Original Research Article

The Egyptian Journal of Immunology

The Egyptian Journal of Immunology
Volume 30 (4), 2023: 93–100.
www.Ejimmunology.org

Cluster of differentiation 4/cluster of differentiation 8 ratio of T-lymphocyte subsets in Egyptian patients with severe pre-eclampsia

Tarek A. Karkour[1], Eman A. Abdelfattah[1], Tamer M. Abdel-Dayem[1], Dina A. Kholeif[2], and Mohamed M. Elssaidi[1]

[1]Department of Obstetrics & Gynecology, Faculty of Medicine, Alexandria University, Alexandria, Egypt.

[2]Department of Medical Microbiology & Immunology, Faculty of Medicine, Alexandria University, Alexandria, Egypt.

Corresponding author: Dina A. Kholeif, Department of Medical Microbiology & Immunology, Faculty of Medicine, Alexandria University, Alexandria, Egypt.
Email: dina.kholeif@alexmed.edu.eg.

Abstract

This study was designed to evaluate the immunological role of CD4+Tcells, CD8+ T cells in the pathogenesis of severe pre-eclampsia. Consequently, we estimated their blood levels and the CD4+/CD8+Tcells ratio among patients with pre-eclampsia. The study included 50 primigravid patients in third trimester, recruited from El-Shatby Maternity University Hospital. After obtaining informed written consents, they were divided into two groups: Group A included 25 patients with severe pre-eclampsia, and Group B included 25 normal pregnant women. All patients underwent thorough history taking, complete clinical examination and ultrasound evaluation for fetal condition. Then the percentages of blood CD4+ T cells and CD8+ T cells were estimated via flow cytometry and CD4+/CD8+ T cells ratio was calculated. Patients with severe pre-eclampsia in Group A revealed an increase in CD4+ T cells and a decrease of CD8+ T cells together with an increase in CD4+/CD8+ T cells ratio in comparison with the normal pregnancy (Group B). These differences were statistically significant (p=0.041, p=0.0001 and, p=0.0001, respectively). In addition, there was a positive correlation of blood CD4+ T cells, CD8+ T cells, CD4/CD8 T cells ratio and severe pre-eclampsia. In conclusion, estimation of the percentage of CD4+ T cells, CD8+ T cells and their ratio may be used as a marker to predict pre-eclampsia and confirm its severity.

Keywords: CD4+T cells, CD8+T cells, CD4+/CD8+ ratio, pre-eclampsia

Date received: 18 May 2023; **accepted:** 30 August 2023

Introduction

Pre-eclampsia (PE) is a phenomenon unique to pregnancy that results in extensive vasospasm and vascular endothelial dysfunction. It happens after 20 weeks of pregnancy and can appear up to 4–6 weeks following delivery. Clinically, it is indicated by proteinuria and hypertension, with or without pathologic edoema.[1]

The “National High Blood Pressure Education Program” (NHBPEP) and the American College of Obstetricians and Gynecologists (ACOG) state that pre-eclampsia is defined as the occurrence of a systolic blood pressure (SBP) equal to or

more than 140 mm Hg or a diastolic blood pressure (DBP) more than or equal to 90 mm Hg on two occasions, each at least four hours apart.[1,2] Alongside the increase in blood pressure, proteinuria greater than or equal 0.3 g in a 24h urine, a protein/creatinine ratio of 0.3 or more, or a 1+ proteins by urine dipstick is necessary to identify pre-eclampsia.[3,4]

The pathophysiology of pre-eclampsia is still unknown, but it has recently been suggested that systemic widespread inflammation may play a role. This theory was supported by the presence of activated circulating leukocytes, enhanced release of reactive oxygen species, elevated inflammatory cytokine production, and increased activation of the clotting cascade in preeclampsia compared to healthy pregnancies. Additionally, it is thought that pre-eclampsia is associated with a decrease in the trophoblastic infiltration into the mother decidua, which causes chronic inflammation inside the placenta.[5]

As members of the immunoglobulin superfamily, CD4+ and CD8+ molecules promote adhesion to major histocompatibility complex (MHC) class II and class I molecules, respectively. Additionally, through the T-cell receptors, CD4 and CD8 molecules increase stimulatory impulses.[6,7]

Among the diverse subsets of CD4+T cells there exist T regulatory cells, T helper 1 (Th1), T helper 2 (Th2), and T helper 17 (Th17). Compared to women with normal pregnancies, women whose pregnancies are affected by PE have higher Th1 and Th17 subsets and decreased Th2 and T regulatory subsets.[8]

