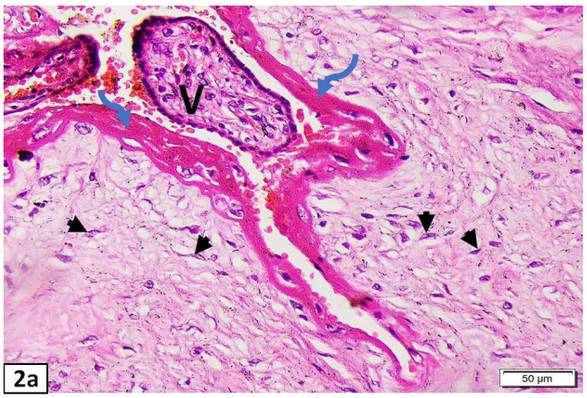
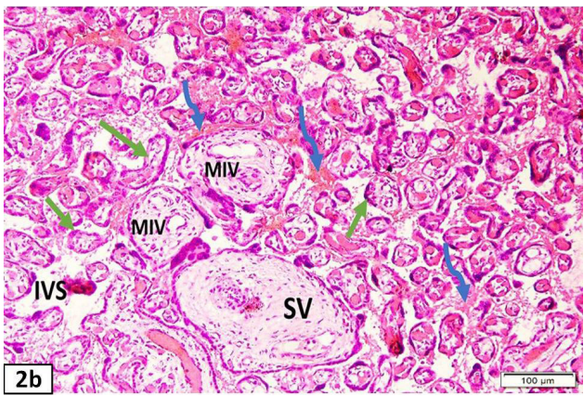
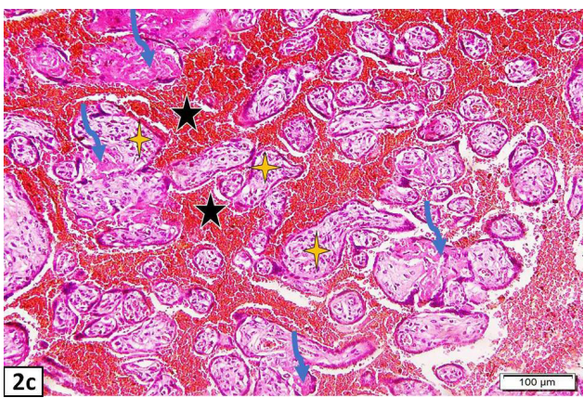
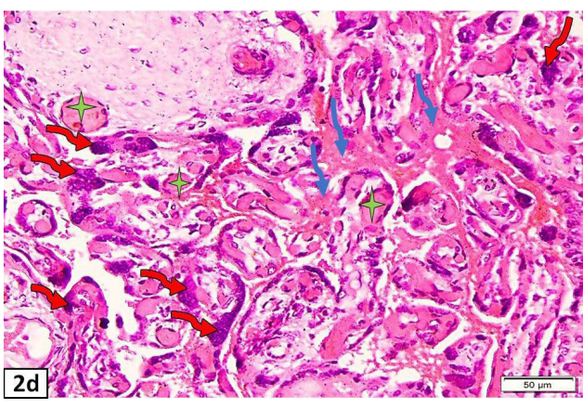


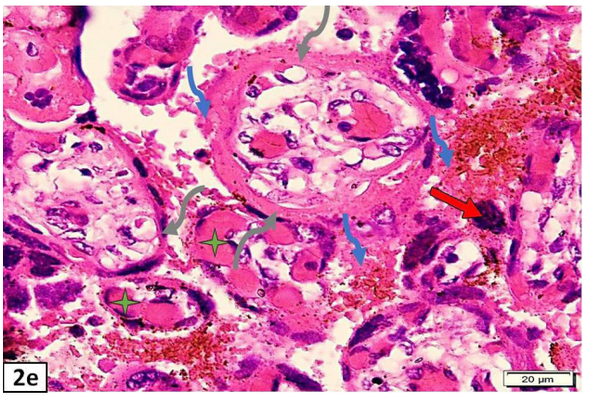
Fig. 1: Photomicrographs of placental sections from group I (control) stained with H&E. a) Chorionic plate (CP) at the fetal side faces chorionic villi (V) and consists of extraembryonic mesoderm containing fibroblasts (black arrowheads) and blood vessels (black asterisk) and covered by STB (black arrows). Also the amnion (AM), amniotic epithelium (orange arrow) and mesenchymal cells (orange arrow heads) in its stroma are seen. Chorionic cavity (blue star) is present between CP and AM (X200). b) Basal plate of placenta at the maternal side reveals decidual cells (black bifid arrows) within decidua basalis, anchoring villi (black stars) and Rohr's fibrinoid (curved blue arrows) (X200). c) Different types of villi with intervillous space (IVS) are seen; stem villi (SV), mature intermediate villi (purple arrows), and terminal villi (green arrows). Fbrinoid deposition in the periphery of stem villous (blue curved arrow) is detected (X100). d) Shows syncytial knots (red arrow), STB (black arrows), remnants of cytotrophoblasts (blue arrows) and fetal blood capillaries (black asterisk) lined by flat endothelial cells (black curved arrows). Intervillous space (IVS) containing maternal blood (red star) and vasculosyncytial membrane are seen (gray curved arrows) (X400).











