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Complement protein and Immunoglobulins Serum levels in Normal Pregnant and Spontaneous Aborted Women

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Abstract: ***Disorder of maternal immune responses during pregnancy triggers immunological rejection of fetus antigens by maternal immune components, contribute to spontaneous abortion or miscarriage. The study was designed to concentrated on immunoglobulins (IgM, IgG and IgA) and complement elements (C3 and C4) serum levels changes in normal pregnant and abortion women. Study groups were classified into normal pregnant women (20), spontaneous abortion (30) and non-pregnant women (16) as a control group, attending to Shahid Dr.Khalid Hospital/Department of Gynecology and Obstetrics/Koya city. Serum levels of immunoglobulins (IgG, IgM, and IgA), complement proteins (C3 and C4) were determined and analyzed for normal pregnant, abortion and control groups by using Single Radial Immunodiffusion (SRID) technique. The results demonstrated that concentration of IgG levels in abortion differed significantly in compare to normal pregnancy (p ≤0.05), while there were no significant differences in IgM and IgA serum levels among groups (p >0.05). Also, statistical analysis revealed that serum levels of C3 and C4 significantly decreased in abortion group compared to normal pregnant and non-pregnant groups (p ≤0.05). Concluded that complement proteins (C3 andC4) are a good defense line during normal pregnancy, sometime activation (hyper-consuming) of complement elements may provoke spontaneous abortion, while immunoglobulins are a little role in inducing of miscarriage in pregnant women.***

Keywords: Pregnant, Abortion, IgG, IgM, IgA, C3, C4.

1. INTRODUCTION

Pregnancy is one of the most important periods in human life with hormonal, immunological, vascular, metabolic and psychological changes. Abortion is a common complication of pregnancy and may either be spontaneous or induced [1]. The causative agent for approximately 50% of pregnancy is unknown, it is postulated that the mother immune system invades the paternal antigens on the feto-placental part, which affect placenta development or deterioration of endometrium resulting in abortion of fetus [2].

Several investigations on women with recurrent pregnancy losses demonstrated that the numbers of uterine natural killer (uNK) cells elevated in the peripheral blood of women during or prior of pregnancy in compare to fertile healthy pregnant and non-pregnant women, it's also been reported that the levels of $CD56^+$ NK cells are a causative agent of miscarriage in the pregnant women. [3,4,5]. $CD56^+$ cells produce cytokines and growth factors required to provoke regional immunosuppression andassist fetus developments [6,7]. Imbalance of Th1/Th2 toward Th1 lead to more production of pro-inflammatory cytokines by Th1 cells (IFN-gamma, TNF-α, and IL-2), activation of macrophage and NK cells resulting in thrombosis in the maternal and placental blood circulation by increasing production of fg-12. Immunoglobulins production stimulated by Th1 cytokines are able to activation of complement cascade which participate in the abortion process [8].

Complement system contributes in non-specific immunity by direct lysis target cell through the membrane attach complex (MAC), followed by activation and infiltration of inflammatory cells [9]. Normally complement proteins play a critical role in protecting both fetus and mother against harmful agents, while hyperactivation of complement system due to infection by pathogens can lead to pathogenesis and be very dangerous to embryo [10]. Regulation of complement activation by regulatory proteins, such as decay accelerating factor (DAF) and complement receptor 1-related gene protein/y (Crry) is a critical factor for a successful of pregnancy [11]. Recognition of paternal antigens on the surface of syncytiotrophoblast by mother immune system could activate complement proteins, contributing in death of trophoblastic cells [12]. In animal model deficiency in Crry regulatory protein, result in spontaneous deposition of C3 protein on the surface of tropoblast, and fetus loss, for that reason, it has been proposed that restriction of complement system is required for normal pregnancy [9, 13].

