

The role of Th1 and Th2 cytokines among women with recurrent spontaneous miscarriage

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ABSTRACT

To examine the concentrations of Th1 (IFN- γ , TNF- α and IL-2) and Th2 (IL-4, IL-6 and IL-10) cytokines in the trophoblastic tissue during curettage of recurrent spontaneous miscarriage (RSM) and compared with normal pregnancy at time of delivery. This cross-sectional comparative study which was carried out at Basrah University from February 2012 to May 2014. 172 women, aged between 16 – 41 years, were included in the current study and were further classified into three categories: Group A- Recurrent spontaneous miscarriage (RSM): n= 65 women, with a mean age of (25.2 \pm 7.28). Group B- Non- recurrent spontaneous miscarriage (non-RSM): n= 36 women, with a mean age of (26.61 \pm 6.97). Group C- Control (normal pregnancy): n=71 women, with a mean age of (26.17 \pm 7.01). A trophoblastic tissue placenta sample from each curettage patient and control subject was collected and placed in a wide mouth container and homogenized by mortar and pestle method then detection by human tissue lysate ELISA kit. There was a significant increasing in the mean concentration of Th1/Th2 in miscarry groups than control group. Results show high concentration of Th1 cytokines (134.17 \pm 24.37), (432.16 \pm 51.7), (100.46 \pm 9.94) of (IL-2, TNF- α and IFN- γ) respectively was detected in case of RSM group rather than control and non-RSM groups, while high concentration (49.97 \pm 8.82), (180.7 \pm 15.03), (94.34 \pm 7.68) for (IL-4, IL-6 and IL-10) respectively in case of control group when compared with patient groups with highly significant differences.

Conclusion: The results of present study demonstrate that RSM was associated with increasing concentration of Th1 cytokines in the first trimester of pregnancy while normal pregnancy was associated with increase concentration of Th2 cytokines, indicating Th2 bias in normal pregnancy and a Th1 –bias in unexplained RSM

Index Terms __Th1 cytokines, Th2 cytokines, Recurrent spontaneous miscarriage.

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INTRODUCTION

Miscarriage is spontaneous termination of pregnancy prior to the fetus can survive. (Lu Li, 2014), Which is happen in about 8% to 20% of all recognized pregnancies (Petrozza, 2014). Immunological causes may be associated with up to 60% of recurrent spontaneous miscarriage (Verena *et al.*, 2006). It was initially consideration that pregnancy confers general immunosuppression to confirm tolerance of

the semiallogeneic fetus. The fascinating growth and development of a semi allogeneic conceptus within an immunologically competent mother depends on the manner in which pregnancy alters the immune factors that govern tissue rejection, proposed a shift from Th1 to Th2 immunity through pregnancy (Constantin *et al.*, 2007). The role of cytokines (IL-4, IL-6 and IL-10) that represent Th2

(helper2) and Th1 (IL-2, and TNF- α and IFN- γ) in trophoblastic tissue polarized immune response contributes to the survival of the fetus . Th2 cells stimulate B lymphocytes, increase antibody formation , and suppress the cytotoxic T-lymphocyte reaction, decreasing the strength of cell-mediated immunity. A shift to Th2 immunity is responsible for altered responses to auto antigens in pregnancy and could explain the severity of infections, in

which cell-mediated immunity is essential.(Ostensen *et al .*, 2002; Jamieson *et al.*, 2006; Pazos *et al.*, 2012)

A strong association between maternal Th1/Th2 ratio either in the normal pregnant or recurrent spontaneous miscarriage , Th₂ type immunity impetuses successful pregnancy, whereas Th1 type immune reactivity is associated with pregnancy loss.

METHODS

A total of 172 women, aged between 16 – 41 years, who admitted to the Maternity and Children Hospital in Basrah because of incomplete first trimester miscarriage, in addition to women with normal pregnancy at time of delivery during the period from February 2012 to May 2014 were included in the present study then they were divided into 3 groups

Group –A included (65) women with recurrent spontaneous miscarriage (RSM) during the first trimester , with a mean age of (25.2 \pm 7.28) years .

Group –B included (36) women with incomplete first trimester miscarriage and had at least three previous normal pregnancy (non- recurrent spontaneous miscarriage) with a mean age of (26.61 \pm 6.97) years .

Group C- Control (normal pregnancy): n=71 women with a mean age of (26.17 \pm 7.01) years .

