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Comparative Histological and Immunohistochemical Study in Normotensive and Preeclamptic Human Placental Tissue

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Abstract:

Background: The leading causes of maternal and neonatal death and morbidity globally continue to be preeclampsia (PE) and eclampsia. **Aim of the work**: present study was designed to compare histopathological changes, cell to cell adhesion and apoptotic changes in both normotensive and preeclamptic human placenta, using histological, immunohistochemical and morphometric methods. **Patients and methods**: Sixty pregnant women were included; 30 were normal clinically and considered the control group (group I) and the other 30 were included in the preeclampsia group (group II). Placental tissue was taken from pregnant women aged 25 to 35 who had had a caesarean section in order to end their pregnancy at Obstetric and Gynecology Department, Faculty of medicine, Beni-Suef University Hospital. Sections of placenta were processed and stained with Masson's trichrome, H&E, and immunostained for Bcl-2 and IL-10. **Results**: Group II (PE) revealed marked elevation in the number of syncytial knots, significant elevation in area percent of collagen fibers, and significant elevation in area percent of IL-10 immunoexpression as compared to group I. But there was marked reduction in the area percent of Bcl-2 immunoexpression. **Conclusion**: It can be concluded that Preeclampsia is associated with marked inflammatory and immune responses. Bcl-2 and IL-10 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.

Keywords: Placenta, preeclampsia, Bcl-2 , IL-10,Immunohistochemistry.

1. Introduction:

Throughout the prenatal period, the placenta, fetus, and mother are still creating a composite functional balance. Each of them can be impacted by the malfunction of the others (1). The placenta continues to provide the most accurate assessment of the prenatal experience of the child. It is a special and magnificent organ which develops from nothing and has a direct relationship to the fetus's growth and development within the mother (2).

The placenta increases the levels of lipids, glucose, and estrogen which is responsible for fetal weight; in the mother's blood as well as relaxin, which relaxes the cervix during labour, and human chorionic gonadotropin (hCG) (3).

Preeclampsia is widely understood to be the onset of hypertension and proteinuria in a previously normotensive woman after 20 weeks of gestation (4). Eclampsia is the development of 1 or more generalized tonic-clonic convulsions in pregnant women with hypertension that are unrelated to other medical diseases (5).

Preeclampsia and eclampsia, which account for 12–15% of all direct maternal fatalities globally, continue to be the leading causes of maternal and perinatal mortality and morbidity (6).

Preeclampsia's pathogenesis is still poorly understood, but a latest study found that excess calories and carbohydrates, as well as deficiencies in zinc, fat, and calcium, as well as vitamin C and vitamin E, were all linked to a higher risk of developing preeclampsia and eclampsia (7).

Systemic endothelial dysfunction in preeclampsia is the main cause of maternal clinical symptoms. Hypertension results from endothelium-dependent modulation of vascular tone and vasoconstriction, whereas elevated capillary permeability causes fluid loss into the third cavity, hemoconcentration, and edema (4).

Proteinuria is caused by elevated glomerular permeability, while extensive intravascular coagulation is caused by the coagulation process. Numerous cytokines can be produced by endometrial cells, trophoblasts, and immune cells during implantation. Preeclampsia can potentially occur due to endothelial malfunction or issues with placenta development brought on by a cytokine environment disease (8).

In the feto-maternal interface, interlukin-10 (IL-10) is regarded as a key immunomodulatory agent and the primary modulator of the inflammatory reaction. IL-10 is produced by the natural killer (NK) cells and trophoblast decidual macrophages (9). The feto-maternal interface between extra villous cytotrophoblasts in the early stages of pregnancy and villous cytotrophoblasts in the late stages of pregnancy is where IL-10 is highest expressed (10).

The death-promoting protein Bcl-2-associated X protein (Bax) activates cell apoptosis by forming a heterodimer with the B-cell lymphoma-2 (Bcl-2) protein. Apoptosis is avoided by Bcl-2, a member of the Bcl-2 family. This protein, which is found in the mitochondrial outer membrane, prevents the activity of proteins that promote apoptosis (9). The Bcl-2 gene, which has two promoters, three exons, and two introns, is located on chromosome 18 (11).

Aim of the work:

The current research was designed to compare histopathological changes, cell to cell adhesion and apoptotic changes in both normotensive and preeclamptic human placenta, using histological, immunohistochemical and morphometric methods.

2. Patients and Methods:

Subjects

Between October 2020 and December 2021, 60 placentas were collected from pregnant women, with an average age of 25 to 35, who were hospitalized to the obstetric department of Beni-Suef University Hospital to have a caesarean section to end their pregnancies. Pregnant women who presented with pre-eclampsia were given 30 placentas (Pre-eclamptic group), and 30 placentas were extracted from normal pregnancies (Control group). The faculty of medicine's ethics committee gave the study the approval. Approval number FMBSUREC\ 06072021\ Raafat Beni-Suef University, all participants gave their fully informed permission after explaining the objectives of the study and they were informed that their participation is voluntary.

