Expression of certain activation markers, CD45RA, CD45RO and CD11b on the surface of peripheral blood lymphocytes isolated from patient with idiopathic preterm labour.

Nidhal Abdul Muhymen[1] *PhD*, **Maha M. Al-Bayati**[2] *MBChB* ; *CABOG*, **Thoraya Hosaam Al-din**[3] *MBChB*.

Abstract

Background: preterm labor (PL) is remaining the leading cause of non-anomalous prei natal mortalities.

Objective: is to determine the association of PL on the expression of certain activation markers on the surface of peripheral blood lymphocytes (PBLs).

Patients and methods: Thirty patients with idiopathic pre term labour (IPL) (group A) in addition to 30 healthy pregnant women of comparable gestational age groups (group B) were enrolled in this study. Blood samples were taken from both groups and lymphocytes were separated and stained with fluorescent labeled monoclonal antibodies against CD45RA, CD45RO and CD11b.

Results: results indicated that there were a significant increase in the percentage of CD45RA in group A and reduction in the percentage of both CD45RO and CD11b in the same group.

Conclusions: patients with IPL have a less tendency of the activity of lymphocytes.

Key words: Idiopathic premature labour, activation markers, CD45RA, CD45RO, CD11b.

IRAQI J MED SCI, 2010; VOL.8 (3):20-24

Introduction

Preterm labour (PL) is the major cause of prenatal mortality and morbidity [(1)]. It is one of the most serious problem facing obstetrician and other perinatal health care [(2)]. About 90% of births occur between 37 and 42 weeks, this period is called term [(3)]. The etiology of PL is multifactorial, but in majority of instances the precise cause are unknown [(4)]. This is known as idiopathic PL which makes up at least 75% of the cases. The uterus is not immunologically privileged site; it is well vascularized with good lymphatic drainage and can reject foreign tissues [(5)].

The human decidua contains an un-usually high proportion of lymphocytes, mainly NK and T cells, which are potentially cytotoxic to trophoblast when they are stimulated with certain cytokines [(5)]. It was found that there are a higher proportion of dicidual and peripheral lymphocytes that expressed activation markers in spontaneous abortions than in elective termination of pregnancy [(6)].

Hence in the current study we intended to study some of activation markers that expressed on the surface of PBLs in patients with IPL in comparison with healthy pregnant women.

Materials and methods

Thirty blood samples from IPL women (group A), attending the Department of Obstetrics and Gynecology in AL-Khadhemia teaching hospital were collected. Other thirty blood samples taken from healthy pregnant women with

[1]Dept. Medical Microbiology, College of Medicine, Al-Nahrain University, [2] Dept. Gynecology & Obstetrics, College of Medicine, Al-Nahrain University, [3] Dept. Gynecology & Obstetrics\Al-Kadhimiya Teaching Hospital.

Address Correspondence to: Dr. Nidhal Abdul-Mohymen.

E- mail: dr.nidhalmohammed@yahoo.com

Received: 3th November 2008, Accepted: 18th June 2009.

comparable gestational age with no evidence of PL.

Lymphocyte separation and staining: Blood sample (Five ml venous blood) was aspirated from all patients and controls. Blood was collected in pyrogen-free silicone-coated tubes with heparin. The blood samples were used for lymphocyte separation according to Isopaque-ficoll technique (originally described by Boyum in 1968).

Mouse monoclonal Ab (primary Ab) specific for human CD45RA; CD45RO and CD11b and biotinylated secondary antibody (anti-mouse Ab) were used.

Slides were examined under 400X-magnification power of light microscope. The dark brown (homogenous or membranous) staining identified positive labeled cells.

Statistical analysis: chi – square test and students t –test were used to analyze the results.

Results

As shown in figure 1, the percentage of CD45RA antigen on PBMC of group A was significantly higher ($p<0.00069$) than that of the percentage expressed by group B, while the expression of the other molecule (CD45RO) on these cells was significantly lower ($p<0.0005$) in group A than that expressed by group B, as shown in figure 2.

Mean while, the expression of CD11b molecule was significantly reduced ($p<0.0001$) on the surface of PBMC of group A compared with that of group B, as shown in figure 3.

