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Study of histopathological changes and the levels of TNF-α in Preterm Preeclamptic women

Alaa Saadi Abbood[*1] , Anwar M. Lazm[2] , Ahmed F. Hasan[1]

[1] *Department of Biology, Al-Farabi university college.,* [2] *Department of Nursing, Al-Farabi university college, Baghdad, Iraq*

[*] Corresponding author e-mail: alaas@alfarabiuc.edu.iq

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Abstract

Preeclampsia (PE) is a significant contributor to maternal morbidity and mortality that manifests as new-onset hypertension after 20 weeks of pregnancy with signs of uteroplacental or maternal organ dysfunction or proteinuria. This condition is linked to intrauterine growth restriction, preterm birth, placental abruption, fetal distress, and fetal death in utero. The findings of the recent study can be summed up as follows: Hematoxylin and eosin staining of placenta tissues revealed a significant area of calcification in the villous stroma. Additionally, increased collagen fibers around blood vessels and varying degrees of mononuclear cell infiltration are caused by capillary and vein congestion. The Masson's Trichrome stain revealed deposited fibrin between decidual cells and extra-villous cytotrophoblasts, which were surrounded by mature intermediate and terminal villi. Congestion of veins with increased fibrin deposits around veins and arterial vessels with increased fibrocytes When the data was statistically analyzed, it was discovered that PE pregnant women had significantly $p<0.05$ higher levels of serum TNF- α , than normotensive women (NT). Finally, the study found a link between preterm PE and women's pregnancy, as well as histopathologic and immunological parameters in preterm PE and NT women.

Key words : Preeclampsia, preterm birth, Trichrome stain, TNF- α .

1. Introduction

Preeclampsia (PE) affects approximately 5% of pregnant women worldwide, accounting for 25% of perinatal and maternal mortality (Moussa *et al*., 2014) . In addition to perinatal death, PE is linked to Preterm Birth (PTB) and fetal growth restriction, both of which have long-term neurological and cardiometabolic consequences in adults (Guid *et al.*, 2018). The primary and worst possible effect of PE, which usually occurs at or around 20 weeks of gestation, is maternal and neonatal morbidity, and PE has been proven to be the most significant in causing it (Brown *et al* .,2018). It quickly progresses to a high blood pressure, protein, and protein-containing urine. Binding a mother or child's umbilical cord increases the risk of both maternal and child morbidity and death-based disability for years (Fisher, 2015). Inadequate trophoblast invasion results in incomplete remodeling of the uterine spiral arteries, which causes PE. As a result, the placenta experiences ischemia, hypoxia, and oxidative stress. Placental hypoxia triggers a biochemical cascade of angiogenic/antiangiogenic factors, leading to endothelial cell dysfunction and the associated maternal PE signs and symptoms (Verlohren, 2014).

The cause of PTB is still unknown. Numerous recent studies have found a link between immune changes during pregnancy and delivery timing, but few of these studies

have been conducted in low-income countries, where PTB rates are highest (Black and Horowitz , 2018). It has been discovered that TNF- α overexpression and secretion in the placenta go hand in hand with elevated IL-1 in the maternal plasma. Endothelial structural and functional changes such as oxidative stress, complement activation, leukocyte infiltration, and high thrombin levels are all promoted by IL-1 and TNF- α. All of these findings involve the pathological processes that lead to PE (Du *et al* ., 2017). As a result, TNF- α plays a role in many of the local and systemic changes associated with PE. TNF- α (lymphotoxin) increases leptin levels, according to PE research. Surprisingly, the PE microarray gene expression analysis revealed that one of the most upregulated transcripts was found in the placenta (Gray *et al*., 2018) . The current study focuses on some histological and immunological changes in preeclampsia women.