Inclusion criteria of the pre-eclamptic group:

1. Age range: 25 to 35.
2. Gestational age: 34-40 weeks.
3. There is just one surviving fetus
4. After 26 weeks of pregnancy, the blood pressure was over 140/90 on two consecutive occasions, six hours apart.
5. No previous history of systemic hypertension or any other illness that might have affected the study's findings.
6. No prior usage of drugs while pregnant.
7. Urine analysis: A urine dipstick test reveals a protein (albumin) level of at least +1.

Exclusion criteria:

1. Age: more than 35 years old.
2. Gestational age: less than 34 weeks.
3. More than one viable fetus is present.
4. History of chronic hypertension.

Methods:

Histological study

In order to prevent taking samples from apparent calcifications or infarctions, the placentae were extensively evaluated from the maternal surface after delivery. Samples (1.5 X 1.5 X 1 cm in size) were obtained from the centre of the maternal surface, which is opposite the location where the umbilical cord was inserted in the placenta's fetal surface. The collected placental samples were fixed

for 24–48 hours in 10% buffered formalin solution, dehydrated in increasing grades of ethanol, cleaned in xylol, and then embedded in paraffin wax. 5 µm thick serial sections were collected.

Paraffin sections were subjected for the following

1- Hematoxylin and Eosin (H&E) (12).

2- Masson's trichrome stain to demonstrate stromal changes (13).

Immunohistochemical study:

A suitable paraffinized block from each sample was chosen for immunohistochemical staining, and sequential tissue slices (5 µm) were mounted on 3-aminopropyltriethixysaline (APES; Sigma) coated slides. Samples were dewaxed using progressive xylene washes before being completely dehydrated in absolute alcohol. Slides were then rinsed under running water after being submerged in 3% H2O2 for 10 minutes to inhibit endogenous peroxidase. Microwaving (800 W, maximum power) in citrate buffer (pH 6-7) for 2 minutes was used to retrieve the antigen. Slides were given 20 minutes to cool at room temperature. By allowing the incubation in 10% normal goat serum for 10 minutes and then washing in PBS solution (pH 7.6), non-specific background staining was successfully blocked. Slides were kept in humidity chamber. Slides were incubated with monoclonal mouse Bcl-2, Clone 124, (Lab Vision Corporation laboratories, CA 94539, USA, Cat No. (550847) at a 1/400 dilution, at room temperature for one hour (14). Sections were incubated with mouse monoclonal anti-IL-10 antibody (15) (cat no: ab34843, Abcam, Cambridge, MA 02139-1517, USA, 1:100) overnight at+ 4ºC.Biotinylated secondary antibody was applied to the sections for 30 minutes, and then washed. Streptavidin-peroxidase conjugate was applied for 15 minutes, and then washed. Substrate chromogen mixture was prepared and slides were incubated at room temperature for 5-10 minutes. Counterstaining of slides was done using Mayer's haematoxylin. Slides were dehydrated in ascending grades of ethanol (70%, 95% &100%) 5 minutes each. Slides were covered and examined by light microscope.

Morphometric Study:

At Beni-Suef University's Histology Department Faculty of Veterinary Medicine, data were collected utilizing a "Leica Qwin 500 C" image analyzer computer system Ltd. (Cambridge, England). With a video camera, live images from sections seen under a light microscope were recorded. A color video camera, an Olympus microscope, a colored display, an IBM personal computer's hard disc attached to the microscope, and "Leica Qwin 500 C" software were all components of the image analyzer. Slides were studied using a light microscope.

All measurements were taken in 10 non overlapping fields for each specimen for all groups:

1. The mean area percent of syncytial knots
2. The mean area percent of collagen deposition stained blue with Masson's trichrome
3. The mean area percent of Bcl-2 immunoreactivity
4. The mean area percent of IL-10 immunoreactivity

Statistical analysis:

All collected data were revised for completeness and logical consistency and items were then transferred to the Statistical Package of Social Science Software program, version 25 (SPSS) to be statistically analyzed (16). Graphs were utilized to demonstrate simple information Suitable statistical tests were used (Chi-square (χ^2) and Independent T-test). P-values equal to or less than 0.05 were regarded statistically significant.