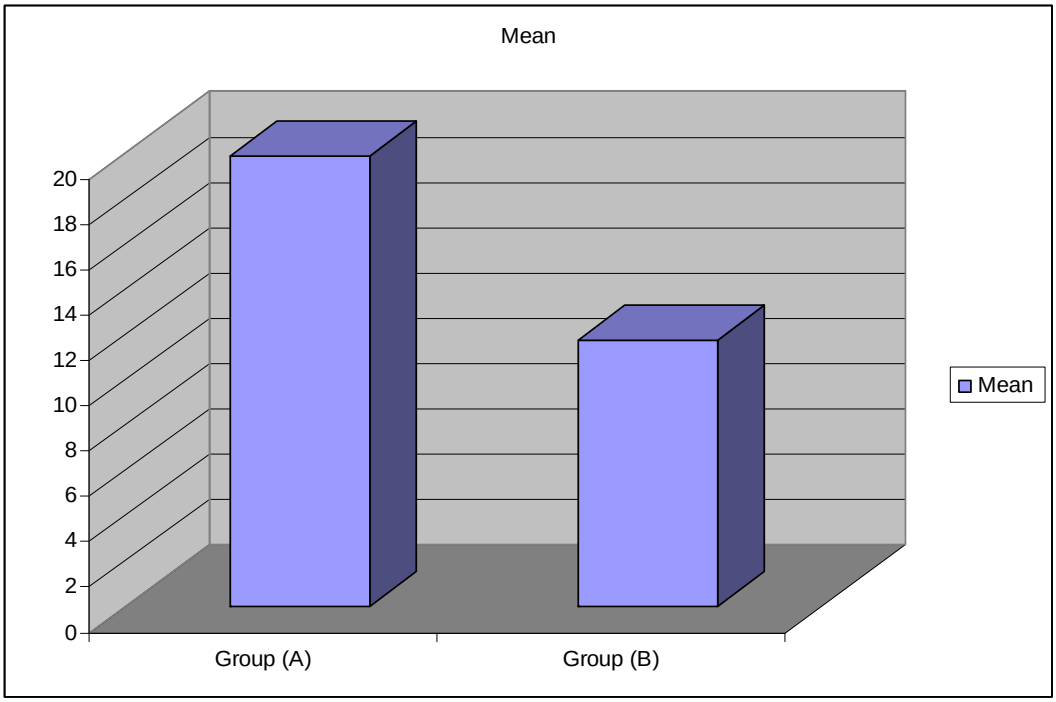

Figure 1: Percentage of naïve cells in group (A) compared with group (B).

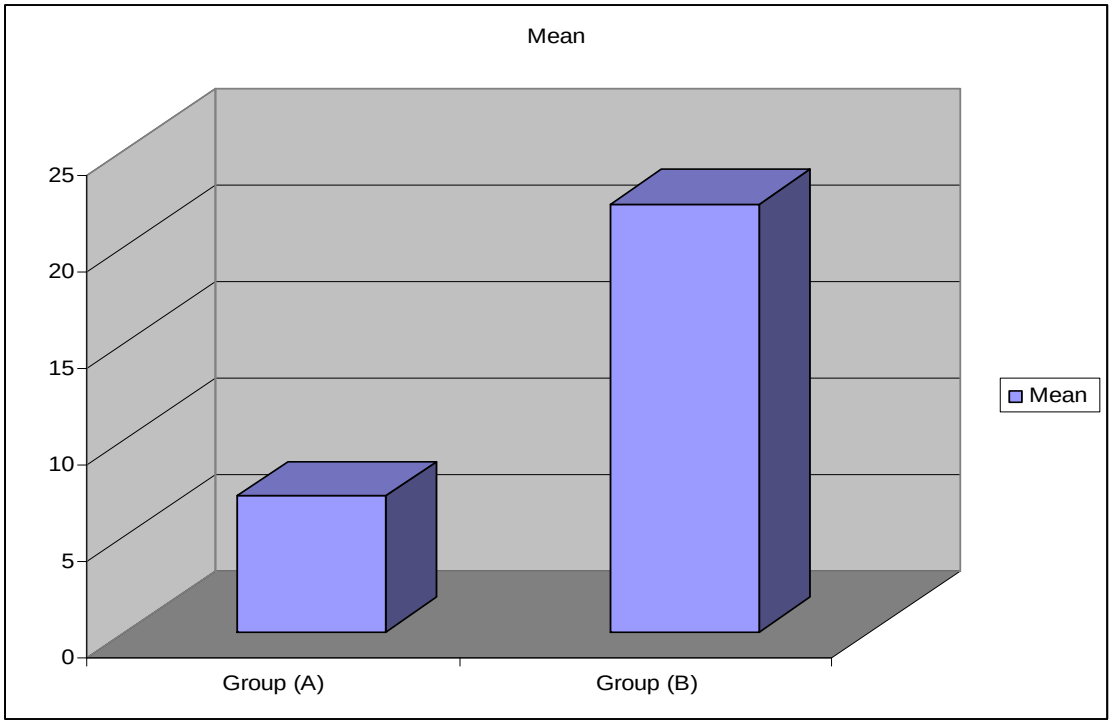

Figure 2: Percentage of memory T-cells in group (A) compared to group (B).