A trophoblastic tissue placenta sample from each curettage patient and control subject was collected and placed in a wide mouth container then homogenized by mortar and pestle method for ELISA test. Human Interleukin ELISA kit is an *in vitro* enzyme-linked Immunosorbent assay for the quantitative measurement of human interleukin for tissue lysate. To examine the concentrations of Th1 (IFN- γ , TNF- α and IL-2) and Th2 (IL-4, IL-6 and IL-10) cytokines in the trophoblastic tissue during curettage of recurrent spontaneous miscarriage (RSM). Statistical Package for Social Science (SPSS) was used to analyze the data. ANOVA and t-test were used to assess the significance of differences between groups. .

RESULTS:

Regarding the obstetrical history of the studied groups Table (1) shows the gravidity, parity and the residency. The mean number of pregnancies for RSM (3.846±1.946) with significant difference with non- RSM –groups (2.83 ± 1.61) (p<0.05) and highly significant (p<0.001) than those in the control groups (2.21± 0.99) .

On the other hand the mean of parity was high in non RSM- groups (4.02± 0.97) and control (2.8 1 ± 1.09) rather than in the RSM- groups (1.4 ± 1.04) with high significant difference (P<0.001) between them . The residency of Three groups in current study shows higher percent in rural area rather than urban region .

Characteristics	RSM-group	Non-RSM group	Normal pregnant(control) group
Gravidity Mean ± SD Range	3.85±1.946 2-9	2.83 ± 1.61 3-7	2.21± 0.99 1-5
Parity Mean ± SD Range	1.4 ± 1.04 0-3	4.02± 0.97 3-6	2.8 1 ± 1.09 1-5
Residence Rural	39 (60%)	22 (61%)	29 (40.85%)
Urban	26 (40%)	14 (38.88%)	42 (59.2%)

Table 2: Shows the level of cytokines IL-10, IL-6 and IL-4 of these was detected from placental tissues by ELISA test among the three groups (A, B, and C), the highest percent of IL-10 was found in control group(C)(94.34±7.68), while the lowest percent was found in the group(B) with no recurrent spontaneous miscarriage (40.64±9.81) as shown in Fig. 1. IL-6 highest percent was found in the control group(C)(180.69±15.03),the lowest percent was found in recurrent miscarriage group(A) (99.01±9.43) as illustrated by Fig. 2, and the highest

percent of IL-4 was found in the control group(C) (49.97 ±8.82) , while the lowest percent was found in the recurrent miscarriage group(A) (42.82±7.57) (Fig .3) .

Variable		RSM	Non-RSM	Control	Sig. between groups		
		A	B	C	A/B	A/C	B/C
IL-10 Pg/ml	Mean + SD	42.21±8.55	40.64± 9.81	94.34±7.68	0.373	0.000**	0.000**
IL-6 Pg/ml	Mean + SD	99.01± 9.43	110.14±13.91	180.7±15.03	0.000**	0.000**	0.000**
IL-4 Pg/ml	Mean + SD	44.63±6.41	42.82±7.57	49.97±8.82	0.264	0.000**	0.000**

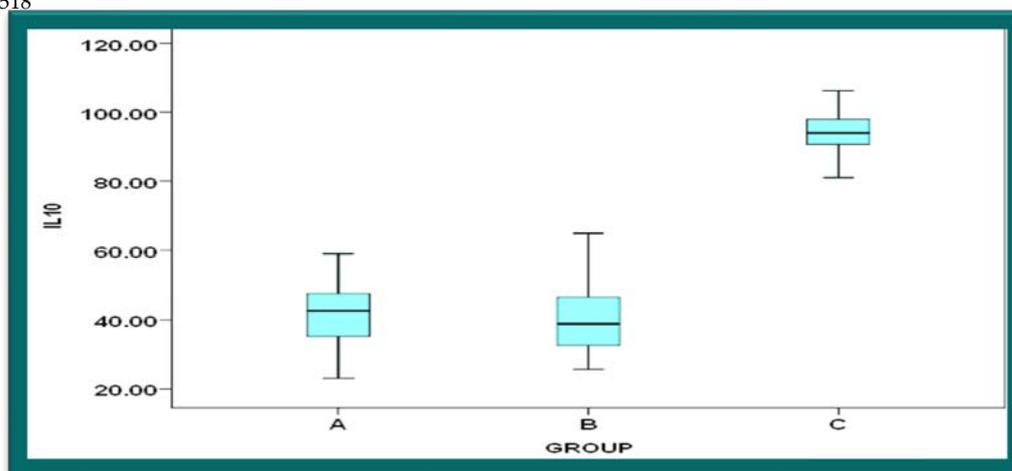


Figure 1: Levels of IL-10 in the three groups of cases (A, B and C).

