The Role of CD3 and CD8 in Preterm Preeclamptic women by using Immunohistochemical technique

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Abstract

Preterm birth (PTB) and PE are the two major causes of perinatal mortality, morbidity, and long-range neurological disability. PTB defined as delivery before 37 weeks gestation, has become an epidemic in developed countries, Indeed PTB and PE are leading causes of maternal and neonatal death worldwide. The immunohistochemical study show: - The staining intensity for PE immunohistochemical showed the greatest (+2) intensity was recorded at 52% for anti-CD3 lymphocyte biomarker. Also, followed by 28% of (+1) Intensity was comparable biomarkers. (12%) of +3 and 8% for 0 intensity when compared with Tonsil control positive and placental tissue control negative. On the other hand, the staining intensity for PE immunohistochemical shows the greatest frequency of (0) intensity was recorded for anti-CD8 cytotoxic T-cell biomarker with 52 % then +1 (28%) and 20% for +2 when compared with skin control positive and negative.

1- Introduction

PE is a very significant cause of morbidity and mortality in pregnancies and the early stages of gestation (**Ahmed, M. *et al.*,2019**), and in addition, is frequently involved in maternal and perinatal health issues (**Ali1, Z. *et al.*,2020**). When there is a correlation between arterial hypertension and proteinuria or when there is evidence of severity, the diagnosis is made (Alsaeed**, S. *et al.*,2020**). Given the risk of developing maternal complications versus the risk of adverse perinatal outcomes associated with

prematurity, PE has been diagnosed (Armistead**, B. *et al.*,2020**). Multiple factors have been shown to contribute to PTB, but PTB, in particular, has been associated with preterm delivery before term (**Bartal, M . *et al.*,2020**), including infection and inflammation (**Bellos, I. *et al.*,2018**). CD3 is a protein complex and T cell co-receptor that plays a role in activating both cytotoxic T cells and T helper cells (**Black, K. *et al.*,2018**). Because CD3 is expressed at all stages of T-cell development, it serves as an immunohistochemical marker for T cells in tissue

(Buchrieser**, J.** *et al***.,2019**). Lymphocytes are critical components of inflammation. Numerous investigators have examined the counts of lymphocyte subsets in PE patients to understand why these patients' immune responses differ (Burton**, G.** *et al***.,2019**). It indicates changes in the immune system of the mother linked to pregnancy (**David, A and Denise, M. 2020)**. CD8 exists as an α, β chain, or two α chains, disulfide-linked dimer (**Drs, E.** *et al***.,2020**). The N-terminal 113 amino acid crystal structure of human CD8 has shown homology of variable areas of immunoglobulin (ig) with nine β strands divided into two β-plate strands, one out of four and one out of five strands (Feig**, D. 2018**). CD8 cells also differ in various subpopulations The subset of CD8 T cells may play important roles in maintaining ordinary pregnancy (**Gerasimova, E.** *et al***.,2019**). Therefore, these findings show that the recruitment of these large granular uterine lymphocytes is under hormonal control and not a local response to the presence of the trophoblast (**Heerema-McKenney, A. 2020**). the aim of this study detection of CD3 and CD8 in the placenta by Immunohistochemistry technique (**Jingzhu, L.** *et al***.,2018**).

2- Material and methods

Between December 2019 and June 2020, this study was conducted in the Department of Obstetrics and Gynecology in Al- Elwiya and the Al- Yarmouk Teaching Hospital. Each lady in the study received a questionnaire sheet to complete. This study included 20 women with normal blood pressure and 25 women with preeclampsia and preterm labor. Fresh placentas were obtained from women who gave birth vaginally or by cesarean section at hospitals' Departments of Obstetrics and Gynecology shortly after delivery. Anti CD3 and Anti CD8 kits (PathnSitu Biotechnologies/UK) have also been used. After the formalin had been fixed, the immunohistochemical

staining was done. A 5m thick segment was cut from paraffin-embedded tissue blocks. All slides were deparaffinized in xylene and then incubated in phosphate-buffered saline with decreasing ethanol grades. Retrieval of antigen as required by the main antibody. The steps of staining protocol with monoclonal antibodies toward CD3 and CD8 from (PathnSitu Biotechnologies) are as follows:

- Step 1: endogenous peroxidase blocking
- Step 2: primary antibody incubation
- Step 3: poly HRP conjugate incubation
- Step 4: substrate / chromogen
- Step 5: counterstaining
- Step 6: Mounting

The intensity of positive staining with CD3 was graded as Negative 0: <25% of lymphocyte cells showed positive staining. Weak (+1):10- 20 <50% lymphocyte cells showed positive staining. Moderate (+2):20-50-75% of cells showed positive staining. Strong (+3) :>50 % of cells showed positive staining.

