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Immune modulations during gestation periods

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ABSTRACT

Immune alteration during pregnancy is associated with many complications including fetus abortion, anogenital abnormalities, and autoimmune disease. This study aimed to observe immunomodulatory effects for pregnant women during their pregnancy periods. A total of 150 pregnant women were taken for the study which was divided into three groups. Pregnant women in the third and fifth months of gestation provided samples for the first and second groups, respectively. Finally, samples were taken from eighth-month-pregnant women for the third and final group. The one hundred and fifty samples were drawn from pregnant women aged between 30 to 40 years old. The concentration of immunological markers such as TNF-α, IFN-γ, IL-17A, anti-phospholipid (APL) (IgM, and IgG), and anti-cardiolipin (ACL) (IgM, and IgG) was estimated using the ELISA method, which utilizes enzyme-linked immunosorbent assay. Furthermore, blood tests for serum immunoglobulins (IgM, IgG, IgA) were investigated by using Single Radial Immuno Diffusion (SRID). Also, the Westergren method was performed to measure the Erythrocyte sedimentation rate (ESR) level. The concentration of immunological markers such as TNF-α, IFN-γ, IgA immunoglobulin, anti-phospholipid (APL) IgM antibody, APL IgG antibody, and anti-cardiolipin (ACL) IgG antibody and ESR level were higher in 3rd trimesters compared to 1st trimester gestational periods. Our result demonstrates that the 3rd trimester can show the highest risk for obstetric complications due to the highest immunological markers responses compared to the first and second trimesters.

1. INTRODUCTION

Pregnancy loss has become increasing in the world, genetic factors and environmental factors including alcoholism, smoking, medication, thromboembolism, and infection in the uterus are the main factors in the developing fetus abortion [1- 4]. Immune alteration during pregnancy exerts many complications including

fetus abortion, anogenital abnormalities, and autoimmune disease [1], [4], [5]. Infectious diseases are a threatening population, especially pregnant women, and children are at higher risk due to susceptibility to infection. Immunological changes are the main reason that adversely affect on the pregnant women's health which results in various diseases and fetus abnormalities [2- 5]. Fetal-intrinsic or extrinsic abnormalities are developed from all stages of pregnancy, especially implantation and placental development [1- 5]. There are many factors including genetic, endometrium immunological imbalance, endometrial abnormalities, and abnormal blood clotting which can be distributed to the implantation failure [6], [7]. Immunological alterations during pregnancy have become a global disease in the world. The immune modulation has many adverse impacts on women's health and their fetus including autoimmune diseases for instance rheumatoid arthritis, systemic lupus erythematosus and autoimmune thyroid disease, and women's infection resulting in abortion [8].

Neonates with placental infection present high levels of cytokines including Interleukin (IL) -1, IL-6, IL-8, and Tumor necrosis factor-alpha (TNF-α), which are involved in the development of fetal inflammatory response syndrome. Fetal pro-inflammatory cytokines, including TNF-α, and Interferon gamma (INF-γ), may have roles in fetus abnormalities. In the neonatal periods, inflammatory response syndrome and fetus morphological abnormalities may lead to the development of autism, schizophrenia, neurosensorial deficits, and psychosis [2]. IL-17A is a cytokine produced by T-helper (Th)17 cells, and involved in the influx of leukocytes to the site of infection, and, it has a significant role against infection, autoimmune physiology, and cardiovascular disease [9]. Antiphospholipid Antibody Syndrome or APS is an autoimmune disorder, normal components of blood and/or cell membranes can be recognized as foreign substances and produces antibodies against them resulting in blood clots, including heart attacks and strokes, and miscarriages. Antiphospholipid antibodies are initiated due to an innate inflammatory process. It has been shown that anticardiolipin phospholipid IgM and IgG were increased in pregnant women resulting in loss of pregnancy, and venous and arterial thrombosis [10], [11].