2. Material and Methods

From Al-Yarmouk Teaching Hospital and Al-Elweyia Governmental Teaching Hospital, 45 pregnant women were chosen to represent the study's samples. Two groups of women were formed, with the first group (25 samples) represent preeclamptic women and preterm birth with ages ranging from 19 to 46, while the second group (20 samples) represents pregnant women with normotensive (NT) whose ages range from 20 to 41. Serum were used for sandwich ELISA to measure TNF- α and placental tissues(Tissue samples were prepared for histopathological technique include (Fixation, Dehydration , Clearing, Embedding, staining) were gathered between December 2019 and June 2020.

3. Results and discussion

3.1 Gravidity and Delivery mode

Regarding gravidity, there was no significant difference between the two group subjects. The delivery mode, however, differed significantly with the majority of PE patients (96%) undergoing cesarian section while most of the NT (60%) had normal vaginal deliver as shown in Table-1.

Table 1 : Comparison of gravidity and delivery mode between PE patients and NT.

		PE	NT	P-value	
Gravidity	Primigravida	28%	30%	0.906	N.S
	Multigravida	72%	70%		
Delivery mode	Vaginal delivery	4%	60%	0.000	***
	Cesarian section	96%	40%		

Results are expressed as a percentage, N.S :Non significant , *** :Very highly significant, P<0.001

3.2 Histopathological study

The Histological study using H & E stain in placental tissue of NT pregnancy after delivery Figure 1-A shows normal placental villi and intervillous spaces also show syncytial knots ,The villous stroma (VS) shows blood vessels . The histopathological changes in the PE preterm placenta of pregnant women showed the following changes like Large area of calcification in the villous stroma Figure 1-B and also Congestion of capillaries and veins. Staining with trichrome gives identical results of fibrin deposition. Figure 1-C showed non-deposits fibrin in microvilli in placental tissue of NT pregnancy and the villous stroma is composed of connective tissue core and shows vein vessels and intervillous spaces. The histopathological changes in placental tissues showed the following changes such Deposited fibrin between decidual cells (DC) and extra-villous cytotrophoblast (EC) surrounded by some mature intermediate and terminal villi Figure 2-A and Congestion of veins with increased deposits of fibrin around veins and arterial (A) vessels with increased fibrocytes Figure 2-B also Excess amounts of fibrinoid deposits within and around villi Figure 2-C.