Th1 cells produce pro-inflammatory interleukin-2 (IL-2) and interferon (IFN), which are engaged in cellular defenses, whereas Th2 cells produce anti-inflammatory IL-4, IL-5, and IL-13, which are essential in humoral immunity. A dominating Th2 profile and decreased Th1-type immunity are associated with normal pregnancy, whereas PE is related with an elevated ratio of Th1:Th2 cells.[9-11]

Controversy surrounds CD8+ T cells' role in a healthy pregnancy. After being stimulated by an antigen, CD8+ T cells can develop into one of numerous CD8 + T cell subsets, each of which has distinct activities. At the fetal-maternal interface, CD8+ T cells appear to be highly differentiated and activated, albeit the precise processes underlying CD8+ T cell activation and differentiation are still unknown. The differentiation process of CD8+ T cells is subject to a variety of influences. In order for CD8+ T cells to differentiate into complete effector cells, the intensity and persistence of the T cell antigen receptor signal are crucial; otherwise, the cells die through programmed cell death or neglect.[12,13] This study aimed to evaluate the immunological role of CD4+, CD8+ T cells and CD4+/CD8+ T cells ratio in the pathogenesis of severe pre-eclampsia.

Subjects and Methods

The study included 50 primigravid women, presented to El Shatby University Maternity Hospital in the last trimester of pregnancy. After an informed and written consent for participation they were categorized into two groups; Group A included 25 patients with severe pre-eclampsia, while Group B included 25 normal pregnant patients as controls. Patients with severe pre-eclampsia were diagnosed according to the criteria of NHBPEP working group on high blood pressure in pregnancy and ACOG. All cases had thorough history taking, thorough clinical examinations, and essential laboratory tests. Full blood count data were obtained from hospital records.

CD4+ T cells and CD8+ T cells estimation from peripheral blood samples was done by using flow cytometry using an automated immunoassay analyzer (BD FACS Canto II flow cytometry, BD Biosciences, USA). The typical baseline reference range of the CD4+/CD8+ ratio was (> 1.0), with CD4 lymphocyte counts (500–1,200/mm3) and CD8 lymphocyte counts (150–1,000/mm3), according to western laboratory references. For immune-competent people, a CD4+ T cell percent of 38–46% and a CD8+ T cell percent of 31–40% were used as the usual range values.[14]

For the assay, a fresh EDTA-blood sample was used. A 50 µl sample of blood was vortexed forcefully after being incubating with 5 µl of each of the anti-CD4 fluorescein isothiocyanate (FITC) (BD-cat. #345768, BD Biosciences, USA) and anti-CD8 PE (BD-cat. #555367, BD

Biosciences, USA) antibodies. The supernatant was removed after incubation and after adding the lysing solution (BD-cat. #349202, BD Biosciences, USA), and the pellet was then suspended in 500 µl of sheath buffer before the sample was collected for analysis using a flow cytometer (BD FACS Canto II flow cytometry, 6 colors, BD Biosciences, USA).[14]

Statistical Analysis

Utilizing the IBM Statistical Package for the Social Sciences (SPSS) software package, version 24.0, data were input into the computer. Qualitative data were described as numbers and percentages. The Chi-square test was used to compare two groups in terms of categorical variables. For normally distributed data, the mean and standard deviation were used to explain quantitative data. For abnormally distributed data, the median, minimum, and maximum were used to communicate the data. The independent t-test was used to compare two independent populations of properly distributed data. Results from significant tests are expressed as two-tailed probability. At the 5% level, the significance of the results was determined. Using the receiver operating characteristic (ROC) curve analysis, an optimal cut-off point value was defined. The sensitivity and specificity for each conceivable value of the cut-off point between cases and controls was determined by the ROC curve. Summary measurements like the area under the ROC curve (AUC) and/or the partial area under the ROC curve are frequently used to assess a biomarker's capacity for diagnosis and predictive usefulness.

Results

Comparing Group A, consisted of 25 primigravid severe preeclamptic patients, and Group B, consisted of 25 normal pregnant women as a control group, showed marked differences as regards ultrasonographic features and fetal growth restriction. (Tables 1 and 2)

Table 1. Comparison of the ultrasonographic (US) findings in the two study groups.

