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Evaluation the Physiological Effect of Pregnancy on Some Immunological Parameters for Pregnant Women

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Abstract: *The present study was designed to focus on some immunologicalchanges accompanied pregnancy. The sample included 120 women who were attending Al-Batool hospital for Gynecology and Children in Baqubah city during the period from September 2014 to May 2015. Women divided into two groups: Pregnant women include 60 pregnant women with apparently healthy pregnancy; their ages range between 17-37 years, Age Mean of them was 26.95 years. Non-pregnant women include 60 women their ages range between 17-37 years. Age Mean of them was 27.13 years, and they considered as control group. The immunological parameters studied were included (IL-2, IgA, IgG, IgM, C3, C4, WBC, Neutrophils, Monocytes and Lymphocytes). The results showed the following features: A significant (P<0.001) decrease in the serum levels of (IL-2, IgA, IgG, C3, C4 and Lymphocytes) in pregnant women compared to control group, also significant (P<0.05) decrease in the level of IgM in the serum of pregnant women as compared with control group.Furthermore there were a significant (P<0.001) increase in the levels of (WBC count, neutrophils and monocytes percentage) in the plasma of pregnant women as compared with control group.*

Key word:*Pregnancy, IL-2, IgG, IgM, C3.*

I. Introduction

Pregnancy is the state of a female after conception until the birth of the baby (Gacek , 2009). The length of pregnancy is considered to be 280 days, or 40 weeks after the onset of the last normal menstrual cycle or, more accurately, 266 days or 38 weeks after fertilization (Sadler , 2012).Normal pregnancy is characterized by profound changes in almost every organ and system to accommodate the demands of fetoplacental unit (Ifukor*et al.* , 2013). Pregnancy is one of the most important periods in human life with hormonal, immunological, vascular, metabolic and psychological changes (Akkoca*et al.* , 2014). Pregnancy induces several physiological adaptations to meet the needs of the developing fetus and the health requirements of the mother (Jiang *et al.* , 2012). With 50% of its genetic material derived from its father, Successful pregnancy has been considered a biologic example of semiallogeneic graft acceptance, in which the semiallogeneic fetus is protected from immune attack from the mother (Chen *et al.* , 2012), therefore pregnancy represent a unique immunological period for the mother (Oliveira *et al.* , 2012). The immunological paradox of pregnancy relies on a careful balance of both immune tolerance and immune suppression (Sykes *et al.* , 2012). The maternal immune system undergoes profound transformations already at the very beginning of pregnancy. These prominent changes are directed to protect the fetus from a detrimental immune response (Zenclussen , 2013). The hormonal and immunological changes that occur during pregnancy affect susceptibly to and the outcome of autoimmune and infectious disease (Robinson and Klein , 2012). An increase in infectious disease severity and complications during pregnancy has been reported (Strubl*et al.* , 2012), as well as hospitalization and treatment, may be lower for pregnant women than for other patients (Kourtis*et al.* , 2014). The understanding of these adaptations to pregnancy remains a major goal of obstetrics, and without such knowledge, it is almost impossible to understand the disease processes that can threaten women during pregnancy (Gohel*et al.* , 2013).

The Th1/Th2 hypothesis was the dominating explanation model for immune regulation during pregnancy for at least a decade, and this model still provides, along with more recently added mechanisms, a foundation for explaining fetal tolerance (Ernerudh*et al.* , 2011). Therefore, pregnancy is define as a 'Th2' state or 'Th2' phenomenon (Warning *et al.* , 2011).