3. Results:

Histological results:

Histological observations of group I (control group) stained with HX&E showed mature villi, with a connective tissue core containing many thin walled fetal blood vessels and minimal fibrinoid degeneration **(Fig.1a).** The villi appeared covered with a continuous syncytial layer **(Fig.2a).** Syncytial knots appeared as a cluster of tiny, darkened syncytial nuclei inside the syncytial layer **(Fig.2b).** The histological examination of group II showed patchy areas within C.T appeared acidophilic, hyaline and non-cellular (Fig.1b). The connective tissue core of villi condensed the wall of fetal vessels with moderate to marked thickening **(Fig.1c, 2c).** Degeneration in the wall of the blood vessels, congested blood vessels **(Fig.1d)** and increased syncytial knot formation were also observed **(Fig.2d).**

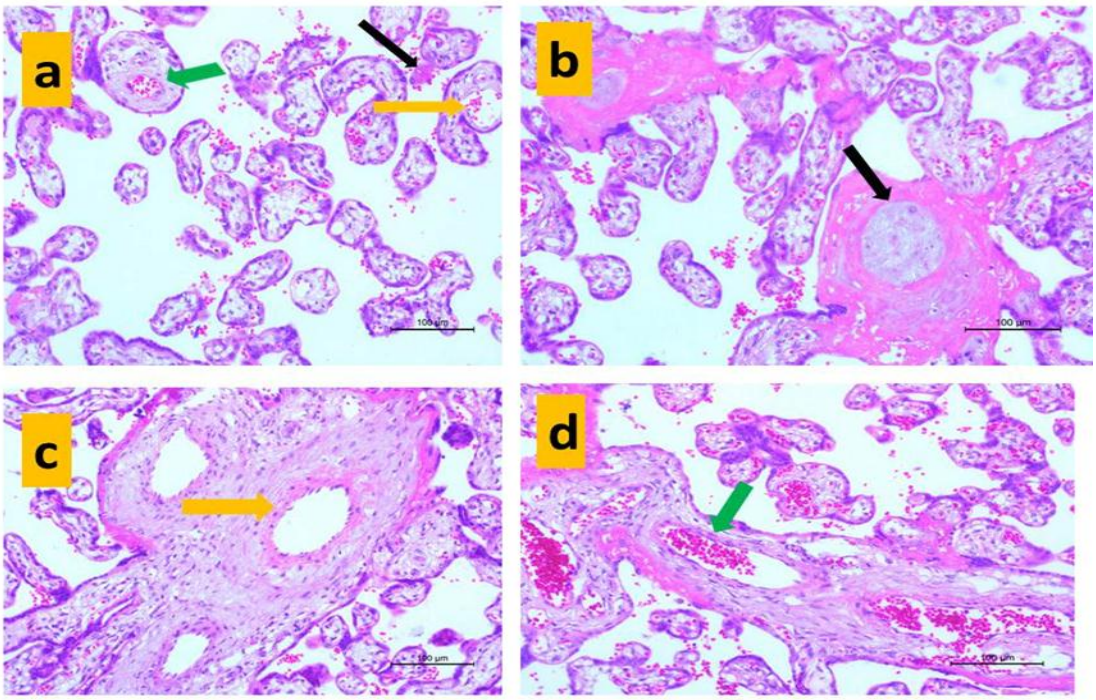


Fig.1 (a) Placenta group I showing mature villi, with a connective tissue core (green arrow) containing many thin walled fetal vessels (yellow arrows) and minimal fibrinoid degeneration (black arrow) .**(b)** section of group II showed patchy areas within C.T appeared acidophilic ,hyaline and non-cellular (black arrow). **(c)** Section of group II showed the connective tissue core of villi condensed the wall of fetal vessels with moderate to marked thickening (yellow arrow). **(d)** Section of group II showed excessive vessel wall degeneration and congested blood vessels (green arrow), (H&E x400).