US findings	Group A "Cases" "n=25"		Group B "Control" "n=25"		*p* value
US gestational age (in weeks)					
Range	26-36		33-39		^{t}0.001
Mean±SD	30.74±2.24		36.61±1.45		
Intrauterine Growth retardation (IUGR)	No	%	No	%	^{x2}0.002
	15	60	0	0.0	
US fetal weight (gm)					
Range	900-2900		2000-3900		^{t}0.001
Mean±SD	1686.40±410.03		3121.60±446.02		
Systolic/Diastolic (S/D) ratio					
Range	3.0-6.1		2.1-3.9		^{t}0.001
Mean±SD	5.03±0.90		3.1±0.58		
US: liquor					
	No	%	No	%	^{x2}0.001
Oligohydramnios	15	60.0	0	0.0	
Average	4	16.0	25	100.0	
Drained	6	24.0	0	0.0	

US: ultrasonographic; *t-test = student t-test, X^2= Chi square test, p value was of significance if ≤ 0.05*

Table 2. Comparison of fetal growth restriction in the two studied groups.

	Group A "Cases" "n=25"		Group B "Control" "n=25"		*p* value
Fetal growth restriction	No	%	No	%	
Absent	3	12	25	100.0	^{X2}0.001
Present	22	88	0	0.0	

X^2= Chi square test, p value was of significance if ≤ 0.05

As regards CD4+ T cells, CD8+ T cells parameters, the study showed a statistically significant difference in CD4+ T cells percentage, CD8+cells percentage and CD4+/CD8+ T cells ratio between group A (cases) and group B (control) (p<0.05) (Table 3, Figures 1 and 2).

Table 3. Percentage of CD4+Tcells, CD8+ T cells in white blood cells (WBCs) and CD4+/CD8+ T cells ratio.

	Group A "Cases" "n=25"	Group B "Control" "n=25"	^{t}*p* value
CD4 + T cells percentage in WBCs			
Range	22-52	18-52	0.041
Mean±SD	40.36±7.57	37.28±8.48	
CD8 + T cells percentage in WBCs			
Range	17-35	19-49	0.0001
Mean±SD	22.44±5.14	35.04±8.88	
CD4+/CD8+ T cells ratio			
Range	1-2.94	0.54-2.63	0.0001
Mean±SD	1.87±0.50	1.17±0.51	

WBC: white blood cells; t-test = student t-test. p value was of significance if ≤ 0.05.

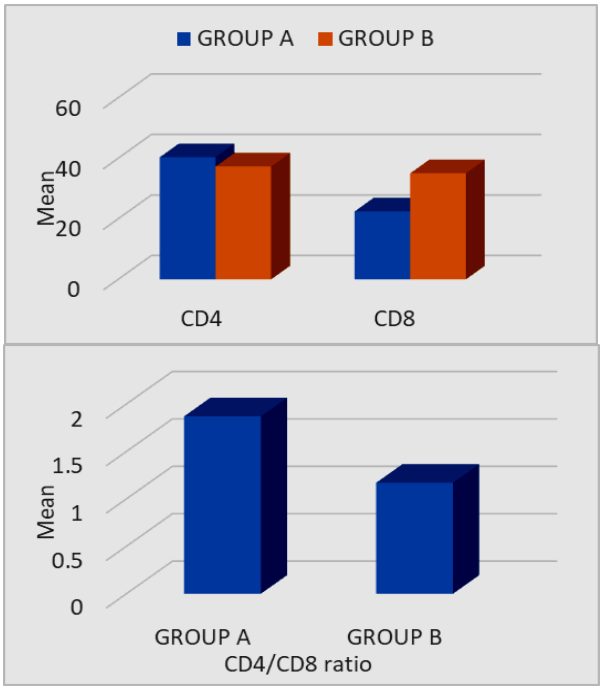

Figure 1. Comparison of CD4+T cell %, CD8+T cell% between the two study groups.

Figure 2. Comparison of the CD4+/CD8+ T cell ratio between the two study groups.

The prediction value of percentage of CD4+ T cells, CD8+ T cells and CD4+/CD8+ T cells ratio in the diagnosis of pre-eclampsia was calculated by the ROC curve analysis. The highest accuracy of prediction was CD8+ T cells, followed by CD4+/CD8+ T cells ratio and then CD4+ T cells (90%, 86%, 62%, respectively). (Tables 4-6, Figures 3-5)

Table 4. Sensitivity, specificity, and accuracy of CD4+ cells in diagnosing pre-eclampsia.

Area Under the curve	Cut off value	Asymptotic Significance	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.617	≥39	0.157	0.460	0.774
Sensitivity		60.0		
Specificity		65.0		
Accuracy		62.0		

Table 5. Sensitivity, specificity, and accuracy of CD8+ cells in diagnosis pre-eclampsia.