IL-2 is a monomer of 15.5kD and consists of 133 amino acids.IL-2, discovered more than 30 years ago in supernatants of activated T cells, is mainly produced by CD4 and CD8 T cells, and to a lesser extent by activated DCs and NK cells. IL-2 acts as a B-cell growth factor, and promotes proliferation and differentiation of NK cells to increase their cytolytic functions cells (Akdis*et al.* , 2011). IL-2 is a potent activator of T cell proliferation and in its absence T cells tend to enter an inactive state. In addition, secreted IL-2 acts as a paracrine growth factor for Tregulatory cells. Therefore, it is important for instigating the proliferation of T cells

and in maintaining both inflammatory and regulatory T cells population. IL-2 can function in synergy with INF-γ or IL-4 to promote the production of antibody by activated plasma cells (Williams *et al.* , 2012).

The aims of the present study were to estimate changes in some immunological indicators in apparently healthy pregnant women compared with apparently healthy non-pregnant women (control) by studying(IL-2, IgA, IgG, IgM, C3, C4, total WBC, neutrophils, lymphocytes and monocytes).

II. Martial And Method

The study included 120 women divided into two groups: Pregnant women were included 60 pregnant women with apparently healthy pregnancy, their ages range between 17-37 years. Age Mean of them was 26.95 years. Non-pregnant women: included 60 non-pregnant women their ages range between 17-37 years. Age Mean of them was 27.13 years, and they considered as control group.Samples were collected from those individuals only if they were not having a history of a chronic or acute illness.

From each woman, 5 ml of venous blood was collected from a suitable vein. Blood sample divided into two parts, 2 ml of fresh blood was put in sterile tube contains Ethylene diamine tetra acetic acid (EDTA) to estimate the total and differential of WBC, and 3ml was transferred to sterile plain gel tube and let to clot and then centrifuged at 5000 r.p.m. for 10 minutes at room temperature to separate the serum, The serum dispensed into two sterile tightly closed Eppendorf tubes and stored at (-20°C) until analysis time to estimate immunological parameters.

The level of IL-2 was measured in serum samples (pregnant and control groups) by using Enzyme linked immunosorbent assay (ELISA) technique , This was performed as described in the leflet of kit provided by Cosabio company.

The method of Mancini et al. (1962) was employed for the measurement of immunoglobulin classes (IgA, IgG, IgM) and complement components (C3 and C4). The examined protein, diffusing in agarose gel containing a specific antibody will form an immune-complex, visible as a ring around the well. The ring diameter is direct proportional to the concentration of the analysis protein. The proportion corresponds to the diffusion time. The square of diameter will be in linear proportion to the concentration of the sample (Mancini and Coll , 1965).Withthe plates are supplied a reference table in which each diameter of the halo is associated a concentration.

Total and differential WBC were makes with automated method by ABX Pentra DX 120 (Horiba Company) as an automatic laboratory analysis system. This automotive test system characterized by speed and exactness, whereas it can estimate blood cells numbers as electric pulses.

Statistical Analysis

Data Analysis was computer aided. Statistical analysis was done using SPSS (Statistical Package of Social Science) version 20 computer software. Frequency distribution and percentage for selected variable were done. The independent t-test was used and P-Value (less than 0.05, 0.001) was considered as the level of significance (Nisi , 2004)

III. Results

Table (1) shows that there were no significant ($p>0.05$) differences in the mean age between the studied groups. The mean age of pregnant women was 26.95 years compared with the mean age of non-pregnant women (control) was 27.13 years.

Table (1): The mean of ages in pregnant and control groups (mean ± SD).

Studied Groups	No.	Age (Years)		P-Value
		Range	Mean±SD	
Pregnant women	60	17-37	26.95± 5.58	> 0.05
Control	60	17-37	27.13± 6.14	

The serum levels of IL-2, C3 and C4 for both pregnant women and control groups are shown in table (2). There is a significant ($p<0.001$) decrease in the mean of serum IL-2, C3 and C4 levels in pregnant women (8.20 ± 5.06 pg/ml, 98.15±16.73, 19.87±8.12 respectively) when compared with the mean of control group (13.27 ± 7.03 pg/ml, 115.92±32.95, 26.60±7.12 respectively).