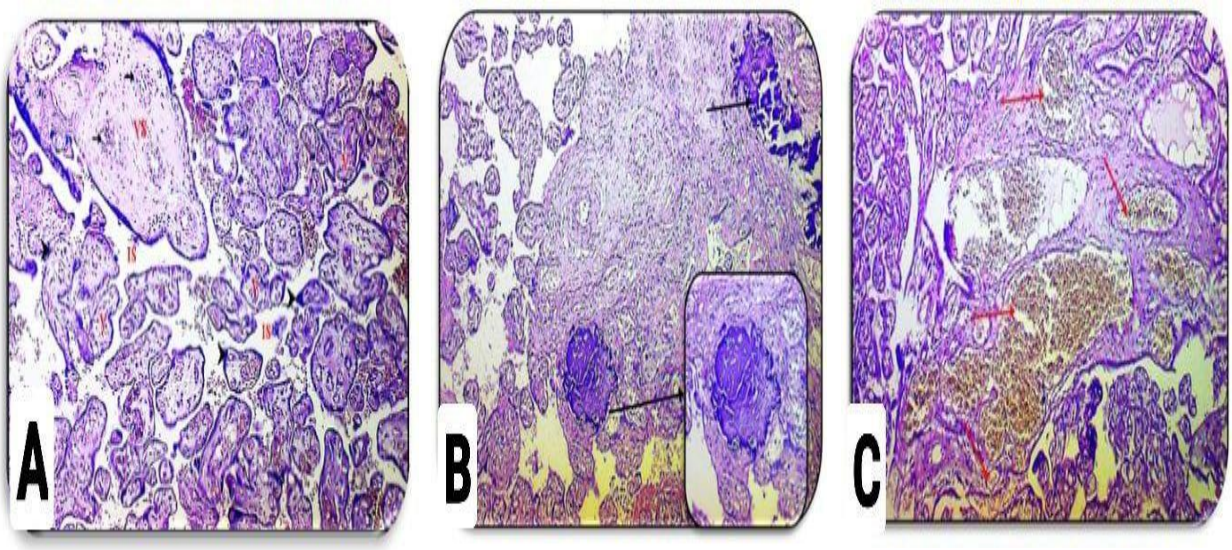

Figure (1): A: Cross-section in the term placenta of NT pregnant women showing microvilli (V), with narrow intervillous spaces (IS) and syncytial knots (head arrows).The villous stroma (VS) shows blood vessels (arrows), (H&E, X10).B: Cross-section in the preterm placenta of PE pregnant women showing large area of calcification in the villous stroma (black arrows) (H & E ,large figure X4, small figure X10). C :Cross-section in the preterm placenta of PE pregnant women showing congestion of capillaries and veins (red arrows) (H & E, X10).

3.3 Immunological studies

TNF- α Serum Levels

Serum TNF-α (pg/ml) levels were normally distributed in all groups, data analysis showed statistically significant higher levels of serum TNF-α in PE patients was (35±20.8 pg/ml) as compare with NT (20.5±1.7 pg/ml) as shown in Table-2 and Figure 3.