Area Under the curve	Cut off value	Asymptotic Significance	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.890	≤ 25	<0.0001	0.801	0.979
Sensitivity		92.0		
Specificity		88.0		
Accuracy		90.0		

Table 6. Sensitivity, specificity, and accuracy of CD4/CD8 ratio to predict pre-eclampsia.

Area Under the curve	Cut off value	Asymptotic Significance	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.844	≥1.5	<0.0001	0.734	0.954
Sensitivity		90.0		
Specificity		82.0		
Accuracy		86.0		

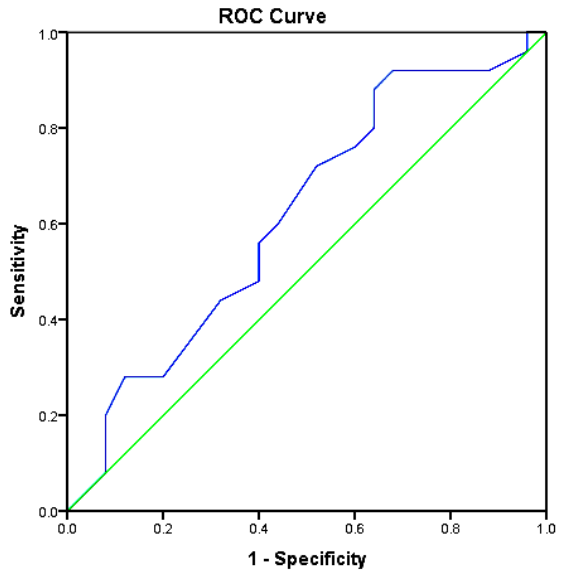

Figure 3. Sensitivity, specificity, and accuracy of CD4+T cells to predict pre-eclampsia.

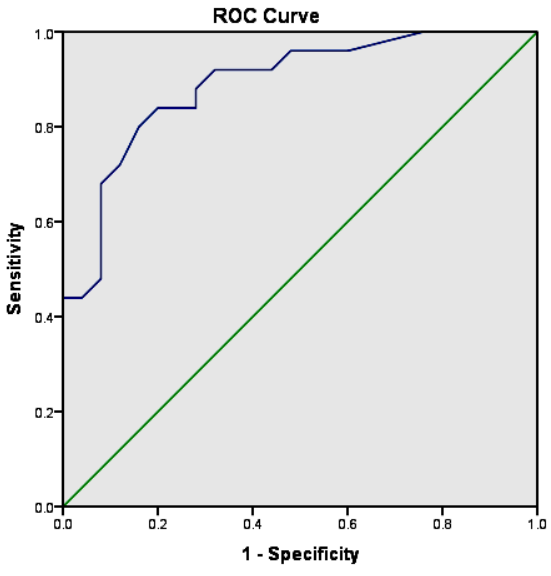

Figure 4. Sensitivity, specificity, and accuracy of CD8+T cells to predict pre-eclampsia.

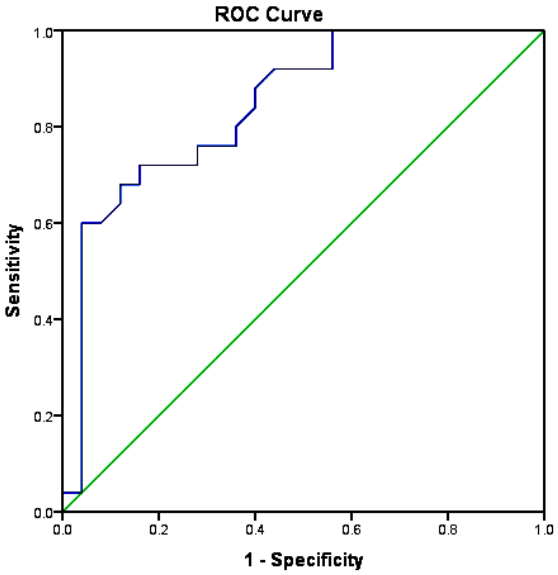

Figure 5. Sensitivity, specificity, and accuracy of CD4+/CD8+ T cells to predict pre-eclampsia.