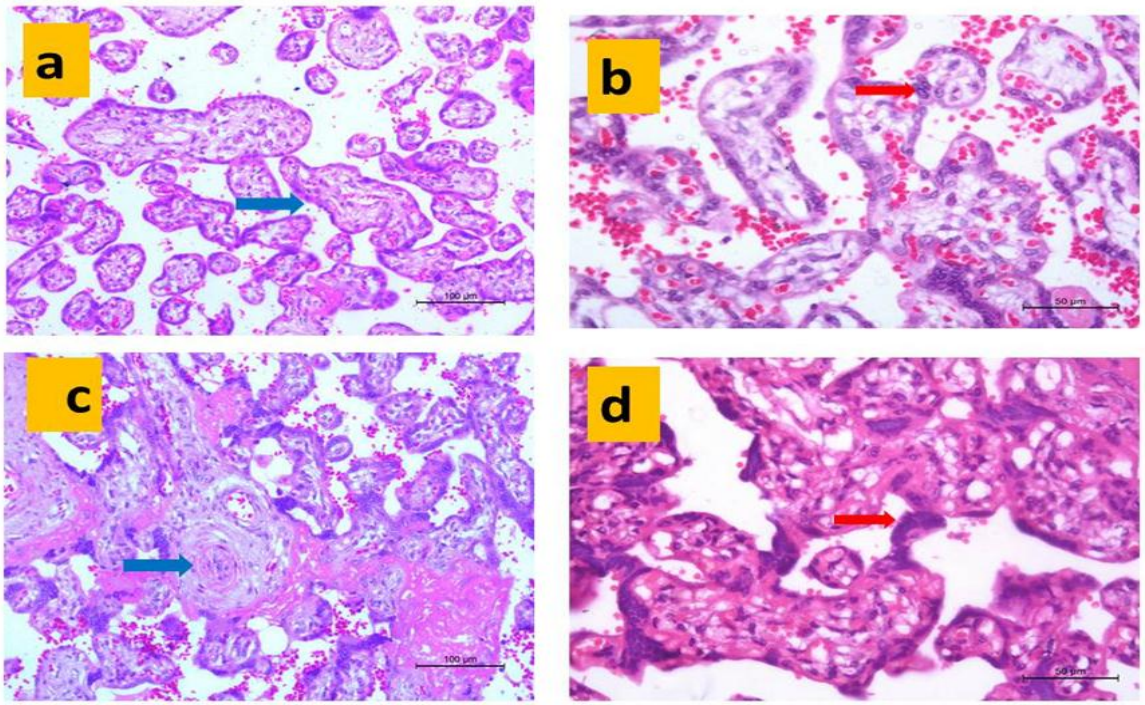


Fig.2 (a) Placenta group I demonstrated how a continuous syncytial layer covered the villous structure, which had a central connective tissue core that included fetal vessels (blue arrow) (H&E X200). **(b)** placenta group I showed syncytial knots as a cluster of tiny, darkened syncytial nuclei inside the syncytial layer (red arrow) (H&E X400). **(c)** Section of group II showed the connective tissue core of villi condensed (blue arrow) (H&E X200). **(d)** Section of group II showed thickening of syncytiotrophoblast basement membrane and increased syncytial knot formation (red arrow) (H&E x400).

Histological examination of Masson's trichrome stained sections revealed that group I presented numerous collagen fibers within the stroma of stem villi around fetal blood vessels and very minimal collagen fibers within terminal villi **(Fig.3 a,b)** .In group II, dense excessive collagen fibers were detected within stem mature intermediate villi and terminal villi **(Fig.3 c, d).**

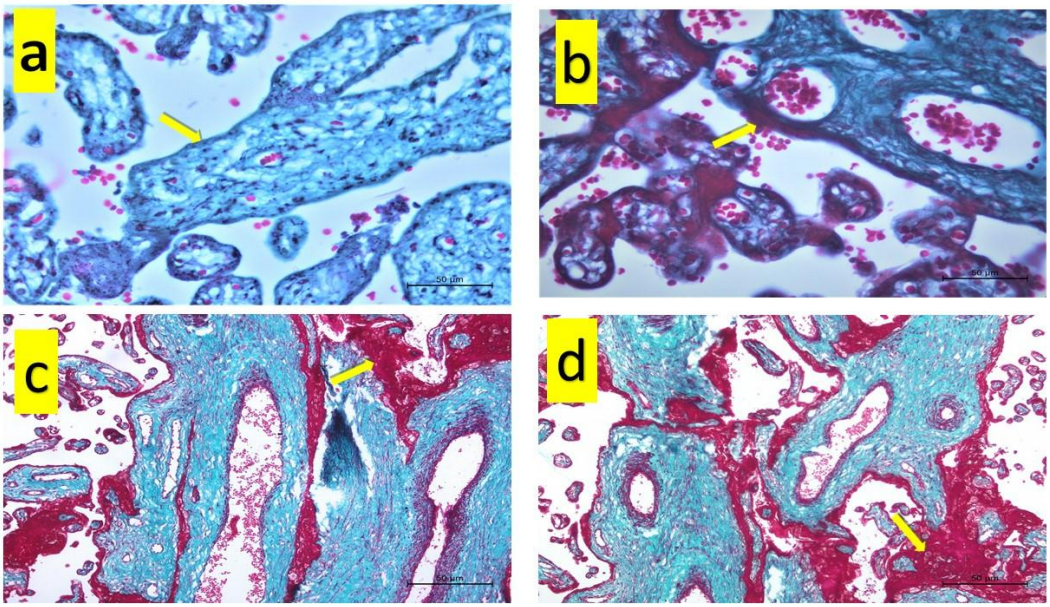


Fig.3 (a) placenta group I showed very minimal collagen fibers within terminal villi (yellow arrow). **(b)** Collagen fibers within the stroma of stem villi around fetal blood vessels (yellow arrow). **(c)** and **(d)** placenta group II showed dense excessive collagen fibers were detected within stem mature intermediate villi and terminal villi (yellow arrow), (Masson's trichrome x400).