Furthermore, endothelial-derived nitric oxide (NO), which is initiated by endothelial NO synthesis, is involved in endothelial function and vascular health. Plasma nitrite levels and an impaired endothelium-dependent vascular response were developed due to the initiation of antiphospholipid antibody syndrome resulting in cordial dysfunction and miscarriage. Endothelial dysfunction is associated with complement activation which leads to placental injuries in female patients and obstetric diseases [12]. This study was performed on pregnant women in Iraq- Baghdad, the aim was to investigate immunological markers changes during different stages of pregnancy and this was to understand the mechanism behind immune modulation and its complication in pregnant women in the first, second and third trimesters.

2. Material and Methods

2.1 Ethical issue

The all-proposed protocol and procedures in the current study were reviewed and approved by the medical laboratory science departments' scientific committee and the director of the research Centre at the University of Raparin in Rania city at Raparin district, Kurdistan Region, Iraq.

2.2 Experimental design

Hundred and fifty pregnant women between the ages of 30 and 40 years old, were participated in this study. During the month of April 2019, blood samples were taken in Baghdad city. All tests were performed in NASA clinical laboratory, Baghdad, Iraq. The study was composed of three groups. Samples were taken from pregnant women in the first, second, and third trimesters, respectively (third, fifth, and eighth months).

2.3 Methods

Under sterile conditions, the samples were collected in plain tubes (8 ml in total from each participant, which was divided into 6 ml for the serological tests and 2 ml for the ESR test). Centrifugation at 1000 g for 30 minutes at room temperature used in the separation of the sera from the blood. Before analysis, aliquots of the samples were prepared and held at a temperature of (-20°C). ELISA technique was achieved to measure the concentration of TNF-a, IFN-γ, IL-17A, ALP (IgG, and IgM), and ACL (IgG, and IgM). In addition, Immunoglobulins (IgA, IgM, and IgG) were performed by using Single Radial Immunodiffusion. Furthermore, the ESR parameter was tested by using the Westergren method.

2.4 Statistical analysis

The statistical analysis was carried out using the Statistical Package for Social Science (SPSS) V22 software package. A one-way analysis of variance was used to evaluate the facts (ANOVA). P value (0.05) was determined statistically significant for all the findings.

3. Results

In the present study, 150 pregnant women were recruited after adjusting for age, weight, and presence of comorbidity. The mean age of the whole study participants was 35.25 years old. Proinflammatory cytokines are increased during pregnancy and they can have a dramatic effect on pregnancy complications [13]. In this study, there were significant increases of the inflammatory mediators, at the level ($P < 0.05$) for TNF-α and IFN-γ, but there was no significant elevation for the IL-17A in the 3rd gestational period compared to the 1st gestational period. In addition, TNF-α, IFN-γ, and IL-17A cytokines did not have significant differences between the 1st and 2nd trimester, also the 2nd and 3rd gestational period (Table 1). Therefore, these data revealed that most of the inflammatory responses in pregnant women will happen in the 3rd trimester compared to the 1st and 2nd trimesters (Table 1).

Furthermore, immunoglobulins changes are linked to the release of pro-inflammatory cytokines [14]. Consequently, immunoglobulins (IgM, IgG, and IgA) were measured. Our study showed that immunoglobulin IgA (Table 2) increased significantly in 3rd gestational compared to 1st and 2nd gestational periods. However, immunoglobulins IgM and IgG did not demonstrate significant differences in the trimesters (Table 2).

Antiphospholipid immunoglobulins (IgM and IgG) are linked with pregnancy complications, and thrombosis [15]. Thus, antiphospholipid immunoglobulins IgM and IgG were investigated. Our result revealed higher levels (significantly different $P < 0.05$) of immunoglobulins IgM and IgG in the 3rd trimesters compared to the 1st and 2nd trimesters (Table 2).