Discussion

The present study aimed to evaluate the immunological role of CD4+, CD8+ T cells in the pathogenesis of severe pre-eclampsia. Consequently, we estimated their blood levels and CD4+/CD8+ T cells ratio among patients with pre-eclampsia and a control group. In this study, there was a statistically significant difference in severe pre-eclamptic Group A in comparison to normal pregnant control subjects Group B as regards increasing CD4+ T cells and decreasing CD8+T cells and increasing CD4+cells/CD8+cells ratio.

Similar to our findings, Bajnok et al., 2017,[15] found that the ratio of CD4+ T lymphocytes expressing CD122, CD62E, and CD62L was elevated in pre-eclampsia compared to normal healthy pregnancy. Additionally, Benzon et al., 2016,[16] reported that there was a statistically significant difference between pre-eclamptic and healthy pregnancies in the overall CD4+ T cell count and the number of villi invaded with CD4+ T cells. According to their study, there was also a statistically significant difference in the CD4+Tcells/CD8+Tcells ratio between the two study groups (rising in pre-eclampsia). However, contrary to our study finding of a decline in CD8+T cells amongst pre-eclamptic patients, the same study did not find a statistically significant difference in CD8+T cells between their study groups.

Our findings agreed to those indicated by a study of Vianna et al., 2016,[17] which reported that pre-eclamptic patients had higher frequencies of CD4+CD25brightFoxP3+ as well as decreased frequencies of CD8+CD28 cells in comparison to normal healthy pregnancies. In addition, line with the current study findings, it also found that pre-eclampsia patients had greater counts of CD4+CD69+ cells than in normal healthy pregnant women.

According to a study by Malinowski et al., 1994,[18] pre-eclamptic pregnant women had a markedly lower fraction of peripheral blood lymphocytes and CD8+cells and a higher percentage of CD3+T cells and CD4+Tcells than normal pregnant women. As a result, the ratio of CD4+T cells to CD8+T cells increased during pre-eclampsia by twofold compared to normal pregnancy which agreed with the results of our study.

In contrast to the current study findings, Musa et al., 2012,[19] observed that levels of CD3+ T cells and CD4+ T cells in pre-eclamptic patients were significantly lower than those of pregnant controls. Additionally, contrary to our findings, CD3+ and CD4+ T cells were lower in eclampsia and pregnant controls as compared to non-pregnant controls. The results of a previous research by Orlovic et al., 2017,[20] indicated that the total number of CD4+T cells in the decidua basalis of severe pre-eclamptic patients was markedly reduced in comparison with the control group, which disagrees with the findings of the current study. On the other hand, the total number of CD8+T cells in the decidua basalis of severe pre-eclamptic patients was significantly decreased in comparison with the control group, which accords with our study findings.

On the other hand, a research study by Paeschke et al., 2005,[21] found no distinction between pre-eclamptic patients and healthy pregnancy in terms of CD4+Tcell and CD8+Tcell counts. In their investigation, peripheral blood samples from pre-eclampsia patients and healthy pregnant women were collected. Surface antigens CD4+T cells, CD25+T cells, CD8+T cells, and CTLA4 were evaluated in these

samples. Their findings were debatable since they observed that pre-eclampsia was unrelated to variations in the number of regulatory T cells in peripheral blood. Comparing pre-eclamptic with normal pregnancy, their study was unable to show any significant variation in the numbers of CD4+T cells, CD8+T cells, or many other T-cell subgroups.

In conclusion, findings of this study indicated that the blood percentages of CD4+Tcell, CD8+Tcell, and CD4+/CD8+ T cells ratios were significantly different in severe pre-eclamptic patients compared to normal control cases, with increased CD4+Tcells and decreased CD8+T cells**.** The percentage of serum CD4+T cells, CD8+Tcells, and the CD4/CD8 T cells ratio were positively correlated with severe pre-eclampsia**.** Therefore, estimation of CD4+Tcells and CD8+Tcells may be utilized as a marker to anticipate pre-eclampsia and validate the severity of the condition.

Author Contributions

TAK, EAA, TMA, DAK contributed to the study concept and design, DAK and MME contributed to the clinical part, material collection, immunological assay, and interpretation of the results. DAK wrote the manuscript draft, TAK, EAA, TMA revised the manuscript. All authors have approved the manuscript as submitted.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) denies receipt of any financial support for the research, authorship, and/or publication of this article.

Ethical approval

The study protocol was reviewed and approved by the Research Ethical Committee of the Faculty of medicine, Alexandria University (approval no. 0106442, dated July 2020).

Informed consent

A signed consent form was obtained from each study participant.