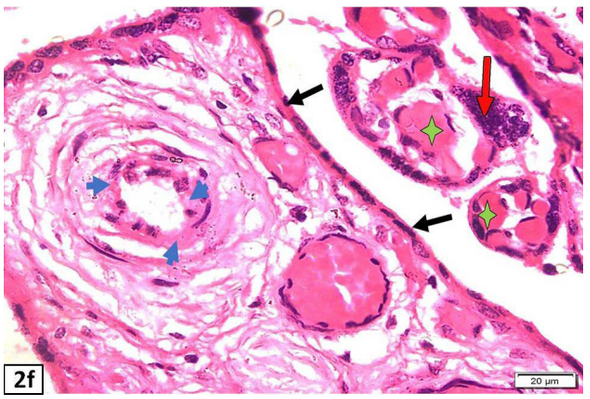


Fig. 2: Photomicrographs of placental sections from group II (PE) stained with H&E. a) Chorionic mesoderm enclosing fibroblasts (black arrows head) is covered by thick Langhan's layer of fibrinoid (blue curved arrow) towards chorionic villi (V) (X200). b) Different types of villi are seen; stem villous (SV), mature intermediate villi (MIV) and terminal villi (Green arrows). They are overcrowded with minimal intervillous space (IVS) and fibrinoid deposition (blue curved arrows) (X100). c) Hemorrhage in intervillous space (black stars) is seen, together with decreased fetal capillaries within the villi (yellow stars) and fibrinoid deposition (blue curved arrows) (X100). d) Reveals overcrowded distorted villi, syncytial knots (red arrows), fibrinoid deposition (blue curved arrows) and hyalinization of fetal blood capillaries (green stars) (X200). e) Syncytial knots (red arrows) and increased thickness of vasculosyncytial membrane (grey curved arrows) are demonstrated, in addition to hyalinized fetal capillaries (green stars) and increased fibrin deposition (blue curved arrows) (X400). f) Fibrinoid deposition is seen in the wall of fetal blood vessel (blue arrows head) and in STB layer (black arrow). Hyalinized fetal capillaries (green stars) and a syncytial knot (red arrow) are detected (X400).