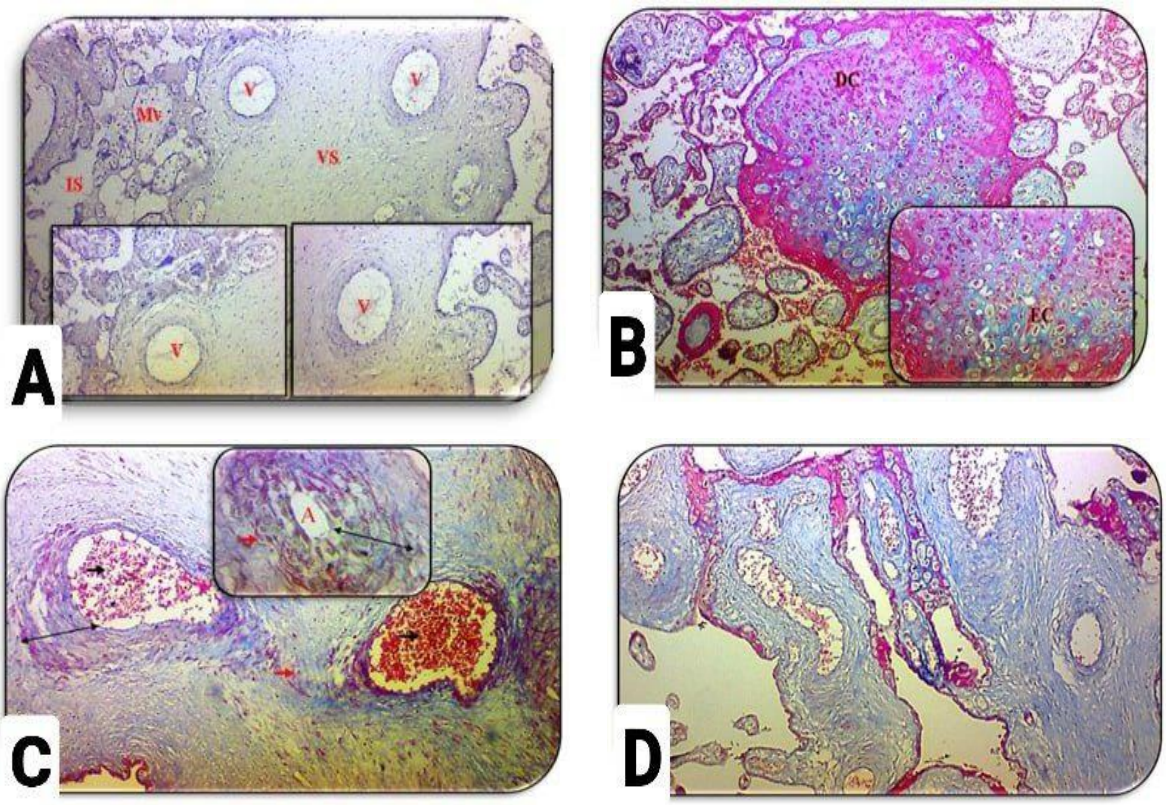

Figure (2): A:Cross-section in the term placenta of NT pregnant women showing non-deposits fibrin in microvilli (MV), intervillous spaces (IS), and villous stroma (VS) shows vein vessels (V), (Trichrome, large figure X4, small figures X10). B: Cross-section in the preterm placenta of PE pregnant women showing deposited fibrin (blue color) between decidual cells (DC) and extravillous cytotrophoblast (EC) (Trichrome, large figure X4, small figures X10).C: Cross-section in the preterm placenta of PE pregnant women showing congestion of veins (black arrows) with increased deposits of fibrin around veins (double arrows) and arterial (A) vessels with increased fibrocytes (red arrows) (Trichrome stain, large figure X4, small figure X40) . D: Cross-section in the preterm placenta of PE pregnant women showing excess amounts of fibrinoid deposits within and around villi (blue color), (Trichrome stain, X4).

Table 2: Serum TNF α levels in preterm PE women and NT.

	PE	NT	P-value
Serum TNF-α (pg/ml)	35±20.8	20.5±1.7	0.037 *

Mean±Standard deviation , * significant , p<0.05

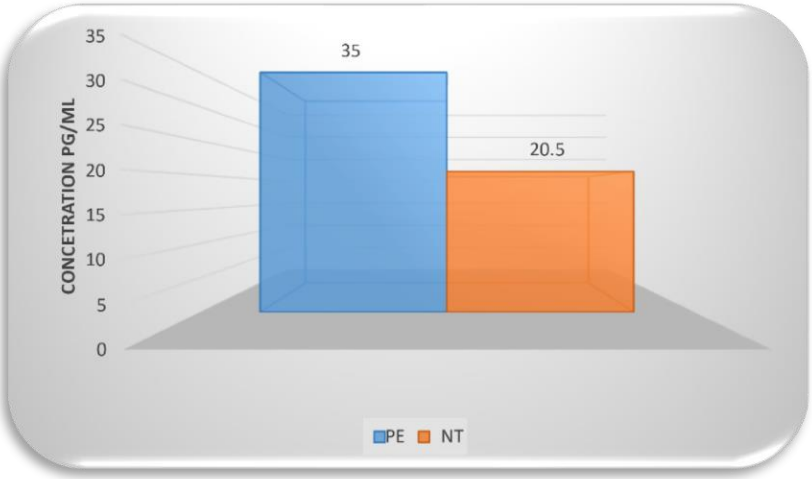

Figure3 : Serum TNF- α levels in preterm PE women and NT .

3.4 Discussion

Compared to primigravida women, multigravida women experience PE more frequently. Numerous factors, such as poor nutrition, environmental factors like smoking, contaminant exposure (chemicals, electromagnetic radiation, pesticides), contraception use, and inadequate prevention, could explain this variation. these outcomes support Youssef *et al*., (2020). Also the results in presenting study are agreement with Sarwor and Iftikhar (2016) The majority of births took place via caesarean section, a delivery method that is discouraged by doctors in order to prevent complications. Heavy bleeding and placental issues are more likely to occur in women who have multiple C-sections, and these issues may require surgical hysterectomy (hysterectomy) (Gill *et al*., 2019).

Placental calcification can be caused by both typical and unusual mechanisms, and it can be brought on by a variety of physiological downstream triggers (Vijayalakshmi *et al*., 2015). In addition to maternal phenotypes and triggers, the risk of PE can also be influenced by subsequent pregnancies, mineral imbalance, or endothelial dysfunction. The phenotypes and triggers of the mother may be associated with placental calcification and cardiovascular disease (Wallingford *et al*., 2018). Unfavorable pregnancy outcomes and poor utero-placental flow are risk factors for early placental calcification. The prevention and efficient treatment of conditions that result in excessive placental calcification can improve fetal outcomes (Goswami *et al* ., 2013).