In addition, anticardiolipin immunoglobulins are linked with autoimmune disease and miscarriages [16]. Hence, anticardiolipin immunoglobulins IgM and IgG were achieved, and the results showed a higher level of IgM (Table 2) in the 3rd trimester compared to 2nd and 1st trimesters (but not significantly elevated); however, anticardiolipin immunoglobulin IgG was significantly different in comparison of 1st and 3rd trimester (Table 2). It appears that antiphospholipid and anticardiolipin immunoglobulins are increased in 3rd trimesters.

Finally, ESR is an inflammation marker and it is level increased during pregnancy as it has adverse effects [17]. For that reason, ESR test was performed, the result showed higher ESR level in 3rd trimesters compared to 2nd and 1st trimesters (Table 2). Which indicates that inflammation is a higher risk during 3rd stage pregnancy.

Table 1. Shows the pregnant women's levels of TNF-, IFN-, and IL-17A (pg/dl) during the study periods.

Pro-inflammatory cytokine markers	**Third month (M±SE)**	**Fifth month (M±SE)**	**Eighth month (M±SE)**
TNF-α) (pg/dl)	10.9933* ±0.57211	13.9744* ±0.64928	15.7544* ±0.76183
IFN-γ) (pg/dl)	31.3878* ±3.37980	39.9144* ±4.57829	48.8511* ±5.52699
IL-17A) (pg/dl)	37.6078 ±3.67030	40.8011 ±3.90623	48.4844 ±4.95682

* Statistical differences were deemed to be significant at P value <0.05.

Table 2. Shows the pregnant women's levels of some inflammation markers during the study periods.

Inflammation markers	**Third month (M±SE)**	**Fifth month (M±SE)**	**Eighth month (M±SE)**
IgM (mg/dl)	75.7000 ±11.85737	80.0222 ±13.10344	84.3000 ±13.06211
IgG (mg/dl)	671.1656 ±155.47804	722.1311 ±166.30220	755.4645 ±169.00511
IGA (mg/dl)	50.2000* ±4.59031	81.0678 ±5.14660	96.9678* ± 6.49470
APL (IU/ml) (IgG)	9.1000* ±0.67746	11.2000 ±0.67835	12.3000* ±0.68730
APL (IU/ml) (IgM)	12.1000* ±0.67746	11.4344 ±0.98199	14.4344* ±0.43345
ACL (IU/ml) (IgM)	8.4344 ±1.30196	9.4345 ±0.98199	11.0000 ±0.10000

ACL (IU/ml) (IgG)	7.3333* ±0.33333	8.3333 ±0.88192	10.3333* ±0.66667
ESR (mm/hr)	10.0000* ±1.93216	15.0000 ±0.57735	30.0000* ±3.88599

* Statistical differences were deemed to be significant at P value <0.05.

4. Discussion

Immunological alterations during pregnancy have led to popular conditions in the world. There are many outcomes of immunological disorders including rheumatoid arthritis, systemic lupus erythematosus, autoimmune thyroid disease, fetal despise, and obstetric complications [12]. One hundred and fifty pregnant women were investigated, fifty women for each 1st (three months), 2nd (five months), and 3rd (eight months) trimesters were compared to each other. Immunological and inflammatory markers were investigated including TNF-α, IFN-γ, and IL-17A, and sera's IgM, IgG, and IgA, also APL (IgM and IgG), and ACL (IgM and IgG), finally, ESR parameters during the mentioned pregnancy periods. The main purpose of this study was to investigate whether these immunological and inflammatory markers will be altered during the first, second, and third trimesters or not and to understand immunological changes risks during each trimester and compared to each other.

In the first and second trimesters, massive immunological changes will commence because of the initiation of implantation and placentation during pregnancy, blastocytes can be implanted and they damage endometrial tissue, also trophoblast is initiated to manage placental fetal blood supply, in addition, the inflammatory response is necessary to remove cellular debris and repair of uterine epithelium. A pro-inflammatory response is developed in the 1st trimester, and an anti-inflammatory response appears during the 2nd trimester, because of that nausea and sickness will gradually disappear in the mother, as the immune alteration will not be affected by the hormonal changes.