Miller in (2009) demonstrated in their investigation, innate immunity markers (Total WBCs and neutrophils) levels are higher in pregnant women, while adaptive

immunity markers (Immunoglobulins and lymphocytes) will be lower in compare to non-pregnant women [14]. A study conducted by Horler*et al.*, (2002) [15], reported the role of complement in pathology during pregnancy on mouse model characterized of anti-phospholipid syndrome (APS), the level of IgG markedly increased against negative charge of phospholipid- protein in patients suffered by APS, also the same study showed that anti-phospholipid (aPL) antibody deposits in the placenta cause necrosis and miscarriage, or retardation of fetus development [14].

Activation of complement cascade is essential and trigger in aPL antibody enhanced injury in fetus, the aPL induced pregnancy loss done following by C3 activation [15]. This study was designed to assess whether abortion is related to immunoglobulins and complement proteins levels in the blood circulation of pregnant women and compare to normal pregnancy.

2. METHODS AND MATERIALS

The present study carried out on (66) women, aged between (20-41) years, they were divided into three groups, first group included (16)non-pregnant married healthy women as control group, the second group was (20) normal pregnant of the first trimester andthird group included (30) cases of first trimester spontaneous abortion. All groups were attended to Shahid Dr. Khalid Hospital/Department of gynecology and obstetrics in Koya.

Five mL of peripheral venous blood samples were collected from, non-pregnant control, patients with first trimester abortion before curettage and so from ongoing first trimester pregnant women in sterile plane tube and left to clot for (15 -30) minutes at room temperature, then centrifuged for 5 minutes at 3000 round per minutes (rpm). Sera were separated and divided into Eppendorf tubes and immediately stored in deep freeze at – 20 ºC. until test.

Serum levels of immunoglobulins (IgM, IgG and IgA) and complement proteins (C3 and C4) determined by using Single Radial Immunodiffusion (SRID) method, according to the instruction of manufacture's (LTA SRI, Milan,Italia).

Statistical analysis was performed by SPSS (Statistical package for social science) version 17.0. Using descriptive statistic, LSD (Least Significant differences) test for multiple comparisons after ANOVA, p value ≤ 0.05 was considered as significant.

3. RESULTS

The study of serum immunoglobulin levels of (30) spontaneous aborted women in comparison to (20) normal pregnant and (16) non-pregnant groups revealed that the mean serum level of IgG significantly increased ($p \leq 0.05$), LSD test showed that this difference is between abortion and normal pregnancy, while there were no significant differences between non-pregnant with normal pregnant and abortion groups ($p > 0.05$), as shown in table and figure (1). However, the data analysis illustrated that the serum concentration of IgM and IgA non-significantly lower in abortion group than other groups ($p > 0.05$), table (2, 3).Statistical analysis reveals a significant decrease of serum complement protein (C3) in abortion group in compare to normal pregnant group ($p \leq 0.01$) as showed in table (4) and figure (3), while there was no significant differences between abortion with normal pregnant group in one hand and non-pregnant with normal pregnant group on other hands ($p > 0.05$).Moreover, the concentration of C4 protein in the sera of abortion individuals differedsignificantly (33.49±2.45 mg/dl) in comparison to normal pregnancy (48.18±2.27 mg/dl) and non-pregnant women (43.5± 3.5 mg/dl) ($p < 0.001$), explained in table (5) and figure (3). LSD- test analysis revealed highly significant decrease in the serum of C4 level of spontaneous abortion compared with normal pregnant women ($p < 0.001$) and with non-pregnant group ($p < 0.05$), but no significant relation was reported between normal pregnant and non-pregnant women ($p > 0.05$).

Table 1: Difference in mean serum level of IgG (mg/dl) between study groups

Study groups	No.	IgG Mean ±SE	P value ANOVA
Abortion	30	1589.11 ±103.41	0.032
Normal Pregnant	20	1235±70.09	
Non-Pregnant	16	1347.21±110.97	
LSD Test			**P value**
Abortion vs Normal Pregnant			0.012
Abortion vs Non-Pregnant			0.104
Normal Pregnant vs Non-Pregnant			0.483