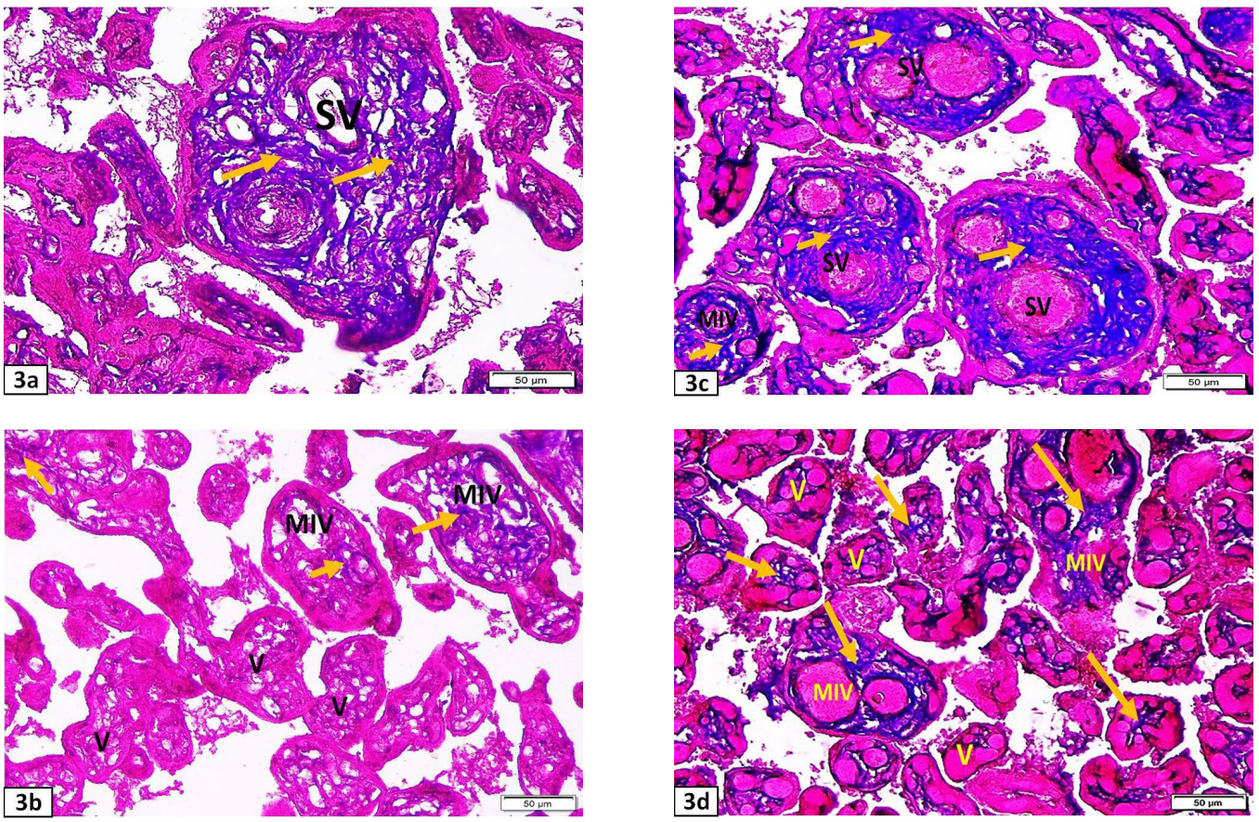


Fig. 3: Photomicrographs of placental sections stained with Mallory's trichrome stain (X200). a and b) Group I: collagen fibers (yellow arrows) are numerous in the stroma of stem villous (SV), delicate in the stroma of mature intermediate villi (MIV) and are not detected in terminal villi (V). c and d) Group II: excessive dense collagen fibers (yellow arrows) are detected in stem villi (SV), mature intermediate villi (MIV) and terminal villi (green curved arrow).

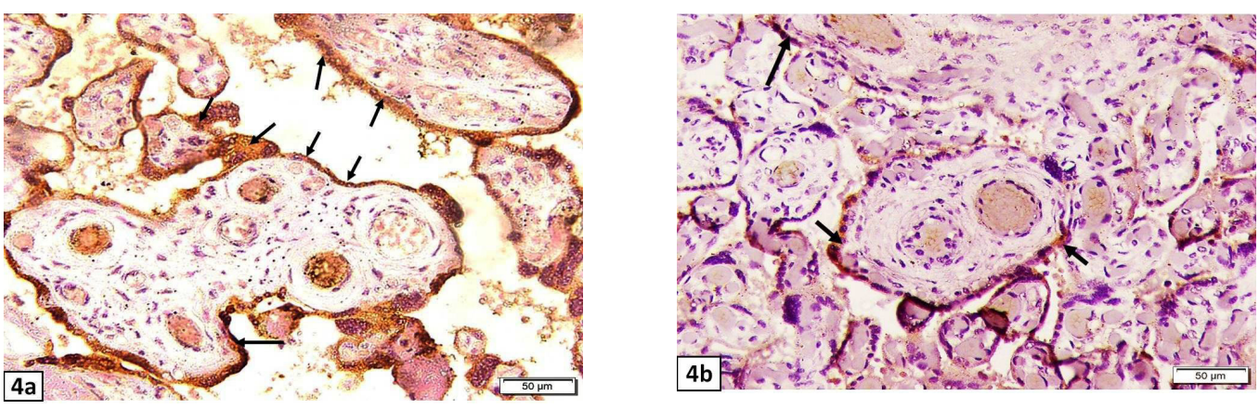


Fig. 4: Photomicrographs of placental sections stained immunohistochemically with Bcl-2 (X200). a) Group I displayed widespread brown cytoplasmic immunoreaction for Bcl-2 in most of STB (arrows). b) Group II shows Bcl-2 immunoreactivity in some STB (arrows).