References

1. American College of Obstetricians and Gynecologists, Task Force on Hypertension in Pregnancy (2013). Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. *Obstet Gynecol.,* 122 (5):1122-31.
2. Bartsch E, Medcalf KE, Park AL, et al. (2016). Clinical risk factors for pre-eclampsia determined in early pregnancy: systematic review and meta-analysis of large cohort studies. High Risk of Preeclampsia Identification Group. *BMJ,* 353: i1753.
3. Morris RK, Riley RD, Doug M, et al. (2012). Diagnostic accuracy of spot urinary protein and albumin to creatinine ratios for detection of significant proteinuria or adverse pregnancy outcome in patients with suspected pre-eclampsia: systematic review and meta-analysis. *BMJ*, 345: e4342.
4. Mattar F, Sibai BM. (2000). Eclampsia. VIII. Risk factors for maternal morbidity. *Am J Obstet Gynecol*, 182(2):307-12.
5. Shakerian B, Irvani S, Mostafavi S, et al. (2022). Quantitative serum determination of CD3, CD4, CD8, CD16, and CD56 in women with primary infertility: The role of cell-mediated immunity. *Turk J Obstet Gynecol.*19(3): 242–5.
6. Chard T, Lilford R et al. (1998). Basic Sciences for Obstetrics and Gynaecology, 5th ed. London: *Springer*, 87-92.
7. Miceli MC, Von Hoegen P, Parnes JR. (1991). Adhesions versus co-receptor function of CD4 and CD8; role of the cytoplasmic tail in co-receptor activity. *Proc Natl Acad Sci.* 88(7): 2623.
8. Saito S, Umekage H, Sakamoto Y, et al. (1999). Increased T-helper-1-type immunity and decreased T-helper-2-type immunity in patients with pre-eclampsia. *Am J Reprod Immunol*, 41(5):297–306.
9. Saito S, Nakashima A, Shima T, et al. (2010). Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy. *Am J Reprod Immunol,* 63(6):601–10.
10. Saito S, Sakai M. (2003). Th1/Th2 balance in preeclampsia. *J Reprod Immunol*, 59(2):161–173.
11. Amaral LM, Cunningham MW, Cornelius DC, et al (2015). Preeclampsia: long-term consequences for vascular health. *Vasc. Health Risk Manag*, 11: 403–15.

12. Scaife PJ, Bulmer JN, Robson SC, et al (2006). Effector activity of decidual CD8+ T lymphocytes in early human pregnancy. *Biol. Reprod*, 75: 562–56.
13. Kaech SM, Ahmed R (2001). Memory CD8+ T cell differentiation: initial antigen encounter triggers a developmental program in naive cells. *Nat. Immunol*, 2: 415–22.
14. Savvidou MD, Lees CC, Parra M, et al. (2002). Levels of C-reactive protein in pregnant women who subsequently develop pre-eclampsia. *BJOG*, 109(3):297-301.
15. Bajnok A, Ivanova M, Rigó J, et al. (2017). The Distribution of Activation Markers and Selectins on Peripheral T Lymphocytes in Preeclampsia. *Mediators of Inflammation*, 80:45-61.
16. Benzon S, Zekić Tomaš S, Benzon Z, et al. (2016) Involvement of T lymphocytes in the placentae with villitis of unknown etiology from pregnancies complicated with preeclampsia. *J. Matern.-Fetal Neonatal Med*, 29(7):1055-60.
17. Vianna P, Mondadori AG, Bauer ME, et al. (2016). HLA-G and CD8+ regulatory T cells in the inflammatory environment of pre-eclampsia. *Reproductio*n, 152(6):741-51.
18. Malinowski A, Szpakowski M, Tchórzewski H, et al. (1994). T lymphocyte subpopulations and lymphocyte proliferative activity in normal and pre-eclamptic pregnancy. *Eur. J. Obstet. Gynecol. Reprod. Biol*, 53(1):27-31.
19. Musa BOP, Onyemelukwe G, Olatunji O, et al. (2012). Serum cytokine levels and T lymphocyte subsets in pregnant women with eclampsia. *Open J. Immunol*, 2:116-24.
20. Orlovic Vlaho M, Tomić V, Vukojevic K, et al. (2017). Decreased expression of MMP-9 in CD8(+) cells in placenta with severe preeclampsia. *Biotech. Histochem*, 92:1-9.
21. Paeschke S, Chen F, Horn N, et al. (2005). Pre-eclampsia is not associated with changes in the levels of regulatory T cells in peripheral blood. *Am. J. Reprod. Immunol*, 54(6):384-9.