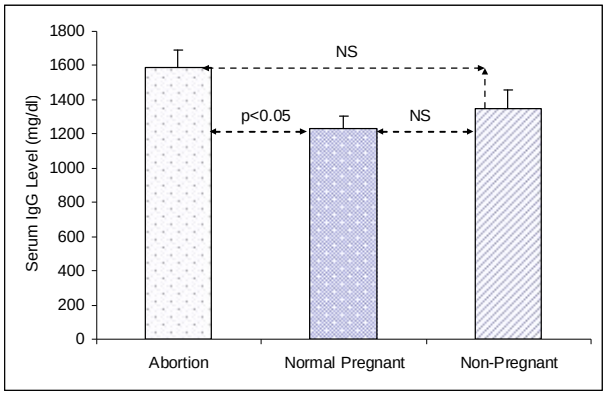

Figure 1: Serum level of IgG in study groups

Table 2: Difference in mean serum level of IgM (mg/dl) between study groups

Study groups	No.	IgM Mean ±SE	P value ANOVA
Abortion	30	242.29±22.91	0.154
Normal Pregnant	20	237.79±24.53	
Non-Pregnant	16	309.13±33.22	
LSD Test			**P value**
Abortion vs Normal Pregnant			0.922
Abortion vs Non-Pregnant			0.079
Normal Pregnant vs Non-Pregnant			0.088

Table 3: Difference in mean serum level of IgA (mg/dl) between study groups

Study groups	No.	IgA Mean ±SE	P value ANOVA
Abortion	30	203.29±14.82	0.93
Normal Pregnant	20	211.88±17.42	
Non-Pregnant	16	207.75±19.55	
LSD Test			**P value**
Abortion vs Normal Pregnant			0.71
Abortion vs Non-Pregnant			0.85
Normal Pregnant vs Non-Pregnant			0.87

Table 4: Difference in mean serum level of C3 (mg/dl) between study groups

Study groups	No.	C3 Mean ±SE	P value ANOVA
Abortion	30	152.09±13.63	0. 023
Normal Pregnant	20	202.96±11.77	
Non-Pregnant	16	186.16±14.03	
LSD Test			**P value**
Abortion versusNormal Pregnant			0.008
Abortion versus Non-Pregnant			0.093
Normal Pregnantversus Non-Pregnant			0.44

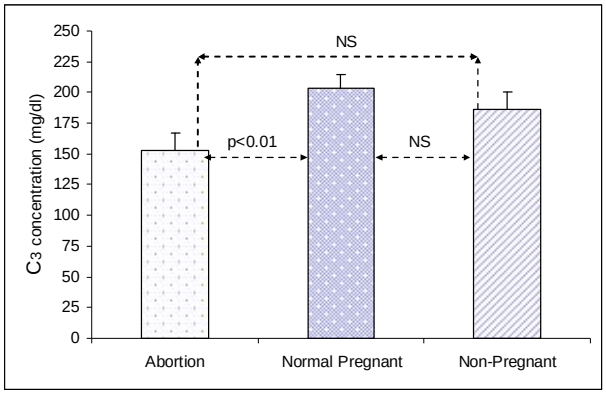

Figure 2: Serum level of C3in study groups

Table 5: Difference in mean serum level of C4 (mg/dl) between study groups

Study groups	No.	C4 Mean ±SE	P value ANOVA
Abortion	30	33.49±2.45	0.001
Normal Pregnant	20	48.18±2.27	
Non-Pregnant	16	43.5±3.5	
LSD Test			**P value**
Abortion vs Normal Pregnant			0.001
Abortion vs Non-Pregnant			0.015
Normal Pregnantvs Non-Pregnant			0.28