3- Results

Immunohistochemical staining method detection of CD3 /lymphocyte in (A) placenta tissue control negative and (B) in Tonsil control positive as shown in figure 1 compared with the staining intensity for PE immunohistochemical show the greatest (+2) intensity was recorded for anti-CD3 lymphocyte marker. With 52% followed by Intensity of (+1) was comparable markers. and (28%) then +3 with 12% and 0 as 8% as shown in table 1and figure 2 and with skin control positive and negative as shown in figure 3 compared with The staining intensity for PE immunohistochemical show The greatest frequency of (0) intensity was recorded for an anti-CD8 cytotoxic T-cell marker with 52 % then +1 (28%) and 20% for +2 as shown in table -2 and figure 4 . and the Differences in immunohistochemical staining intensity in PE women show in figure 5.
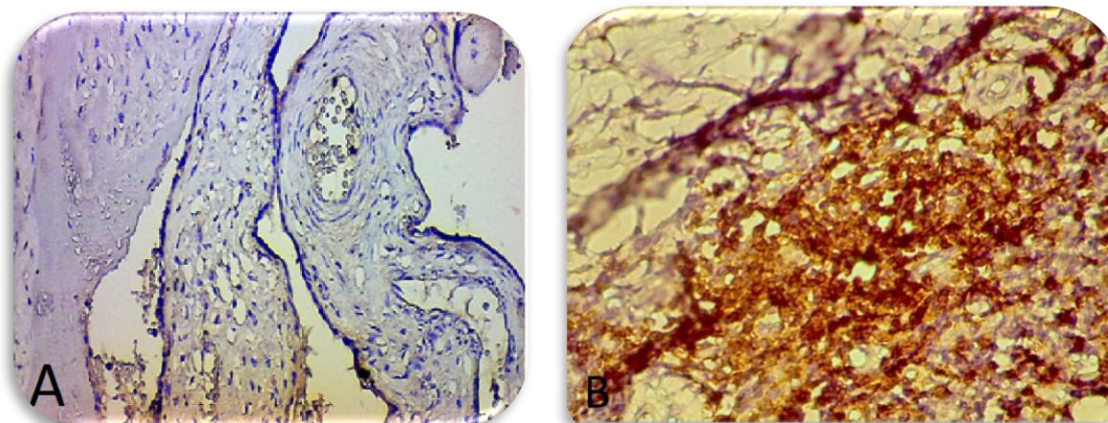
Figure(1): Immunohistochemical staining method detection of CD3 /lymphocyte in (A) placenta tissue control negative and (B) in Tonsil control positive (x10).

Table 1: Intensity of CD3 Lymphocyte expression in placenta tissue with Preterm PE women.

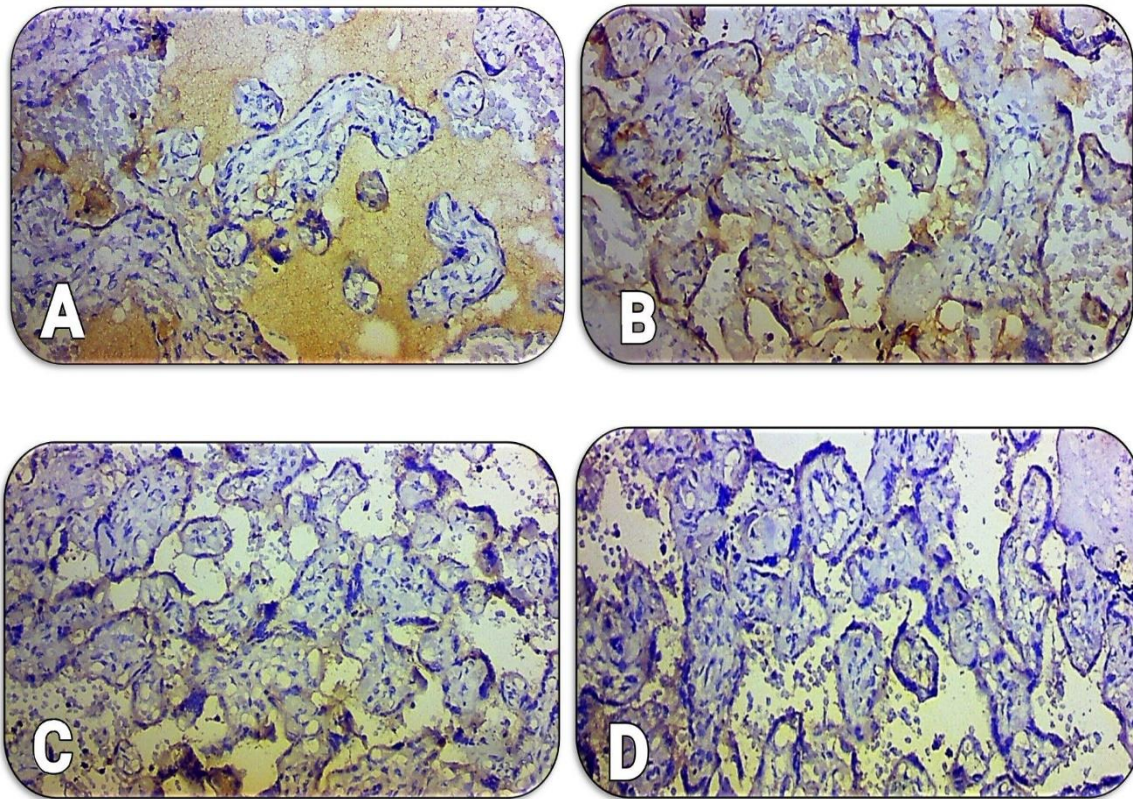| Intensity	No. of CD3 lymphocytes	Anti-CD3 lymphocyte(%)
0	2	8%
+1	7	28%
+2	13	52%
+3	3	12%
Total	25	100%
		Results are expressed as a percentage