Table (2): Serum IL-2, C3 and C4 levels in pregnant women and control groups (mean ± SD)

Parameter	Pregnant (mean ± SD)	Control (mean ± SD)	P-value
IL-2 (pg/ml)	8.20 ± 5.06	13.27 ± 7.03	< 0.001
C3 mg/dl	98.15±16.73	115.92±32.95	< 0.001
C4 mg/dl	19.87±8.12	26.60±7.12	< 0.001

The serum levels of (IgA, IgG, IgM) and count of (WBC, Leucocyte, Monocyte, Lymphocyte) for both pregnant women and control groups are shown in table (3). There is a significant ($p<0.001$) decrease in the mean of serum (IgA, IgG) level and lymphocytes percentage in pregnant women (124.0±47.74 mg/dl , 605.02±177.59 mg/dl , 20.70±4.83% respectively) when compared with the mean of control group (197.13±91.77 mg/dl , 1080.37±235.09 mg/dl , 30.95±5.19% respectively). Also table (3) shows a significant ($p<0.05$) decrease in the mean of serum IgM level in pregnant women (183.73±60.85 mg/dl) when compared with the mean of control group (219.53±83.19 mg/dl). In addition, table (3) shown a significant ($p<0.001$) increase in the mean of (WBC, Neutrophils, Monocytes) in pregnant women (7.43±1.61 X 10^9/L, 69.82±5.44 % 5.82±1.49 % respectively) when compared with the mean of control group (6.13±1.04 X 10^9/L, 59.33±5.90 %, 4.99±0.93 % respectively).

Table (3): Levels of (IgA, IgG, IgM, WBC, neutrophils, lymphocytes and monocytes) in pregnant women and control groups (mean ± SD).

Parameter	Pregnant (mean ± SD)	Control (mean ± SD)	P-value
IgA (mg/dl)	124.0±47.74	197.13±91.77	< 0.001
IgG (mg/dl)	605.02±177.59	1080.37±235.09	< 0.001
IgM (mg/dl)	183.73±60.85	219.53±83.19	< 0.05
WBC (X 10^9/L)	7.43±1.61	6.13±1.04	< 0.001
Neutrophils (%)	69.82±5.44	59.33±5.90	< 0.001
Lymphocytes (%)	20.70±4.83	30.95±5.19	< 0.001
Monocytes (%)	5.82±1.49	4.99±0.93	< 0.001

IV. Discussion

Studies in humans have shown that during pregnancy, peripheral immune responses are changed as compared with peripheral immune responses in non-pregnant women. The present study confirms and extends previous hypothesis showing that the percentage of producing IL-2 is decreased in pregnant women as compared with non-pregnant women. This results in a Th1/Th2 balance, which is shifted toward Th2 cytokine production (Ernerudh*et al.* , 2011 ; Warning *et al.* , 2011 ; Mor and Cardenas , 2010). Results of this study are in agreement with this hypothesis, at less from direction of decline of Th1during pregnancy.

High levels of IL-2 detected in rejecting allografts (Chatterjee*et al.* ,2014). When administered to human subjects, IL-2 elicited multiple toxic side effects (Orvieto*et al.* , 2014). IL-2 can promote the regulatory T cells in playing immunosuppression function. During pregnancy, IL-2 activate T cells to promote secretion of HLA-Class II molecules and variety of cytokines, and also activate natural killer (NK) cells, which can enhance the secretion of inflammatory factors, make the embryo being rejected (Pei *et al.* , 2013). The Th1 cytokines are deleterious, leading to an inflammatory response and placental necrosis, thus it can compromise fetal and/or placental development. While, Th2 cytokines are beneficial for pregnancy, promoting proliferation and differentiation of the trophoblastic cells and placentation, have a protective role on the feto-placental unit, inhibiting the production of Th1 cytokines (Feliciano *et al.* , 2014). Th1 cytokines inhibit trophoblast invasion, stimulate apoptosis of human trophoblast cells, and enhance decidual macrophage activity, all of which result in the production of factors harmful to the embryo (Lee *et al.* , 2011). Cytokines such as IL-2 act directly on the decidual stromal cells and increase myometrial contractility (Desai , 2007), Therefore pregnant women produced lower levels of IL-2 than women with recurrent spontaneous abortion (RSA) (Ernerudh*et al.* , 2011). Whereas reduce IL-2 may play a role in maternal tolerance of the fetal allograft (Sutton et al. , 2004). Furthermore, normal amniotic fluid has been reported not contains levels of IL-2. The IL-2 mRNA is not produced in the utero-placental region. This absence of IL-2 is likely to benefit fetal survival since IL-2 administered to mice causes abortion and IL-2-activated endometrial granulated lymphocytes can lyse human trophoblast in vitro (Searle *et al.* , 2000).