Immunohistochemical results:

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

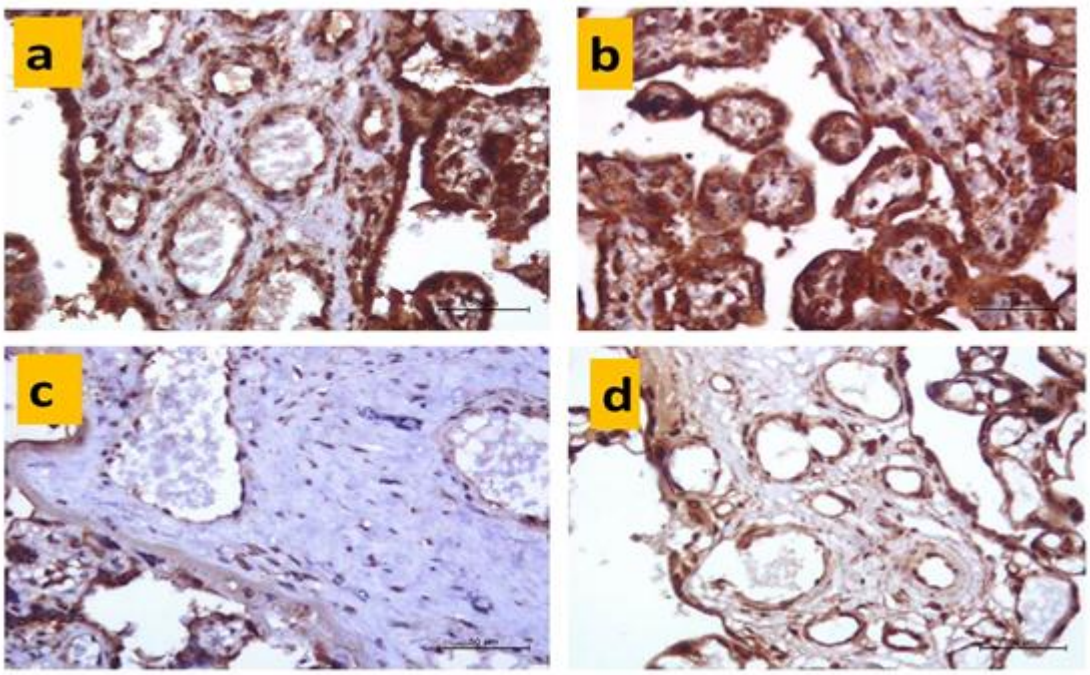


Fig.4 (a) and (b) placenta group I showed widespread immunoreaction for Bcl-2 which appeared as brown cytoplasmic reaction in most of STB. Placenta from group II (c) and (d) exhibited weak Bcl-2 immunoreactivity (Bcl-2 immunostaining x400).

Regarding the IL10: Group I showed that the connective tissue cells and trophoblast cells in floating villi did not express IL-10 **(Fig 5 a, b).** In group II: marked elevated IL-10 expression was observed in subendothelial layers of the medium-sized vessels in the maternal region, inflammed connective tissue areas **(Fig.5 c, d).**

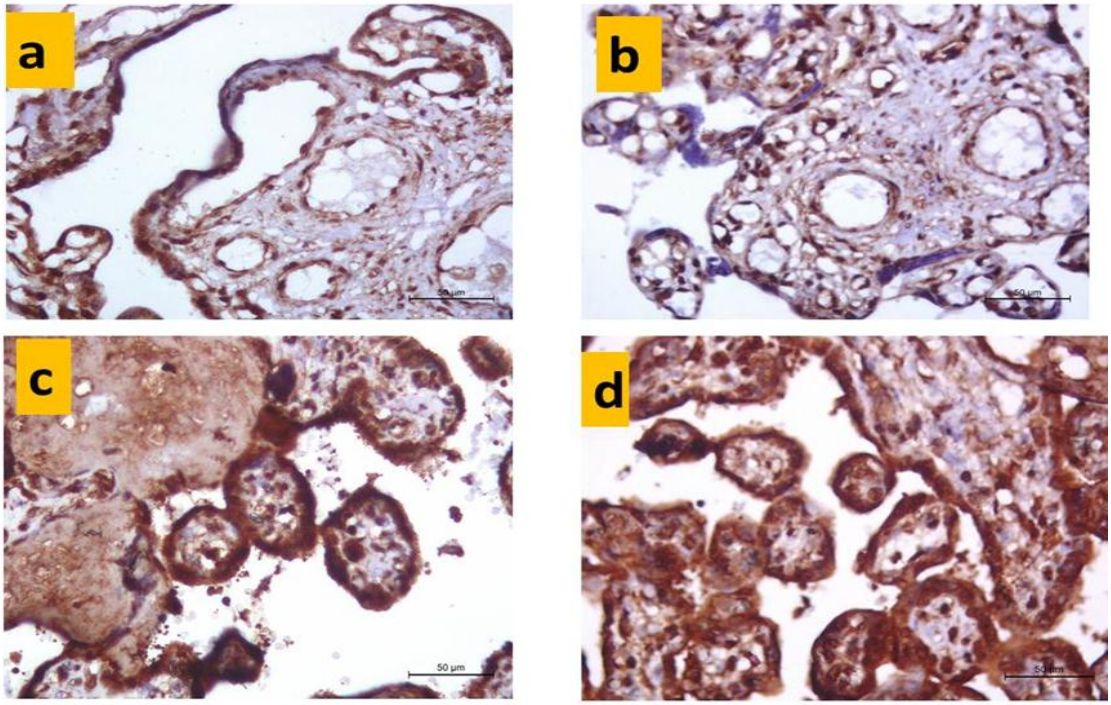


Fig.5 (a) and **(b)** Group I showed that the connective tissue cells and trophoblast cells in floating villi did not express IL-10. **(c)** and **(d)** sections of group II showed marked elevated IL-10 expression was observed in subendothelial layers of the medium-sized vessels in the maternal region, inflammed connective tissue areas (IL10 immunostaining x400).