Incidence of stromal calcification was high in the present study that agreed with Ojha *et al*., (2018**)** then Hyalinized Areas came after that. Because of the narrow lumen, calcifications were mostly found in the villi and basement membrane of the villi, which strongly suggests uteroplacental insufficiency (Kumari and Sinhe, 2016). In primigravida, calcification of the placenta was seen much more frequently. Embryonic The fetus's circulation and development are hampered by excessive placental calcification, which causes uteroplacental insufficiency (Keche ,2015).

When cell death and maternal blood flow are hindered or reduced, congestion results. Blockage or disruption of the blood vessels in maternal or fetal blood can cause acute blood disruption (Siva *et al*., 2017). The most common causes of acute maternal blood flow disturbance in pregnancy are uterine rupture, placental abruption, or maternal blood flow dislocation, while the most common causes of fetal blood flow disturbance are rupture of

the placenta's large fetal vessels or acute umbilical cord blockage (Benton *et al*., 2017). An abdominal injury or the dehiscence of a previous cesarean scar can both result in traumatic uterine rupture. An abrupt, severe fetal hypoxia can result in severe neurologic injury or death, and uterine rupture is frequently linked to life-threatening maternal hemorrhage, necessitating hysterectomy (Agarwal *et al*., 2017). On the pathologic examination, it is possible to determine where the rupture occurs and the uterine wall has been opened, allowing a microscopic examination of the blood passing through the uterus. Prior to delivery, the placental tissue experiences an abruption due to a lack of blood flow. These results are comparable to those of earlier studies that have been published, as has been seen elsewhere (Odibo *et al*., 2014; Falco *et al.*, 2017) .

The receptors and TNF-α play a significant role in pregnancy in women. Each receiver generates a unique set of intracellular signals, but they also interact with one another and have overlapping effects. The majority of TNF's pro-inflammatory functions, especially those that are quick, are carried out by TNF-R1 membrane binding (Gyselaers, 2020). In normal pregnancies, TNF-α receptors are present in the placenta, amniotic fluid, and the coelom cavity. Maternal modifications of TNF and its receptors were linked to early birth, premature membrane rupture, and PE. Future advancements could be beneficial for pregnant women receiving anti-TNF therapy (Jean *et al*., 2017).

The current study found that preterm women with PE had higher mean levels of TNF-α than women who were not preterm. These statistically highly significant increases in TNF-α concentration may be related to the interaction between environmental and genetic factors in PE. Additionally, the findings of this study support Ahmed *et al*., (2019) Immunological results of cytokine concentration in PE was significantly increased ($p<0.05$), compared NT groups.

Shaw *et al*., (2016) It was also argued that PE infections are necessary for both infection and inflammatory responses, and that in regions where subclinical infections are common but patients with severe PE are scarce, inflammatory conditions may be the root cause of PE. Another name for it is endothelial cell dysfunction in the blood supply (Todros *et al*., 2021).

Circulating levels of plasma TNF-α are predictive of PE in the first and second trimester, while (Serina, 2018) has proven that they are useless for early PE prediction. On the other hand, the third trimester of pregnancy is when TNF-α prediction can be helpful. These findings have received consistent support from prior research. Direct and indirect increases in TNF-α can both lead to endothelial dysfunction. The inter-fetal cytokine immune relationship may be dysfunctional during the latent phase of PE, which could compromise the fetomaternal immune system, according to researchers (Mawardi *et al*., 2019).

In PE, TNF-α together with IFNγ, has been shown to cause apoptosis of syncytiotrophoblasts, with impairment of syncytialization, especially under hypoxic conditions in term placenta.