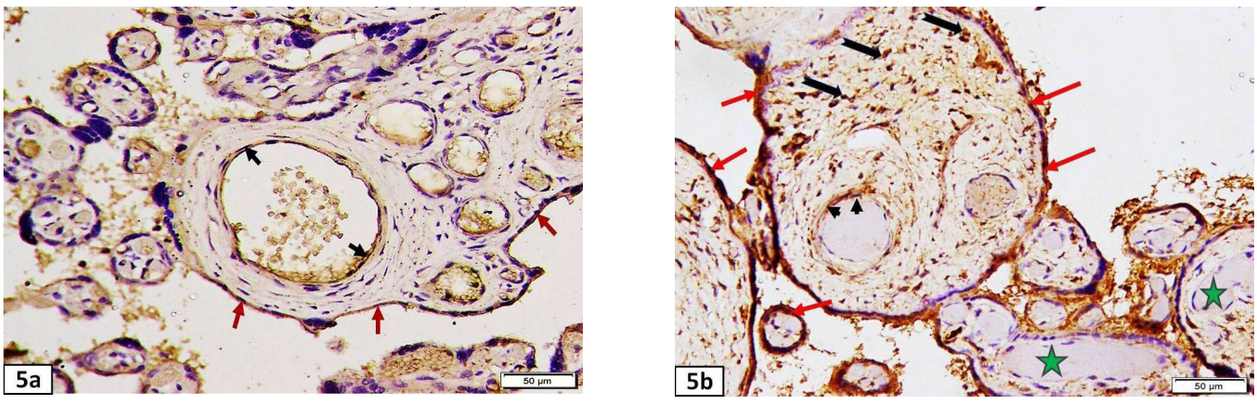


Fig. 5: Photomicrographs of placental sections stained immunohistochemically with VEGF (X200). a) Group I reveals positive cytoplasmic reaction in some STB (red arrows) and in the endothelial lining of fetal blood vessels (black arrows). b) Group II shows widespread VEGF immunoreaction in STB (red arrows) and in some villous stromal cells (black bifid arrows). Blood vessels show little (arrowheads) or negative reaction in the hyalinized ones (green stars).

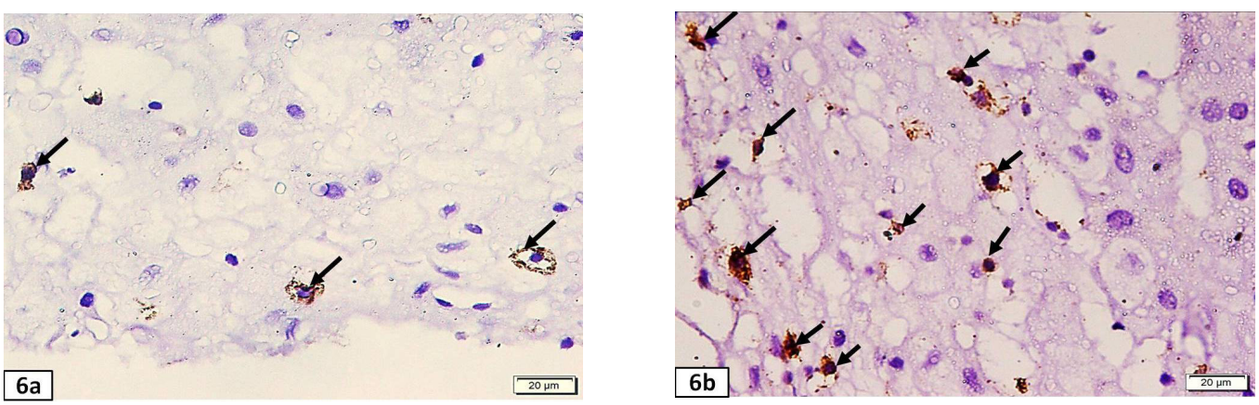
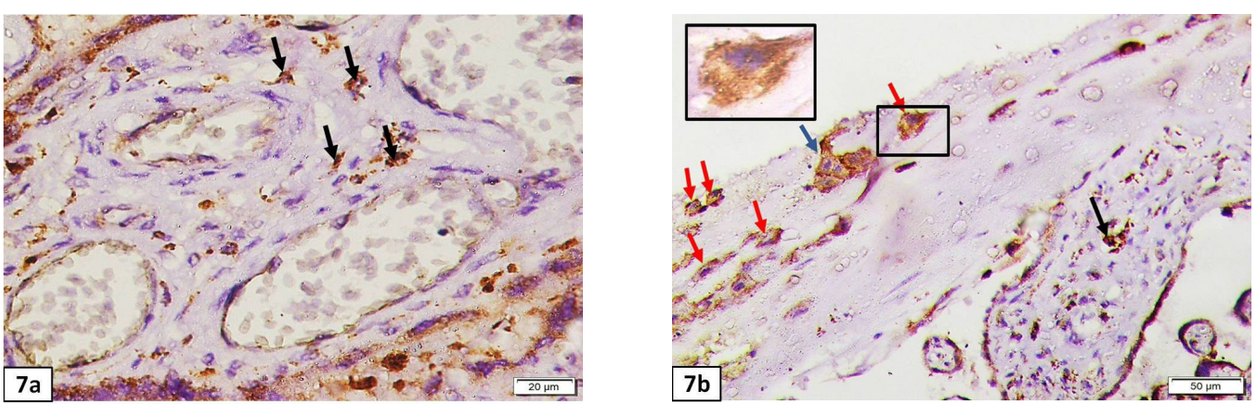