During pregnancy Th1 cytokine production is down-regulated, whereas IL-2 production is under genetic control in humans (Pei *et al.* , 2013). The decrease level of Th1 cytokines associated with the presence of factors that inhibit the production of Th1 cytokines (IL-2) and these factors are important in the proliferation and differentiation of the trophoblastic cells and placentation and play a protective role on the fetal-placental unit (Feliciano *et al.* , 2012). Also steroid hormones are reportedly involved in modulating cytokine secretion by different T cell subsets. Progesterone and estradiol are proposed to influence the Th1/Th2 balance favoring aTh2 pre- dominance at the fetal- maternal interface in humans and mice. Hormonal effects are mainly mediated via their classical receptors expressed by human and murine T cells (Schumcher*et al.* , 2014 ; Zenclussen , 2013). Moreover depression of pro-inflammatory cytokines was associated with high HCG serum levels. The effect of HCG on cytokine production is not entirely clear (Kruse *et al.* , 2000). Furthermore Th1 and Th2 cells are mutually inhibitory to each other; when Th1 reactivity is high, Th2 reactivity is usually low and vice versa (Aris*et al.* , 2008).

The complement activation is correlated with poor pregnancy outcomes such as preeclampsia and preterm birth, leading to the proposal that C inhibition is an ‘‘absolute requirement’’ of normal pregnancy (Girardi*et al.* , 2011). C activation, in particular, has emerged as a common event in recurrent pregnancy loss (Girardi*et al.* , 2006). It has been proposed that C activation during pregnancy leads to a pro-inflammatory, pro-coagulant and tissue damaging environment surrounding the fetus (Denny *et al.* , 2013). C activation in the absence of infections would seem to be in direct conflict with physiologic needs of both the mother and the fetus during pregnancy, since C activation can promote inflammation, cell lysis and anti-angiogenesis (Denny *et al.* , 2013 ; Girardi*et al.* , 2006). Thus, while C plays a key role in protecting both the mother and fetus from potential infection (Girardi*et al.* , 2011), excessive C activation due to infection can contribute to disease pathogenesis and be very dangerous to the fetus (Conroy *et al.*, 2011 ; Holmberg *et al.* , 2012). Delicate regulation of complement activation is critical for a successful pregnancy (Gilbert *et al.* , 2012). Thus, disturbances in C regulation can lead to such catastrophic consequences. Central to these is C attack against endogenous tissue structures, endothelial cells and blood cells that can lead to vascular damage and organ failure, notably in kidneys. Pregnancy is a well-known potential trigger for such syndromes (Lokki*et al.* , 2014). Placenta is particularly susceptible to C-dependent damage because trophoblast exposure to maternal blood and extensive tissue remodeling in maternal decidua promote C activation during physiologic pregnancy (Bulla *et al.* , 2012). Recognition of paternal antigens on the surface of syncytiotrophoblast could activate the complement cascade, resulting in death of trophoblastic cells (Thellin*et al.* , 2000). Complement dependent damage of trophoblasts may promote cell destruction or more likely increased permeability of the barrier, opening the way to bacteria, virus, and other toxic molecules that compromise fetal survival. No wonder if decidual cells have developed strategies to prevent C-mediated damage (Varea*et al.* , 2014). Trophoblast cells have protective mechanisms which allow them to avoid complement activation consisting in the expression of C regulatory proteins (Crry) (Tincani*et al.* , 2010). Cytotrophoblasts express the three regulatory proteins of the complement, these being decay-accelerating factor (CD55), membrane cofactor protein (CD46), and CD59 which avoids the formation of the MAC and subsequent cell lysis. Thus, excessive complement activation is prevented in successful human pregnancies thanks to the presence of these three regulatory proteins in trophoblast membranes (Varea*et al.* , 2014). CD46 and CD55 inhibit C3 convertase activity while CD59 inhibits Membrane Attack Complex (MAC) formation, thus protecting the foetus from complement mediated maternal reactions (Zen *et al.* ,2010). Thus, evolutionary balance between protecting the mother and fetus from infection, and protecting the fetus from the effects of C activation will be necessary to succeed pregnancy.