While, in the 3rd trimester, proinflammatory response redevelops due to complete fetus status and it is required to be delivered. Therefore, it appears that the proinflammatory response can be necessary to expulse the fetus and reject the placenta. In addition, protein C helps to control blood clotting, but the deficiency of protein C may cause blood clots to form in veins, and this activated protein C system resistance which is linked to venous thrombosis, also may be the reason for repeated miscarriages [18].

Thrombin level elevation is associated with the increase of pro-inflammatory cytokines such as TNF-α because of the alteration of the activated protein C system [19].

Inflammatory markers such as TNF-α, and IFN-γ were increased significantly in the third trimester compared to 2nd and 1st trimesters. Moreover, TNF-α, and IFN-γ were increased significantly in the 2nd trimester compared to the 1st trimesters.

Furthermore, IL-17A tended to be highest in the 3rd trimester compared to the 1st and second trimesters. Obtained results from this study were similar to the previous studies that measured cytokines levels which were increased significantly in pregnant women. This revealed that immune modulation is starting from the 3rd month of pregnancy period including the increase of cytokines such as IL-6, and IL-1, and reaches the top in the late 3rd month's pregnancy [20], [21].

A clear reason is that immune modulation and switching macrophage properties from anti-inflammatory to pro-inflammatory response in the pregnancy period, and the prevalence of the highest level of cytokines in the 3rd trimester including IL-17A, TNF alpha, and INF gamma due to infection in the pregnant women and time of the parturition. The increase of the cytokines contributes to the uterus muscle contraction which is important for the childbirth delivery process [21], [22].

There is evidence for the elevation of inflammatory cytokines including IL-1β and IL-8 in the maternal-fetal tissues, TNF signaling pathway production, Nod-like receptors production, and activation of NFκB during the parturition process [23], [24]. It has been shown that anti-cardiolipin and antiphospholipid antibodies (IgM and IgG) were increased in pregnant women resulting in loss of pregnancy, autoimmune disease, and thrombosis [15], [25]. This coincides with our result showing antiphospholipid antibodies, anti-cardiolipin IgM, and IgG increases significantly in the 3rd trimester compared to the second trimester and 1st trimester, respectively.

This is probably due to the highest level of immune modulation in the partition time and can be assessed that autoimmune diseases and loss of fetal are highest in the late 3rd pregnancy trimester. It was concluded that there is a potential for the investigation of immunological markers to study autoimmune diseases, thrombosis, and fetal abortion, our result demonstrates that 3rd trimester can be the highest risk for obstetric complications due to the highest immunological markers.

However, the current study has some limitations that must be taken into consideration, such as the low number of recruited pregnant women, techniques such as Real-time polymerase chain reaction, and few other immunological markers. For instance, IL-10 is also an anti-inflammatory marker and is not included in this study.

5. References

[1] Kwak-Kim, J., J.W. Kim, and A. Gilman-Sachs, Immunology and Pregnancy Losses, in Immunology of pregnancy. 2006, Springer. p. 303-315.

[2] Mor, G. and I. Cardenas, The immune system in pregnancy: a unique complexity. American journal of reproductive immunology, 2010. 63(6): p. 425-433.

[3] Yockey, L.J. and A. Iwasaki, Interferons and proinflammatory cytokines in pregnancy and fetal development. Immunity, 2018. 49(3): p. 397-412.

[4] Moore, K.L., T. Persaud, and M. Torchia, Human birth defects. The Developing Human: Clinically Oriented Embryology. Saunders. 2013, Elsevier, Philadelphia, PA.

[5] Festin, M., G. Limson, and T. Maruo, Autoimmune causes of recurrent pregnancy loss. The Kobe Journal of Medical Sciences, 1997. 43(5): p. 143-157.