Fig. 6: Photomicrographs of placental sections stained immunohistochemically with CD56 (X400). a) Group I shows few CD56+ve dNK cells (Black arrows). b) Group II exhibits numerous CD56+ve dNK cells (Black arrows).



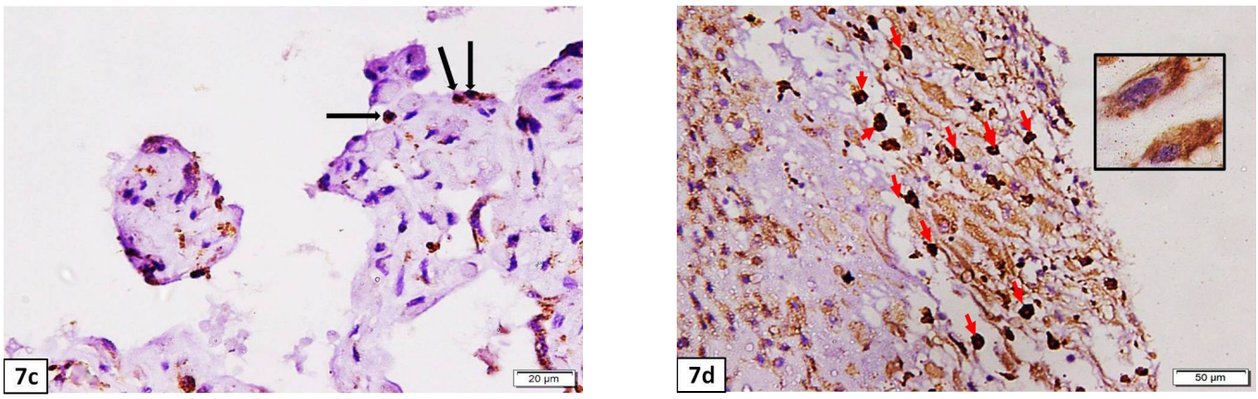


Fig. 7: Photomicrographs of placental sections stained immunohistochemically with CD68. a) Group I shows some CD68+ve fetal macrophages (black arrows) within the stroma of a chorionic villous (X400). b) Group I exhibits some CD68+ve decidual macrophages (red arrows), giant multinucleated CD68+ve macrophage (blue arrow) and CD68+ve fetal macrophages (black arrow) (X200). The inset is higher magnification for a CD68+ve decidual macrophage (X400). c) Group II presents some CD68+ve fetal macrophage (black arrows) within the villous stroma and among STB (X400). d) Group II shows numerous decidual CD68+ve macrophages (red arrows) (X200). The inset is higher magnification for CD68+ve decidual macrophages (X400).

Morphometric results

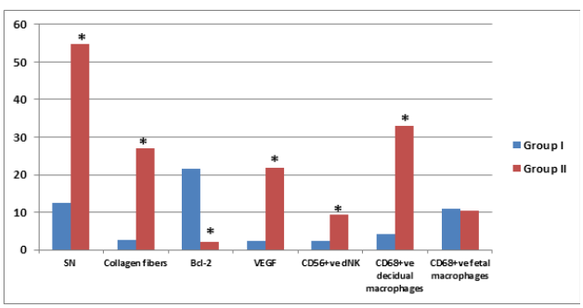
These are illustrated in Table 2 and Histogram 2.

Table 2: Morphometric results for the control and PE groups.

Measurements	Group I	Group II
Percentage of SN	12.39±4	54.7±9.1 *
Area% of collagen fibers	2.74±1.33	26.95±4.33 *
Area% of Bcl-2	21.7±2.65	2.19±0.79 *
Area% of VEGF	2.5±0.57	21.9±3.4 *
Number of CD56+ve dNK	2.5±1.57	9.4±2.2 *
Number of CD68+ve decidual macrophages	4.2±2.2	33.1±6.2 *
Number of CD68+ve fetal macrophages	11±2.9	10.4±3.5

* Significant compared to group I ($P < 0.05$).