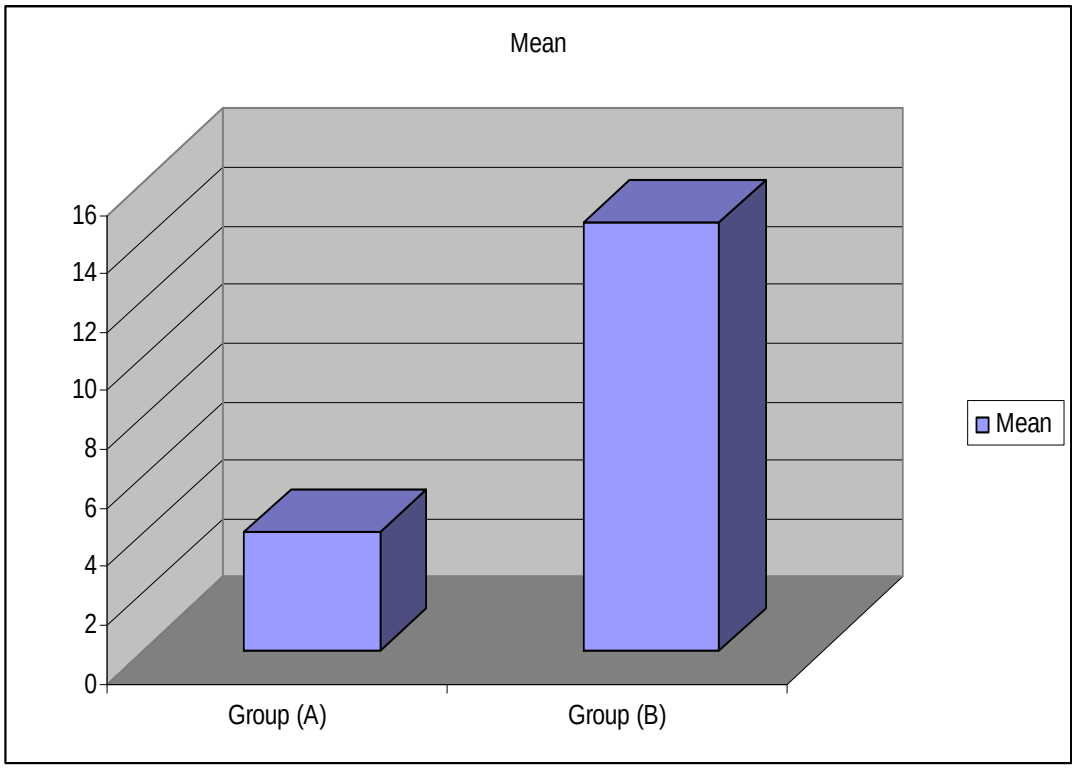

Figure 3: Percentage of intercellular adhesion molecule receptor (CD11b) in group (A) compared to group (B).

Discussion

There is growing interest in the use of mononuclear cells surface markers for the diagnosis of different disorders syndromes [8]. Understanding the impact and physiologic factors, such as age, pregnancy and stress on PMNC surface markers, is essential for appropriate interpretation of results.

Based on the results, equilibrium between CD45 isoforms (RO and RA) exist on the surface of PMBC. Total CD45 phosphatase activity in a cell is determined by this equilibrium, which in turn controlled by isoform expressed Naive T cells express CD45RA isoforms indicating a resting cells [9], while the expression of CD45RO isoforms mean a shifting to activated T cells [10]. So in the case of our patients the predominant type of cells were in its naive form this result can be explained by the fact that HLA-G (MHC-lb) can suppress proinflammation of T lymphocytes [11, 12] beside a membrane -bound HLA-G, a soluble counterpart (sHLA-G) may play an important in the immunological establishment of pregnancy by affecting peripheral immune cells and modulating their function for the benefit of pregnancy [13, 14]. It was found that embryos which secreted sHLA-G gave rise to successful pregnancy [15] by the above finding we can conclude that the embryos of our patients may have low levels of sHLA-G which have an effect on PBMC. Concerning the results of CDllb expression which has been reported herein to be decreased in comparison with group B. this antigen (CDllb) form a hetero dimer with CD 18 and both will be the receptor for complement component fragment receptor. C3b and will be called CR3. CR3 is important in adhesion (It is ligands for ICAM,intra cellular adhesion molecules and phagocytosis[16]. CDllb expression has been reported to be normal or increased in pregnant women [17-21].

However, pregnant women delivering prematurely have consistently shown a higher expression of CDllb [22, 23].But we can explain the decrease in the expression in our patients, by the fact that this antigen has an extensive, intracellular storage pool, which could be released to the surface with activation or excessive manipulation.

References

1. Sviges JM, Robinson R. Threated and actual preterm labour, high risk pregnancy: management options, 2nd edition, 1999; p: 999-1010.

2. morrison J J. Prediction and prevention of preterm labour, Progress in Obstet. and Gyn., volume 12,1996; P. 67-85.