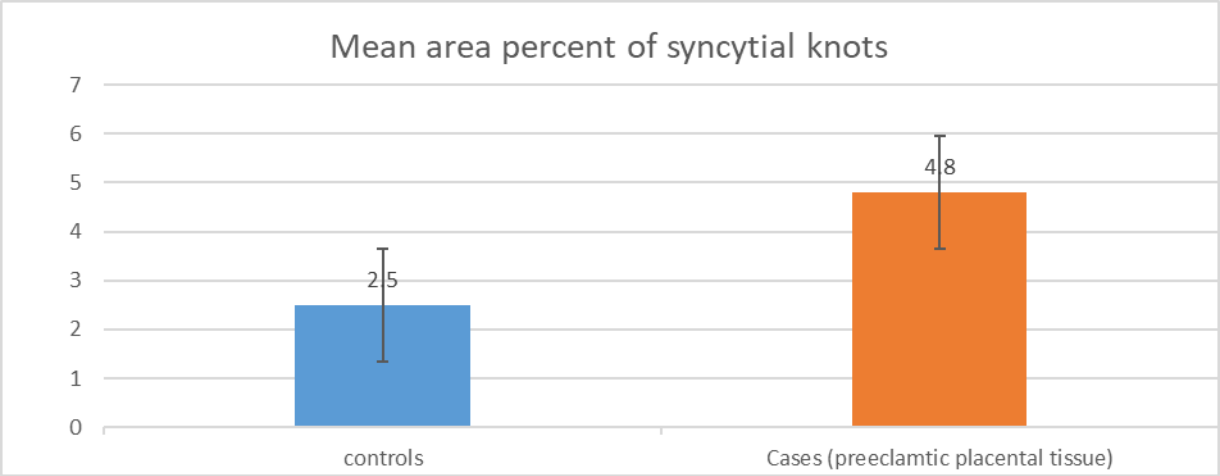
Morphometric results:

Table1: Comparison between the studied groups regarding mean area percent of syncytial knots of placenta:

	Control group	Cases (preeclamsia group)
	(mean ± SD)	**(mean ± SD)**
Mean area percent of syncytial knots	2.5000 ± 1.22474	4.8000 ± 3.30517

**P-value is significant at <0.05*independent T test*

The mean area percent syncytial knots is higher among cases than controls with statistically significant difference (P-Value *<0.05*).



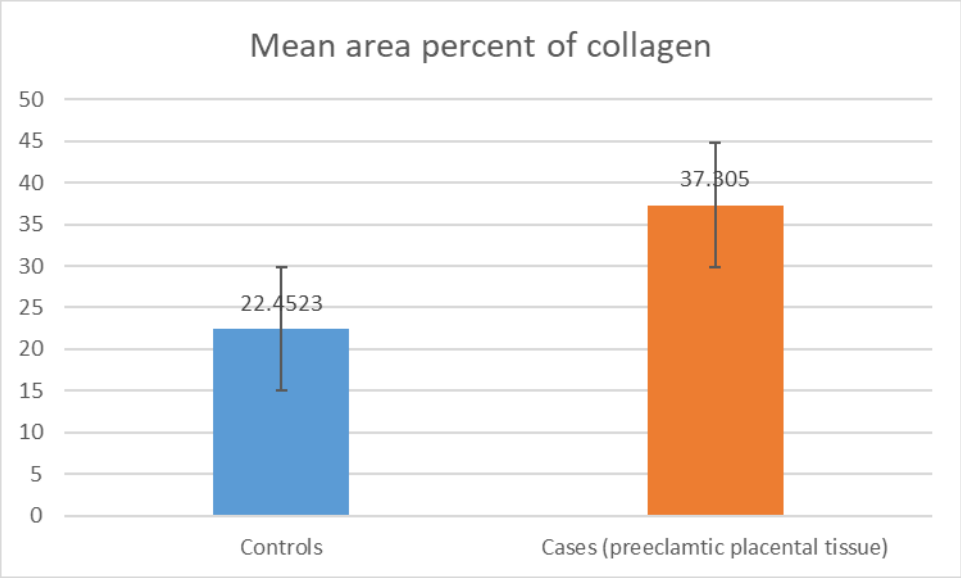
Histogram1: Mean area percent syncytial knots difference between the studied groups

Table 2: Comparison between the studied groups regarding mean area percent of collagen:

	Control group	Cases (preeclamsia group)
	(mean ± SD)	**(mean ± SD)**
Mean area percent of collagen	22.4523 ±3.72346	37.3050 ± 8.46187

**P-value is significant at <0.05*independent T test*

The mean area percent of collagen values are statistically significant in comparison to controls (P-Value*<0.05*).