Histogram 2: Morphometric results for the control and PE groups.



* Significant compared to group I ($P < 0.05$).

DISCUSSION

In the present study, H&E stained sections from group II (PE group) showed chorionic plate with lost syncytiotrophoblast (STB) layer and thickened layer of fibrinoid. Also increased fibrin deposition was detected within and around the villi and in the intervillous space, which coincides with other studies[2,18]. Replacement of the villus by fibrin results in fibrinoid necrosis of placental villi that leads to their distortion with STB degeneration[2]. In addition, interruption of nutrient and gas exchange between fetal villous circulation and maternal sinusoidal circulation by fibrin intervenes with perfusion and nutrient/ gas exchange in the intervillous space. Intervillous fibrin can be the consequence of a repair mechanism for the disrupted trophoblast lining with re-epithelization of the damaged STB[18]. From the factors sharing in reducing materials exchange across the placenta is the increased thickness of vasculosyncytial membrane by increased fibrinoid deposition that was also detected in this study and goes in line with Jaiman *et al*.[19]. Furthermore, fibrinoid deposition was present in the walls of fetal blood vessels replacing the vessel muscular wall by dense fibrinoid necrosis and this was described by other researchers[2,18,20,21], and with losing smooth muscles from the arterial walls, blood flow cannot be directly controlled by the growing fetus or the mother[20]. Copious fibrinoid deposit in the fetal vessels walls was the earliest hint towards a probable immunological origin for pathogenesis of PE; as fibrinoid necrosis has been found in vessels from rejected kidney transplants and it is also a usual feature of autoimmune vasculitis, leading scientists to consider immunological etiology for PE[21].

Increased fibrinoid formation might be due to increased resistance to activated protein C; where micro lesions of the STB make the mesenchyma in contact with maternal blood

resulting in distorted function of the haemostatic factors interacting with activated protein C in the placenta[22]. Additionally, it might be attributed to increased activity of plasminogen activator inhibitor type 1; the major inhibitor of fibrinolytic system[23]. Also, it has been proposed that hypoxic villi or trophoblast damage could give rise to abnormal activation of coagulation in the intervillous space[19].

Signs of accelerated maturation; in form of overcrowded villi and increased number of syncytial knots (SN), were noticeable in sections from PE group. This was supported morphometrically by significant increase in the percentage of SN relative to the villi, and is in harmony with former studies[23,24,25]. Accelerated villous maturation is proposed to be a compensatory mechanism to fetal hypoxia; where defective remodeling of spiral arteries leads to placental malperfusion, endoplasmic reticulum and oxidative stress with decreased surface area for nutrient and gas exchange[19].

Sections from PE group revealed intervillous hemorrhage that could be attributable to retroplacental hemorrhage resulting from separation of placenta (placental abruption) because of high blood pressure. Besides, hypertension provokes placental ischemia by vasoconstriction that decreases the oxygen tension in blood, leading to disorganized muscular media of blood vessels. Moreover, severe elevation in blood pressure can directly damage the vessels causing severe necrotic damage with consequent hemorrhage in intervillous space[26].

Hyalinization of fetal blood vessels together with hyalinization of the villi themselves was observed in the sections from PE group, which goes in line with Ojha *et al*.[2] who defined hyalinized villous as being the hypovascular one. This results from fetal vascular malperfusion; initially ischemia causes necrotic damage and karyorrhexis of fetal endothelium and red corpuscles producing disruption of vessel wall and discharging necrotic cellular fragments inside the villous stroma that goes through degenerative changes itself. This is known as "villous stromal-vascular karyorrhexis". After that, the villi rapidly transform to hyalinized avascular villi once all the vessels disappear and the stroma turns out to be uniformly collagenized[27]. Furthermore, hyalinization might be due to an immunological reaction within the villous tissue; since it resembles the amyloid deposition present in immunological disturbance, so some researchers have stated that hyalinization results from immune attack against trophoblastic tissue[28].

Apoptosis has been illustrated in human placenta; as being crucial for trophoblast invasion, survival and differentiation. Nevertheless, increases apoptosis has been reported in complicated pregnancies such as diabetes, PE and fetal growth restriction. There are several pro- and anti-apoptotic molecules associated with apoptosis. One of the most significant apoptosis regulators is the anti-apoptotic Bcl-2, which is localized in STB cytoplasm to maintain syncytial integrity in normal pregnancy[29,30]. This finding was evident in this study; where sections from PE group demonstrated a significant decrease in the mean area percent of Bcl-2 immunoreaction compared to the control and this is in agreement with earlier studies[29,30].