One possible mechanism for diminished neutralization capacity of C due to dilution of C factors that occurred as a result of the 40–50% increase in blood volume that occurs during pregnancy. Other possible mechanism by which C alterations might occur during pregnancy and postpartum is through changes in sex hormones such as progesterone and estrogen, both of these sex hormones peak during pregnancy at 20–30 times normal peak cycling levels (Mayer and Parks , 2014), the up-regulation of C3 appeared to be influenced by estrogens (Richani*et al.* , 2005).

Hypothesis texted that markers of innate (nonspecific) immunity will be higher, and adaptive (memory) immunity markers will be lower in pregnant women compared with non-pregnant women (Miller , 2009).

Results of present study are in agreement with this hypothesis, whereas this results show an increase in biomarkers related to innate immunity (total WBC, neutrophils and monocytes) and a decrease in biomarkers related to adaptive immunity (IgA, IgG, IgM and lymphocytes) during pregnancy. Thus this study support and agreement with this hypothesis.

There are several possible explanations for this dual pattern (increase nonspecific and reduce specific immunity). First, it could allow the entire immune system to retain desirable levels of immune system reactivity, neither underreacting nor overreacting to threatening pathogens (Kourtis*et al.* , 2014). It may also help prevent a systemic immune overreaction to the fetus. There may also be a second energetic explanation. Research indicates that the innate immune system is less energetically costly to maintain than the adaptive immune system (Lochmiller and Deerenberg , 2000). Mothers may increase the activity of the innate immune system and decrease the activity of the adaptive immune system to reserve energy for pregnancy. In sum, since pregnancy is accompanied by a vast array of immunological changes that allow the mother to tolerate the fetus, the present dual pattern of immunological activity supports the hypothesis that the immune system regulates itself to maintain homeostasis. The function of these homeostatic immune responses is unknown. Mothers accommodate increases in their innate immune system activity by lowering the ability of the adaptive immune system to respond to infection (Miller , 2009).

WBC count is used as a clinical marker of innate immune function, the increase in total WBC was due to an increase in the number of circulating neutrophils, granulocytes, and monocytes (Faas*et al.* , 2014). Alteration in total and differential count of leucocytes, which may indicate the physiological compensation of the body’s defense mechanism through nonspecific immunity, exerted by neutrophils the migratory phagocytes and other leukocytes like monocytes and eosinophil in different trimesters of pregnancy, this alteration in innate

immunity represent a tries to compensate at least partly, the weakened specific immunity of the mother's body (Pramanik*et al.* , 2005). This unique dysregulation between different components of the immune system plays a central role in the maternal adaptation to pregnancy (Ifukor*et al.* , 2013).