3. Steer PJ. Preterm labour, Dewhurst's text book of Obstet. and Gyn.., Sixth edition, 1999; P. 291-297.

4. Bennett P, Edwards D. Use of magnesium sulphate in obstetrics. Preterm labour: recent advances in understanding of pathophysiology, Use of antibiotics to prevent preterm birth. Am J Obstet Gynecol 1997; 177:375-380

5. Enrique G. Olivares, Raquel Munoz, German Tejerizo, Maria Jose Montes et.al, Decidual Lymphocytes of Human Spontaneous Abortion Induce Apoptosis but Not Necrosis in JEG-3 Extravillous Trophoblast Cells, Biology of Reproduction, 2002,67,1211-1217.

6. Bulmer JN. Immune cells in deciduas. In: Kurpisz M, Fernandez N (eds.), Immunology of Human Reproduction. Oxford: Bios Scientific Publishers Ltd.; 1995: 313-334.

7. Boyum A, Lymphocyte separation, Scan J. Clin. Lab. Invest. 1968: 21, (97).

8. Tarek M. Elghetany and Francis Lacombe. Physiologic variations in granulocytic surface antigen expression: impact gender, pregnancy, race, and stress. Journal of Leukocyte Biology. 2004; 75:157-162.

9. Hermiston ML, Xu Z. and Wees A. CD45: A critical regulator of signaling thresholds in immune cells, Ann. Rev. of Immunol. 2003: 21, 7-137.

10. Bleesing J J. and Fleisher TA, Human B cells express a CD45 isoform that is similar to murine B220 and is downregulated with an acquisition of the memory B cell marker CD27. Clin Cytometry, 2003. 51:1-8.

11. Bainbridge DR, Ellis SA, and Sargent I L, HLA-G suppresses proliferation of CD4 (+) T-lymphocytes. J. Reprod. Immunol, 2000: 48, 17-26.

12. Kanai T, Fujii T, Unno N, Yamashita T, Hyodo H, Miki A, et al.Human leukocyte antigen-G- expressing cells differently modulate the release of cytokines from mononuclear cells present in the decidua versus peripheral blood. Am. J. Reprod. Immunol, 2001: 45, 94-99.

13. LeBouteiller P, Solier C, Proll J, Aguerre-Girr M, Fournel S and Lenfant F. Placental HLA-

G protein expression in vivo: where and what for? Hum. Reprod. Update, 1999: 5, 223-233.

14. Solier C, Aguerre-Girr M, Lenfant F, Campan A, Baerrebi A, Rebmann V, Grosse-Wilde H, and Le Bouteiller P. Secretion of proapoptotic intron 4-retaining soluble HLA-GI by human villous trophoblast. Eur. J. Immunol., 2003: 32, 3576-3586.

15. Fuzzi B, Rizzo R, Criscuoli L, Noci I, Melchiorri L, Scarslli B, Bencini E, Menicucci, A, and Baricordi OR. HLA-G expression in early embryos is a fundamental prerequisite for the obtainment of pregnancy. 2002: Eur. J. Immunol., 32,311-315.

16. Veenstra van Nieuwenhoven A L,.Heineman M J and Faas M M. The immunology of successful pregnancy. Human Reproduction Update, 2003; Vol.9, No.4, 347-357.

17. Allan P.KNUSTEN. Complement receptor deficiency.2006, emedcine, from webMED. MIDLINE.

18. Thilaganathan B, Makrydimas G, Plachouras N, Nicolaides K H. Neutrophils and monocytes p2-integrin expression in maternal and fetal blood. Am. J. Obstet. Gynecol. (1995)172, 58-62.

19. Esparaza B, Sanchez H, Ruiz M, Barranquero M, Sabino E, Merino F. Neutrophil function in elderly persons assessed by flow cytometry. Immunol. Invest.1996; 25,185-190.

20. Crouch S P M, Crocker I P, Fletcher J. The effect of pregnancy on polymophonuclear leukocyte function. J. Immunol. 1995; 155, 5436-5443.

21. Naccasha N, Gervasi M-T, Chaiworapongsa T, Berman S, Yoon B H, Maymon E, Romero R. Phenotypic and metabolic characteristics of monocytes and granulocytes in normal pregnancy and maternal infection. Am. J. Obstet. Gynecol. 2001; 185, 1118-1123.

22. Gervasi M T, Chaiworapongsa T, Naccasha N,Blackwell S, Yoon BH, Maymon E, Romero R.Phenotypic end metabolic characteristics of maternal monoctes and granulocytes in preterm labor with intact membranes. Am.J Obstet.Gynecol , 2001;1.185,1124-1129

23. Cowland J B. Granules of the human neutrophilic polymorphonuclear leukocytes. Blood,1997; 89, 3502-3521.