Besides its well known role in angiogenesis, VEGF plays a vital role during pregnancy; promoting trophoblasts proliferation, maternal spiral artery remodeling, as well as embryonic vasculature development[31]. It is produced by decidualized endometrial cells and trophoblasts and acts as a chemoattractant for macrophages and monocytes into hypoxic and inflammatory tissues[32]. Herein, sections from PE group revealed widespread VEGF immunoreactivity in STB and some villous stromal cells with significant increase in its mean area percent compared to the control. This is consistent with former researchers who ascribed this to fetal hypoxia, which is a potent stimulant to VEGF production[25,31]. During early placental development, villous stromal cells differentiate into pericytes and endothelial cells, so they express VEGF as contributors in the process of angiogenesis. Therefore, they express more VEGF in PE in response to hypoxia as a compensatory mechanism to enhance angiogenesis[33]. Nevertheless, sections from PE group displayed little or absent reaction in the endothelial cells of fetal blood vessels especially the hyalinized capillaries. Defective angiogenesis could be explained by two possible mechanisms at the receptor level; down-regulation of membrane-bound VEGF receptor-1 in placentas, or overproduction of a competitive soluble form of this receptor suppressing the VEGF effects[34].

Even though fetal extra villous trophoblasts (EVT) invading the maternal decidua express paternal semialloantigens, the maternal immune system must sustain immune tolerance towards them. Differentially dispersed immune cells, including regulatory T cells (Treg), NK cells, macrophages and dendritic cells are vital for sufficient placental growth and their dysregulation may explain the development of pregnancy complications as PE, premature birth or fetal growth restriction[35].

Decidual NK cells (dNK) display many functional and phenotypic differences compared to peripheral blood NK cells. They decrease significantly throughout the course of pregnancy, but still present in both decidua basalis and parietalis. Nevertheless, gene and protein expression profiles recognized several variations between first trimester and term pregnancy dNK. During the first trimester, they have limited cytotoxicity due to failure to polarize their cytotoxic granules to synapse with EVT[36]. EVT do not express HLA-A and HLA-B molecules, but express HLA-C, HLA-E and HLA-G. The HLA-G molecule is a key contributor to maternal-fetal immune tolerance by

limiting NK cells cytotoxicity[35]. Furthermore, cytokines and growth factors released by dNK are not produced in response to HLA-G+ EVT[36].

The current work demonstrated significant increase in number of CD56 positive dNK in PE group compared to the control, which is in agreement with other researchers[3]. There are inconsistent reports regarding the number of dNK cells in PE; either increase or decrease, which is by itself, may not have great role in PE pathogenesis. The functional activity of dNK cells depends chiefly on the balance between activating and inhibiting their killer immunoglobulin receptors (KIR) by the corresponding HLA ligands of EVT. Therefore, PE pathogenesis maybe multifactorial; involving dNK receptors, maternal HLA typing and cytokines present at the placental area[37].

A chief immunosuppressive and anti-inflammatory cytokine is IL-10 that promotes successful placentation, regulates vascular function, and controls inflammation[13]. Its decreased serum level has been reported in women with PE[3,13,38] and this is in harmony with the current study that showed significantly decreased IL-10 in PE group compared to the control. IL-10 modifies maternal reaction to paternal antigens and promotes tolerance to fetal allograft by provoking HLA-G expression and preventing lysis by maternal NK cells[13]. Consequently, low level of IL-10 results in reduced HLA-G and increased dNK cytolytic enzymes and proinflammatory cytokines as IL-12[3], which was also found in this work; where PE group revealed significant increase in serum IL-12 compared to the control group.

Inflammation is a prominent feature of PE and involves cells of innate and adaptive immunity; where women with PE recorded an increase in monocyte-lymphocyte ratio plus absolute monocyte count[39]. Tissue macrophages are divided into pro-inflammatory (M1) or anti-inflammatory (M2) that differ in their markers; as M1 express (CD80 and CD86) and M2 express (CD206, CD209, and CD163)[40]. It was found that M1 counts in PE patients were significantly increased due to elevated levels of pro-inflammatory cytokines[41] and also due to blocked transition to M2 by the reduced level of anti-inflammatory IL-4 and IL-10[40]. The previous data was evident in the present work; as there was significant increase in the number of CD68 immunopositive decidual macrophages in PE group compared to the control, with the appearance of giant multinucleated macrophages and this is in agreement with earlier studies[10,11]. However, group II presented none significant decrease in the number of CD68 immunopositive fetal macrophages (Von Hoffbauer cells) compared to the control, which is in accordance with Yang *et al*.[42] who explained this by the fact that Hofbauer cells present in the fetal chorionic villi proved to be M2 macrophages.