Hemodilution is a well-characterized phenomenon in pregnant women. Decrease in immunoglobulins may be due to hemodilution occurred during pregnancy also maternal immunologic inertness has been postulated as one mechanism of protection fetal allograft. As well as Progesterone action that progesterone suppresses antibody production (Robinson and Kleina , 2012). Also hCG may inhibit antibody production whereas shown that hCG inhibited antibody formation of murine B cells (Schumacher et al. , 2014). Moreover B cells mediate humoral immunity by producing antibodies, and suppression of B lymphopoiesis during gestation had also been observed (Arck*et al.* , 2014). In addition pregnancy represent stress state to women and stress has been found to be associated with lower immunoglobulin production (Cheng and Pickler , 2014).

Table (3) shows, among immunoglobulins greater decrease occurs in IgG, it may be related in some way to passive transfer of IgG from mother to fetus across the placenta. This study is in line with other studies, whereas Miller (2009) found markers of innate (nonspecific) immunity will be higher, and adaptive (memory) immunity markers will be lower in pregnant women compared with non-pregnant women. Decrease in level of IgA and IgG in pregnant women also obtained by Weixin*et al.* (1999), but they recorded higher IgM in pregnant women compared with non-pregnant women.

Decrease in lymphocytes can be illustrated through that, it has been reported that stimulated by oestrogen the adrenal cortex produces increasing levels of total and free plasma cortisol and other corticosteroids from 12 weeks to term. It decreased the circulating lymphocyte count, size of lymph node and thymus by inhibiting lymphocyte mitotic activity (Pramanik*et al.* , 2005).

Increase WBC (Leucocytosis) occurring during pregnancy in spite of hemodilution is due to the physiologic stress induced by the pregnant state and because increased inflammatory response during normal pregnancy, which can be as a consequence of selective immune tolerance, immunosuppression and immunomodulation of fetus (Kaur*et al.* , 2014). The stress probably stimulate the release of certain factors called leucocytosis inducing factor (LIF) and colony stimulating factors (CSF) which are known to increase haemopoietic activities and blood cells mobilization into circulation (Wazir*iet al.* , 2010).

Monocytes arise from precursors in the bone marrow and comprise about 5–10% of the circulating blood leukocytes. They circulate in the blood for a few days before migrating into tissues to become macrophages or dendritic cells. They have important functions in homeostasis, tissue repair, and inflammation. Not only count of monocytes increase but monocytes are functionally changed in pregnant women. This has, for instance, been demonstrated by measuring the production of oxygen free radicals, which is increased in pregnant women. Also monocytes of pregnant women showed increased cytokine production as compared with monocytes from non-pregnant women (Faas*et al.* , 2014).

Neutrophils are the major type of leucocytes on differential counts. This is likely due to impaired neutrophilic apoptosis in pregnancy. The neutrophil cytoplasm shows toxic granulation, neutrophil chemotaxis and phagocytic activity are depressed, especially due to inhibitory factors present in the serum of a pregnant female (Chandra *et al.* , 2012). In the advanced stage of gestation, there is an endogenous adrenaline release which induces the greater mobilization of neutrophils in the circulation resulting in an increase in total leucocyte count (Roy *et al.* ,2010). Leukocyte and neutrophil count increased significantly on day 1 but start decreasing until fifth day postpartum when the value returns back to normal (Kaur*et al.* , 2014).This important finding should always be kept in mind to avoid the unnecessary use of antibiotic in the postpartum period.Decrease in lymphocytes percentage and increase of WBC, neutrophils and monocytes in pregnant women in this study are in agreement with Ifeanyi*et al.* (2014), Ifukor*et al.* (2013), and Pramanik*et al* (2005). In conclusion the level of serum IL-2, C3 and C4 was being found to be lower in pregnant women compared to control group. Also there were increase in biomarkers related to innate immunity (total WBC, neutrophils and monocytes) and a decrease in biomarkers related to adaptive immunity (IgA, IgG, IgM and lymphocytes) during pregnancy.

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