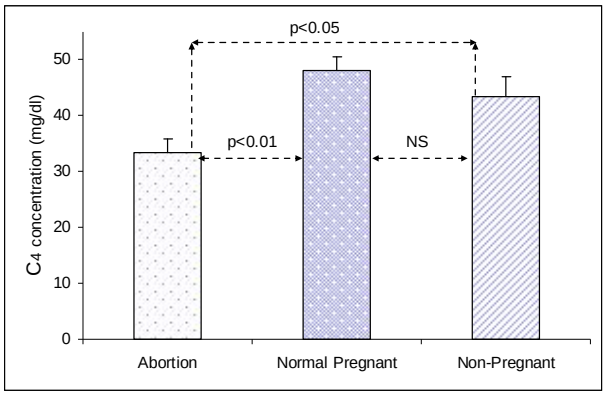

Figure 2: Serum level of C4 in study groups

4. DISCUSSION

Immunological mechanisms are involved in successful implantation. Miscarriage may, therefore, be a consequence of inappropriate humoral or cellular immunological responses towards the embryo that have been investigated is the possible immunologic rejection of paternal antigen by the maternal immune system, resulting in abnormal immune cells and cytokine production [16]. The present study revealed that the concentration of IgG in abortion group significantly increased ($p<0.05$), in comparison to normal pregnancy and non-pregnant groups, while levels of serum IgG among pregnant mother non-significantly decreased compared to non-pregnant women ($p> 0.05$), all groups showed no significant changes in total IgM and IgA sera concentration (table 1, 2). These results are partially in agreement with a study by Saleh *et., al* in Baquba (2015) [17], antibody levels in pregnant mothers decreased, this may be because of hemodiluton, hormonal changes, such as progesterone which inhibit immunoglobulin production by B lymphocytes.

The activity of innate immunity dramatically elevated, while the activity of specific immunity obviously decreased, these dual mechanism reactions is a critical point required for immunotolerance and survive of the fetus. It has been noted that stress is characterized in pregnancy and inhibit antibody productions by B lymphocytes and associated with pregnancy loss. (14,7], another study has shown that there is no relation of IgM, IgA, and IgG serum levels in patients with recurrent miscarriage, it is well characterized that autoantibodies (IgG, IgM, and IgA) against phospholipids are linked with recurrent abortion [18].

We found out that concentrations of C3 and C4proteins significantly decreased in abortion group compare to normal pregnancy group ($p<0.01$), Sugiura-Ogasawara *et al.*,(2006) [18] and Alkhayat*et al.,* (2014) [19], documented that C3 and C4 serum levels were low in recurrent pregnancy loss, and they proposed those complement elements have a role in the pathogenesis of abortion, another investigation determined C3 and C4 protein levels significantly lower in patients with unknown habitual miscarriage with subclinical lower autoimmune diseases than explained habitual abortion. Excessive complement activation from mother circulation or local placenta cell sources interfaces with a hazard of fetal loss [15]. The major structural components of decidual cells are a trophoblast, which associated in the local production of complement components in maternaldeciduas as a basic source of C3 and C4 feto-maternal connection [20]. Placenta is vulnerable by complement proteins because trophoblast encounters maternal blood and tissue alteration in maternal decidua stimulate complement components during pregnancy [11].

Another finding of the present study is that serum levels of C3 and C4 non-significantly elevated in normal pregnancy compared to non-pregnant, Richani and coworkers (2005) have shown similar results [21], normal activation of mother and embryo complement system protected them against infections by pathogens. A local synthesize and release of complement components may participate in tissue destruction, kidney graft rejection done by the C3protein produced in epithelial renal tubules [22]. Activation of complement components by parental antigens presented on syncytiotrophoblast consequence in the destruction of trophoblast then may increase of barrier permeability opening the gate to pathogens, such as bacteria, viruses as well as toxic materials that a risk for fetus survives [23].

The function of complement is crucial to maternal reproductive tract during pregnancy because theimplanted fetus is exposure to invading by microorganisms that habitat in the cervicovaginal area, therefore protection is required to avoid embryo from infectious agents [24].

5. CONCLUSION

Immunological alteration in pregnant women required for fetus survival and successful pregnancy. Break of immunotolerance and attack of immune components of the maternal immune system may result in pregnancy loss. This study demonstrated that the level of immunoglobulins decreased during normal pregnancy, elevation of IgG may contribute in miscarriage. While concentration serum levels of C3 and C4 proteins markedly decreased in abortion, it may refer to high consumption of complement components because of over activation of the complement system, eventually may be associated with the fetus loss. Further studies need to be conducted to explain the role immune components as an etiology and relation with spontaneous abortion.

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