Figure 2: (A) Strong (+3) positive cytoplasmic expression. (B) Positive cytoplasmic expression that is moderate (+2); (C) Positive cytoplasmic expression that is mild (+1). (D) Expression that is negative (0) (x10).
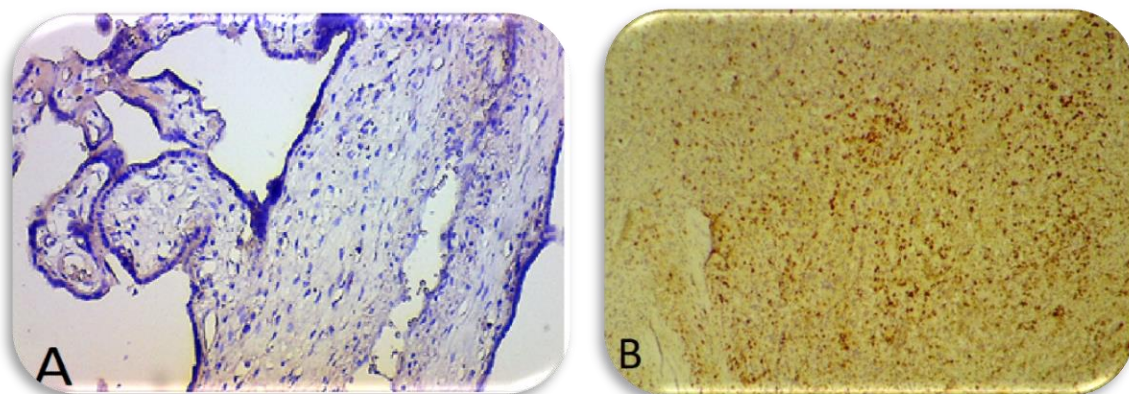

Figure 3: Immunohistochemical staining method detection of CD8 cytotoxic T lymphocyte in (A) placenta tissue control negative and (B) in skin control positive (x10).

Table 2: Intensity of CD8 Cytotoxic T lymphocyte expression in placenta tissue with Preterm PE women.