[6] Aplin, J.D. and P.T. Ruane, Embryo–epithelium interactions during implantation at a glance. Journal of cell science, 2017. 130(1): p. 15-22.

[7] Sadler, A.J. and B.R. Williams, Interferon-inducible antiviral effectors. Nature reviews immunology, 2008. 8(7): p. 559-568.

[8] Adams Waldorf, K.M. and J.L. Nelson, Autoimmune disease during pregnancy and the microchimerism legacy of pregnancy. Immunological investigations, 2008. 37(5-6): p. 631-644.

[9] Griffin, G.K., et al., IL-17 and TNF-α sustain neutrophil recruitment during inflammation through synergistic effects on endothelial activation. The Journal of Immunology, 2012. 188(12): p. 6287-6299.

[10] Meroni, P.L., et al., Pathogenesis of antiphospholipid syndrome: understanding the antibodies. Nature Reviews Rheumatology, 2011. 7(6): p. 330-339.

[11] Derksen, R., et al., How to treat women with antiphospholipid antibodies in pregnancy? Annals of the Rheumatic Diseases, 2001. 60(1): p. 1-3.

[12] Corban, M.T., et al., Antiphospholipid syndrome: role of vascular endothelial cells and implications for risk stratification and targeted therapeutics. Journal of the American College of Cardiology, 2017. 69(18): p. 2317-2330.

[13] Raghupathy, R., Cytokines as key players in the pathophysiology of preeclampsia. Medical Principles and Practice, 2013. 22(Suppl. 1): p. 8-19.

[14] Wilson, R., et al., Abnormal immunoglobulin subclass patterns in women with a history of recurrent miscarriage. Fertility and sterility, 2001. 76(5): p. 915-917.

[15] Di Prima, F.A., et al., Antiphospholipid Syndrome during pregnancy: the state of the art. Journal of prenatal medicine, 2011. 5(2): p. 41.

[16] Spegiorin, L.C.J.F., et al., Prevalence of anticardiolipin antibodies in pregnancies with history of repeated miscarriages. The open rheumatology journal, 2010. 4: p. 28.

[17] Brigden, M.L., Clinical utility of the erythrocyte sedimentation rate. American family physician, 1999. 60(5): p. 1443-1450.

[18] Dean, E. and E.J. Favaloro, The changing face of activated protein C resistance testing—a 10-year retrospective. Ann Blood, 2020. 5: p. 1-7.

[19] Ku, D.-H.W., et al., Circulating levels of inflammatory cytokines (IL-1β and TNF-α), resistance to activated protein C, thrombin and fibrin generation in uncomplicated pregnancies. Thrombosis and haemostasis, 2003. 90(12): p. 1074-1079.

[20] Griffith, O.W., et al., Embryo implantation evolved from an ancestral inflammatory attachment reaction. Proceedings of the National Academy of Sciences, 2017. 114(32): p. E6566-E6575.

[21] Robinson, D.P. and S.L. Klein, Pregnancy and pregnancy-associated hormones alter immune responses and disease pathogenesis. Hormones and behavior, 2012. 62(3): p. 263-271.

[22] Menon, R., et al., Novel concepts on pregnancy clocks and alarms: redundancy and synergy in human parturition. Human reproduction update, 2016. 22(5): p. 535-560.

[23] Christiaens, I., et al., Inflammatory processes in preterm and term parturition. Journal of reproductive immunology, 2008. 79(1): p. 50-57.

[24] Rinaldi, S., et al., Immune cell and transcriptomic analysis of the human decidua in term and preterm parturition. MHR: Basic science of reproductive medicine, 2017. 23(10): p. 708-724.

[25] Opatrny, L., et al., Association between antiphospholipid antibodies and recurrent fetal loss in women without autoimmune disease: a metaanalysis. The Journal of Rheumatology, 2006. 33(11): p. 2214-2221.