There is evidence that the sources of TNF-α production in PE include neutrophils, monocytes, and possibly placenta. According to one theory, the placenta drives monocytes and neutrophils to produce TNF-α, which disrupts the endothelial system, as a possible mechanism for PE (Marijke *et al*., 2018). Therefore, it seemed that increased serum TNF-α might be part of the pathology of PE (Vokalova *et al*., 2020).

Manoj *et al*., (2020) demonstrated that women with intra-amniotic infection and preterm labor have elevated levels of TNF- α, as well as other pro-inflammatory cytokines

such as IL-1, and that these cytokines stimulate prostaglandin synthesis in human tissues.

Pro-inflammatory cytokines like TNF-α are used in conjunction with a higher concentration of soluble receptor to reduce the cytokine response. TNF-α and other pro-inflammatory cytokines were found in the umbilical serum of pregnancies complicated by PE, indicating that they may contribute to intrauterine growth restriction brought on by PE (Schumacher, 2019). Since 11–13 weeks, well before a clinical PE manifestation, proinflammatory cytokines TNF-α and TNF–R have been rising in maternal circulation (Burwick, 2018).

Previous studies have demonstrated that TNF-α is capable of participating in PE pathogenesis and can identify patients with high PE risk.

Conclusions

We can make the following conclusions The majority of multigravida and preterm PE women over 30 undergo cesarean sections. It is thought that the majority of pathophysiological changes that influence the development of PE result in uteroplacental hypoxia and ischemia, which in turn triggers a variety of histopathological changes. Increased serum TNF-a appeared to be a contributing factor to the pathophysiology of PE, according to the immunological parameter.

Acknowledgements

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دراسة التغيرات النسيجية المرضية ومستويات عامل نخر الورم- ألفا في النساء ذوات مقدمة الارتعاج المبكرة

الاء سعدي عبود: كلية الفارابي الجامعة \ قسم علوم الحياة / بغداد - العراق.
انوار محمد لازم : كلية الفارابي الجامعة \ قسم التمريض / بغداد - العراق.
احمد فليح حسن: كلية الفارابي الجامعة \ قسم علوم الحياة / بغداد - العراق.

الخلاصة

تسمم الحمل (Preeclampsia) هو مساهم كبير في إمراضية الام ووفياتها يتجلى في ارتفاع ضغط الدم المستجد بعد 20 أسبوعًا من الحمل مع ظهور علامات تدل على ذلك مثل خلل وظيفي في الرحم أو البيلة البروتينية. هذا الشرط مرتبط بـ تقييد النمو للجنين داخل الرحم ، والولادة المبكرة ، وانفصال المشيمة ، وضيق الجنين ، و موت الجنين في الرحم. يمكن تلخيص نتائج الدراسة على النحو التالي: أظهر تصبيغ Hematoxylin and Eosin لأنسجة المشيمة مساحة كبيرة من التكلس في سدى الزغابات. بالإضافة إلى زيادة أليلف الكولاجين حول الدم الأوعية ودرجات متفاوتة من تسلل الخلايا وحيدة النواة ناتجة عن الشعيرات الدموية واحتقان الوريد. أظهرت صبغة Masson Trichrome عن وجود الفيبرين المترسب بينهما الخلايا الساقطة والأرومة الغاذية الخلوية الزغبية ، والتي كانت محاطة بالناضجة الزغابات المتوسطة والنهائية. احتقان الأوردة مع زيادة رواسب الفيبرين حول الأوردة والأوعية الدموية الشريانية مع زيادة الخلايا الليفية .و بين التحليل الاحصائي أن النساء الحوامل المصابات بـ PE ان مستويات TNF- α $p < 0.05$ اعلى بشكل ملحوظ من مستويات النساء الغير مصابات (NT) . أخيراً وجدت الدراسة وجود صلة بين PE قبل الأوان وحمل المرأة، وكذلك المعلمات النسيجية والمناعة في النساء الخدج PE و NT .