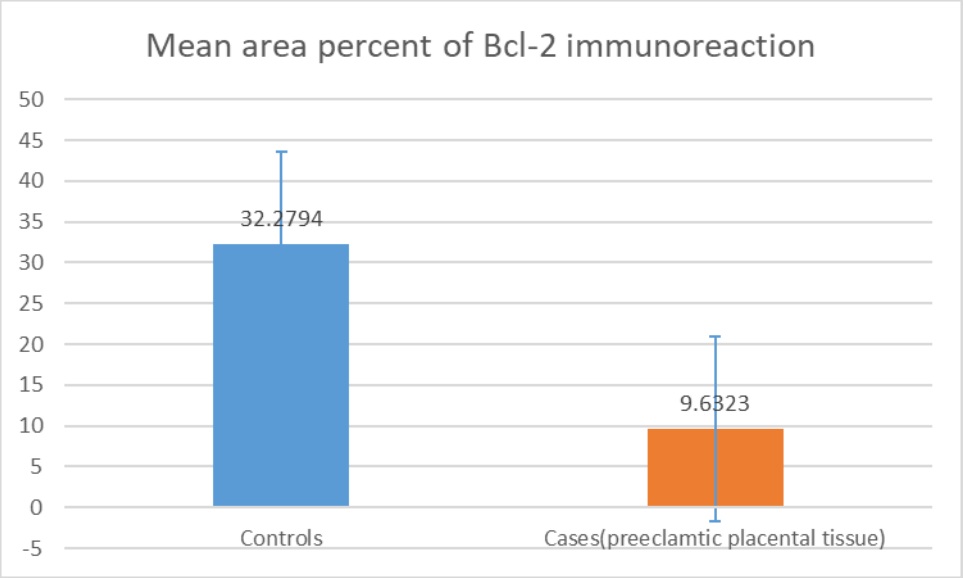
Histogram 2: Mean area percent of collagen difference between the studied groups

Table 3: Comparison between the studied groups regarding the mean area percent of immunoreaction of Bcl-2:

	Control group	Cases (preeclamsia group)
	(mean ± SD)	**(mean ± SD)**
Mean area percent of Bcl-2 immunoreaction	32.2794±6.61545	9.6323±3.90789

**P-value is significant at <0.05*independent T test*

The mean area percent of Bcl-2 immunoreaction in the cytoplasm of syncytiotrophblasts were statistically significant (P-Value *<0.05*) in control group.



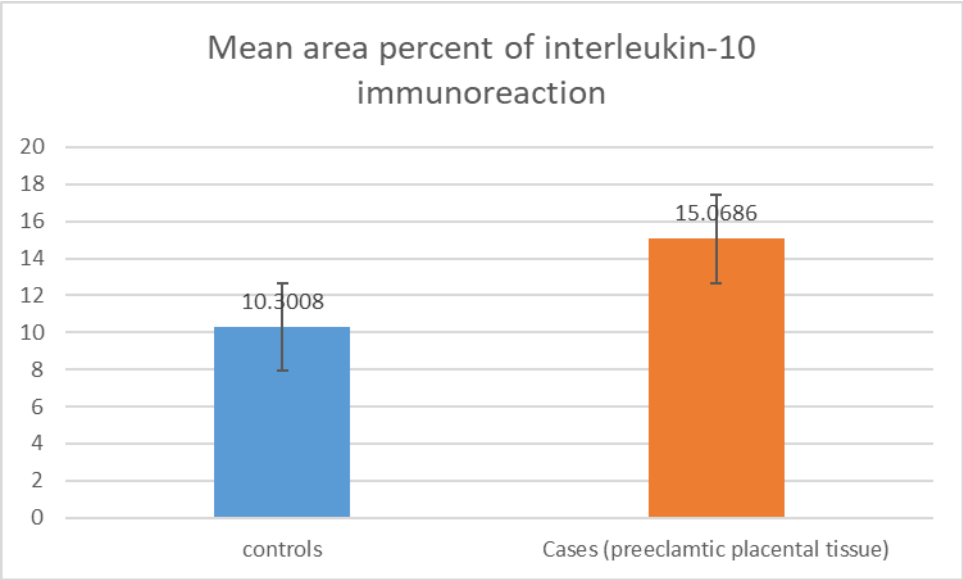
Histogram 3: Mean area percent of Bcl-2immunoreaction difference between the examined groups.

Table 4: Comparison between the studied groups regarding the mean area percent of interleukin-10 immunoreaction:

	Control group	Cases (preeclampsia group)
	(mean ± SD)	**(mean ± SD)**
Mean area percent of interleukin-10 immunoreaction	10.3008 ±2.44409	15.0686 ±4.73751

**P-value is significant at <0.05*independent T test*

The mean area percent of interleukin-10 immunoreactionin the basement membrane of syncytiotrophblasts and connective tissue core values statistically significant in comparison to controls (P-Value<*0.05*).