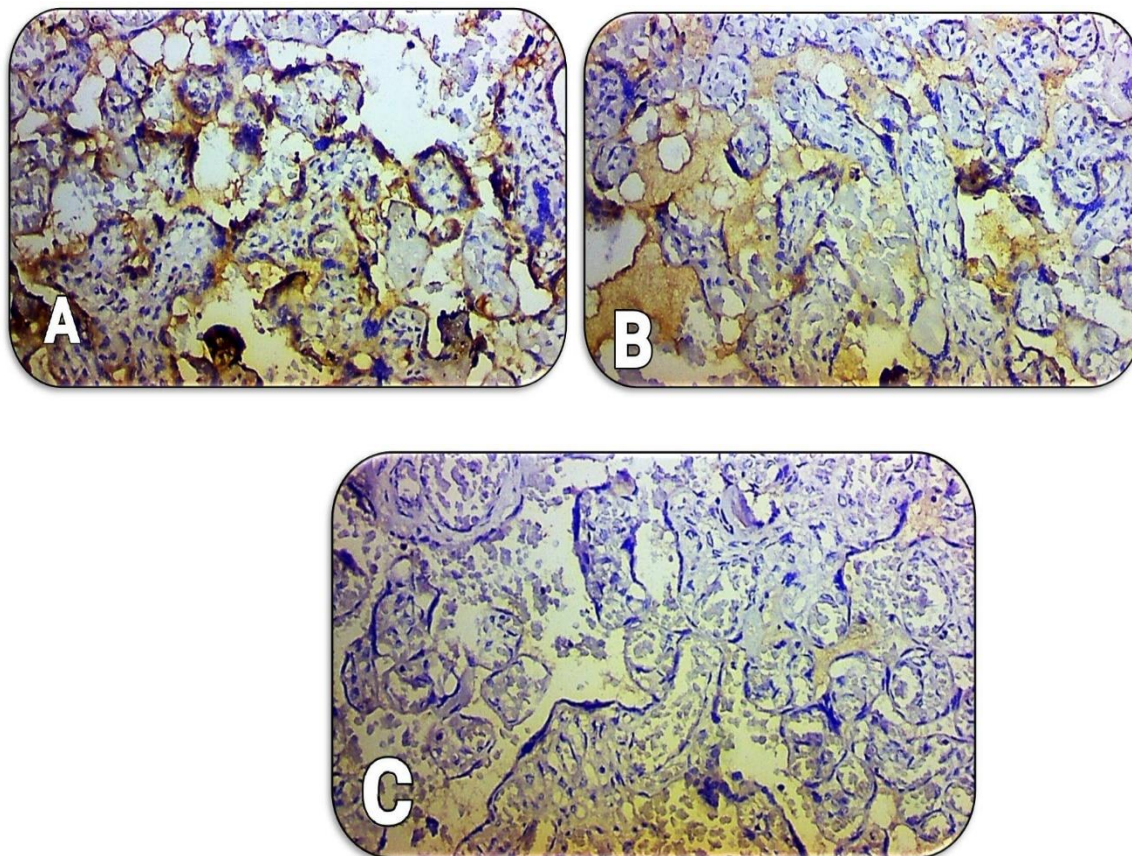| Intensity	Anti-CD8 cytotoxic T-cel1	No of CD8cytotoxic T cel1(%)
0	13	52%
+1	7	28%
+2	5	20%
Total	25	100%
		Results are expressed as a percentage

Figure 4: Immunohistochemical staining method detection of CD8 cytotoxic T lymphocyte in placenta tissue showed: (A) Strong) positive cytoplasmic expression (+2) and (B) Weak (+1)) positive cytoplasmic expression and Negative (0) expression cytoplasmic expression (C).
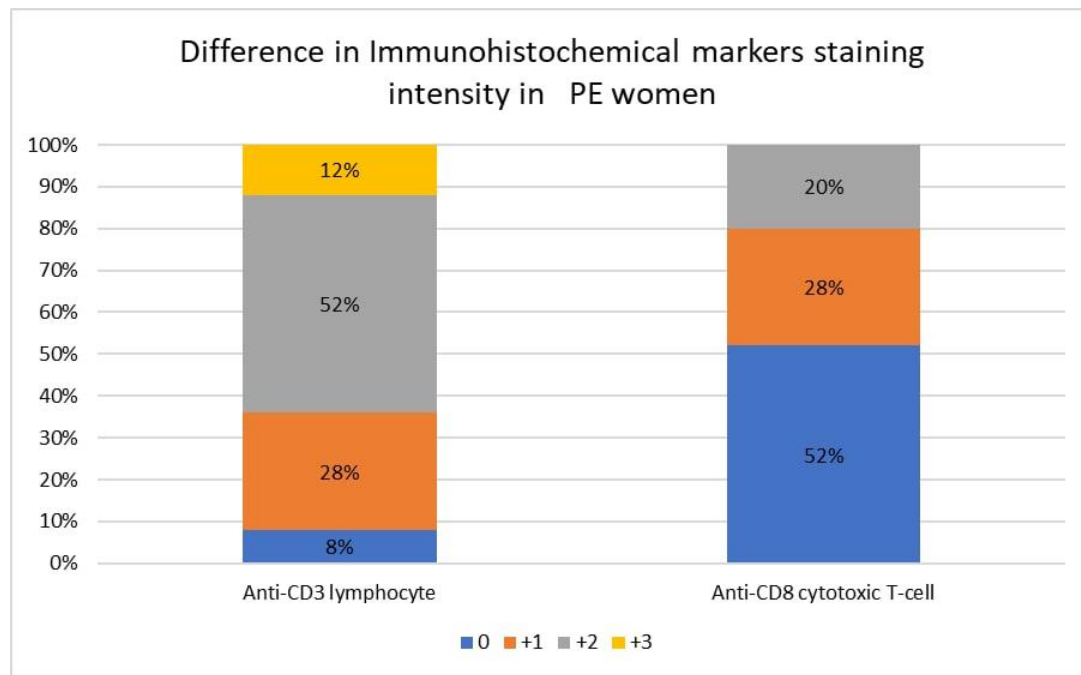
Fig 5: Differences in immunohistochemical staining intensity in PE women