Increased number of M1 and decreased number of M2 macrophages further contribute to the elevated level of IL-12 and to the reduced level of IL-10; as they are secreted by them sequentially[14,42]. Furthermore, increased decidual macrophages leads to increased TGF-β1[10,37], which is also established in this work; as it was significantly elevated in PE group compared to the control. TGF-β1 is an anti-proliferative agent that hinders the ability of trophoblast to invade and remodel spiral arteries[10]. Moreover, it was stated that necrotic trophoblasts, in PE, expelled to maternal circulation are phagocytosed by capillary endothelial cells that are activated to secrete TGF-β1[12]. Additionally, TGF-β1 signaling has been involved in placental fibrosis in PE; it has been accompanied by over production of extra cellular matrix including fibronectin and collagen as the most eminent mechanism of fibroblast activation and fibrosis in the stroma of placental villi in PE is the activity of TGF-β1 attributable to ischemia and hypoxia[43]. This finding is confirmed by the significant increase in mean area% of collagen fibers within the villous stroma and around fetal blood vessels in PE group compared to the control.

CONCLUSION

Preeclampsia is an ischemic placental disorder associated with excessive immune and inflammatory responses. Decidual NK cells and macrophages are extremely crucial in its pathogenesis. Therefore, they could be possible predictors for PE; in addition, therapeutic agents that can modulate the immune system might hold great promise in its early detection and prevention.

CONFLICT OF INTEREST

There are no conflicts of interest.

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الملخص العربى

دراسة هستولوجيه للمشيمة البشرية في تسمم الحمل ودور الخلايا الساقطة القاتلة الطبيعية والخلايا البلعمية فى تطوره

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الخلفية: تسمم الحمل (PE) هي حالة حمل خطرة ، إذا لم يتم علاجها يمكن أن يؤدي إلى مضاعفات خطيرة للأم والجنين.

الهدف: فحص التغيرات الهستولوجية للمشيمة البشرية فى تسمم الحمل PE)) ومشاركة الخلايا القاتلة الطبيعية (NK) والخلايا البلعمية في تطور المرض.

الأدوات والطرق: تم تضمين أربعين امرأة حامل، ٢٠ كانت طبيعية سريريًا واعتبرت المجموعة الضابطة (المجموعة الأولى) وتم تضمين العشرين الأخرى في PE (المجموعة الثانية). تم أخذ عينات الدم قبل الولادة مباشرةً لتقييم إنترلوكينات المصل (١٠- IL و -١٢) وعامل النمو المحول بيتا ١ (TGF-β١). تم صباغة العينات المشيمية بـالهيماتوكسيلين والإيوسين و مالورى ثلاثي الألوان و صبغات هستوكيميائية مناعية ضد بي سي إل-٢ ، عامل نمو بطانة الأوعية الدموية (VEGF)، سى دي ٥٦ وسي دى ٦٨. ثم تم إجراء الدراسات القياسية المترية والإحصائية.

النتائج: أظهرت المجموعة الثانية (PE) انخفاضًا ذو دلالة احصائية في IL-١٠ وزيادة ذات دلالة احصائية في IL-١٢ و TGF-β١ مقارنة بالمجموعة الضابطة. كما أظهرت أيضاً تغييرات نسيجية مختلفة في قطاعات المشيمة بشكل رئيسي في الألواح المشيمية والزغابات. كانت هناك زيادة ذات دلالة احصائية في النسبة المئوية لمساحة ألياف الكولاجين و VEGF ، وفي عدد العقد المخلوية ، والخلايا القاتلة الطبيعية الساقطة الإيجابية ل CD٥٦ والخلايا والخلايا البلعمية الساقطة الإيجابية ل CD٦٨. ولكن كان هناك انخفاض ذو دلالة احصائية في النسبة المئوية لمساحة التعبير المناعى ل بي سي إل-٢.

الخلاصه: يرتبط تسمم الحمل باستجابات التهابية ومناعية ملحوظة والخلايا الساقطة القاتلة الطبيعية والبلعمية هي خلايا ضرورية للغاية في تطوره.