Histogram 4: Mean area percent of Interleukin-10 immunoreaction difference between the studied groups.

4. Discussion:

This study was performed to compare normotensive and preeclamptic human placental tissue. This comparison included histological, immunohistochemical and morphomtric studies. The existence of syncytial knots that were sporadically observed as an accumulation of dark, tiny, pyknotic syncytial nuclei inside the syncytial layer was found in the current study's evaluation of hematoxylin and eosin stained sections from the control placentae group. Syncytial knots may be a useful indicator of villous development because they have been shown to grow with gestational age. (17).

The villus trophoblast layer, villus stroma, and fetal vascular systems were visible in control group placentae and were in usual appearance. However, in preeclamptic placentae, there was fibrinoid deposition and atrophic villi. Other investigations examining the placental histopathology in preeclampsia are similar with these results in preeclamptic placentae (18). Syncytial knots, syncytial bridges, atrophic villus and perivillous fibrin deposition, as well as a considerable thickening of the trophoblast basal membrane, were all significantly increased in the preeclampsia group's placentae (19).

In the present study, H&E stained sections from group II (PE group) revealed chorionic plate with lost syncytiotrophoblast (STB) layer. Also increased fibrin deposition was detected within and around the villi and in the intervillous space, which coincides with

other studies (20). Syncytial knot counts were found to be significantly higher in preeclampsia group as compared to controls. Increase in the number of villi showing syncytial knots. Because the syncytium receives all of its oxygen from the mother, a decrease in villous vascularity has no impact on how well the syncytium is oxygenated.. The findings of the present study correlated well with previous study (21).

As regard histological examination of Masson's trichrome stained sections showed that group I presented numerous collagen fibers within the stroma of stem villi around fetal blood vessels and very minimal collagen fibers within terminal villi .In group II, dense excessive collagen fibers were detected within stem mature intermediate villi and terminal villi. Replacement of the villus by fibrin results in fibrinoid necrosis of placental villi that leads to their distortion with STB degeneration. 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 (22). Intervillous fibrin can be the consequence of a repair mechanism for the disrupted trophoblast lining with reepithelization of the damaged STB (23).

One of the most significant apoptosis regulators is the antiapoptotic Bcl-2, which is localized in STB cytoplasm to maintain syncytial integrity in normal pregnancy. 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 in comparison to the control and this is in agreement with earlier studies (24).

In healthy placental tissue, apoptosis and proliferation coexist dynamically at different phases of pregnancy. The finding of elevated levels of villous trophoblast apoptosis in placental diseases, such as PE and early pregnancy loss, has attracted interest. The number of trophoblast cells decreases within the spiral arteries during PE, which is associated with decreased luminal size and higher apoptosis in more severe PE (25,26).

The present study highlights the role of apoptosis in the morphogenesis and ageing of placenta through the enhanced expression of Bcl-2 in normal placenta. Bcl-2, on the other hand, controls placental apoptosis, maintains syncytial integrity beyond pregnancy, and may be essential because of its significant expression at term. Premature membrane rupture is more common when Bcl-2 levels are lower, according to many independent studies (27).

IL-10 is a chief immunosuppressive and anti-inflammatory cytokine is that promotes successful placentation, regulates vascular function, and controls inflammation (28). By

inducing Human Leukocyte Antigen-G (HLA-G) expression and inhibiting lysis by maternal NK cells, IL-10 modulates the maternal response to paternal antigens and improves tolerance to fetal allograft (29).

Trophoblast invasion into the uterus may change if IL-10 synthesis is abnormal. It is hypothesized that the trophoblast antigen (HLA-G), may safeguard the maternal-fetal tolerance network by regulating decidua cell expression (30).

Select PE patients may have elevated IL-10 expression as a compensatory response to the vicious cycle of hyperinflammation and vascular oxidative stress, which results in elevated IL-10 secretion along with other pro-inflammatory cytokines (31). The enhanced expression of IL-10 by preeclamptic placental tissues is another unique discovery. This theory states that cytokines ordinarily released by T-helper 2 cells, including IL-10, prevent the fetus from being rejected by cytotoxic T cells and macrophages by inhibiting their synthesis (32).

5. Conclusion:

Bcl-2 and IL-10 are extremely crucial in preeclampsia 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. PE development and progression may be facilitated by immune system dysfunction, specifically in relation to the generation of BCL-2 and IL-10.

6. Recommendations:

- More research is urged regarding the role of BCL-2 and IL-10 in the pathogenesis of preeclampsia during pregnancy. Additionally, more research is urged on the use of additional elements in the management or prevention of preeclampsia.
- Further studies are recommended regarding age over 35 years as incidence of preeclampsia is increased with age.
- Pregnancy induced hypertension alters the placental morphology. Early recognition of pregnancy induced hypertension and proper management may therefore be critical to normal placental function.
- Further studies of placental ischemia and inflammation during pregnancy are recommended.

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