4- Discussion

PE is a placental condition that manifests itself in two stages: abnormal placentation during the first trimester, followed by a second stage referred to as "maternal syndrome" during the second and third trimesters Lymphocytes are critical components of inflammation (**Kamel, H. *et al.*,2020**). Numerous investigators have examined the counts of lymphocyte subsets in PE patients to understand why these patients' immune responses differ (Lager**, S. *et al.,*2020**). It indicates changes in the immune system of the mother linked to pregnancy (**Manoj, K. *et al.*,2020**). The reaction of the placenta may result in inflammatory damage to placental villi, which could result in the villus epithelium being disturbed, thus impeding placental to fetal exchange eclamptic patients may cause an increased immunologic and multiorgan dyscrasia (**Mehmet, O. and Oğlak, S. 2020**), resulting in an increased risk of partial rejection of the fetus (**Milosevic, J. *et al.*,2019**). These results indicate that PE is associated with T helper cell activation (**Perry, H.*et al.*,2018**). Infertility, recurrent miscarriage, fetal growth restriction, preterm birth, and PE are all linked to a lack of immunological adaptation during pregnancy (**Rolnik, D.*et al.*,2020**). and PE has a higher incidence of T cells in the decidua, suggesting that inflammation is a key component of clinically apparent PE (**Roy, H. *et al.*,2018**). The higher expression of CD3 lymphocyte cytoplasm was found to be compatible with the current findings (**Stuart, J.*et al.*,2018**). PE isn't the only factor in improper placentation, Poor placement and PE are found in one-third of preterm pregnancies and fetal pregnancies with a restricted growth rate (**Todros, T. *et al.*,2021**). PE may be caused by maternal constitutional variables such as hereditary, acquired, and environmental factors (**Tom, E. *et al.*,2019**). T cell balance imbalance in the decidua's immunological milieu certainly plays a role in PE development (**Vokalova, L. *et al.*,2020**). Normal pregnancy requires a balance of Th1 and Th2 immune responses, whereas dysregulation causes Th1 cytokine production to predominate, resulting in PE and

miscarriage problems (**Wang, Q.** *et al.,***2019**). CD8 cells also differ in various subpopulations The subset of CD8 T cells may play important roles in maintaining ordinary pregnancy (**Wright, D.** *et al.,***2019**). Therefore, these findings show that the recruitment of these large granular uterine lymphocytes is under hormonal control and not a local response to the presence of the trophoblast (Zheng**, L.** *et al.,***2019**). effector CD8cells which are ready to release cytotoxic cytokines or induce apoptosis via cell surface interaction, and a small population of regulatory CD8 cells which exhibit an immune regulatory (**Einbinder, T.** *et al.,***2018**). most CD8 cells die, however, some proliferate into memory CD8$^+$ cells, In the present study, results are agreed with (**Gerasimova, E.** *et al.,***2019**) that decreased in CD8 cells .also present results agreed with (**Katz, M.***et al.,***2019**) in CD8 memory cell populations did not differ between PE and healthy pregnant women or between formerly PE and formerly healthy pregnant women in lowering of CD8 it could be due to selection of the subtype of PE It has become more and more accepted that early-onset and late-onset PE are different subtypes of PE(34). Also, the present study result disagrees with (**Lorie, M.** *et al.,***2017**) that their results increase CD8 cytoplasmic expression. Premature infants who survive are inflicted with many lifelong handicaps including cerebral palsy and mental retardation PTB's etiology is still unknown (**Milosevic, J.** *et al.,***2019**). Numerous recent studies have indicated that immune changes during pregnancy are correlated with delivery timing, but few of these studies have been conducted in low-income countries, where PTB rates are highest (**Serina, Y. 2018**).

5- Conclusion

Most preterm PE women over the age of 30 and multigravida undergo cesarian section. Immunohistochemical analysis demonstrated an important role for the anti-CD3 lymphocyte marker and anti-CD8 cytotoxic T-cell, both of which show greater prevalence in PE pregnant women compared to NT women.

6- Acknowledgments

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