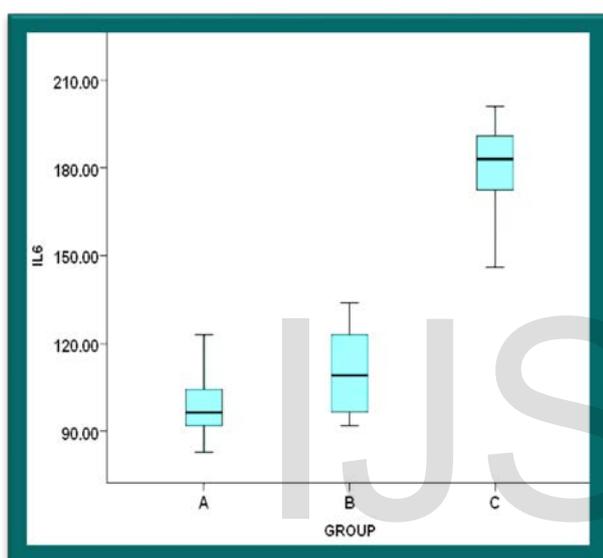


Figure 2: Levels of IL-6 in the three groups of cases (A, B and C).

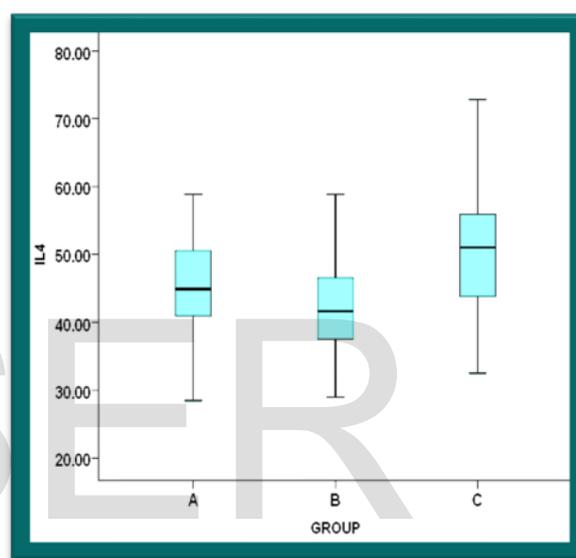


Figure 3: Levels of IL-4 of the three groups of cases (A, B and C)

Table (2) shows that there was no statistical difference ($P>0.05$) in the mean percent of IL-10 and IL-16 between groups (A) and (B) with spontaneous miscarriage . While there was a highly significant difference ($p<0.001$) in the mean percent of IL-10 and IL-6 between group (A) with spontaneous miscarriage and control group. There was a highly significant difference ($p<0.001$) in the mean percent of IL-10 and IL-6 between group (B) with spontaneous miscarriage and control group.

Regarding IL-4, Table (2) also shows a significant difference ($p<0.05$) in the mean percent of IL-4

between (B) and (A) group with spontaneous miscarriage. While there was a highly significant difference ($p<0.001$) in the mean percent of IL-4 between (A) group with spontaneous miscarriage and control group. Also, there was a highly significant difference ($p<0.001$) in the mean percent of IL-4 between (B) group with spontaneous miscarriage and control group.

Table (3) shows that the levels of IL-2, IFN- γ and TNF- α of these cytokines was detected from placental tissues by ELISA . Among the three groups (A, B, and C), the highest percent of

IL-2 was found in the group(A) with recurrent spontaneous miscarriage (134.17 ± 24.37), while the lowest mean percent was found in control group(C) (92.54 ± 9.4678), Figure(4). IFN- γ highest percent was found in the group(A) with recurrent spontaneous miscarriage (100.46 ± 9.94), the lowest percent was found in

control group(C) (55.63 ± 8.82) Figure (5), and the highest percent of TNF- α was found in the recurrent miscarriage group(A) (432.16 ± 51.7), while the lowest mean percent was found in the control group(C) (94.33 ± 14.2) ,Figure (6).

Table 3:IL-2 ,IFN- γ and TNF-α levels in the three studied groups							
Variable		RSM	Non- RSM	Control	Sig. between groups		
		A	B	C	A/B	A/C	B/C
L-2 pg/ml	Mean \pm SD	134.17 ± 24.37	111.45 ± 19.49	92.54 ± 9.47	0.523	0.000**	0.000**
IFN- γ pg/ml	Mean \pm SD	100.46 ± 9.94	86.75 ± 8.07	55.63 ± 8.82	0.540	0.000**	0.000**
TNF- α pg/ml	Mean \pm SD	432.16 ± 51.7	388.01 ± 33.99	94.33 ± 14.2	0.014*	0.000**	0.000**

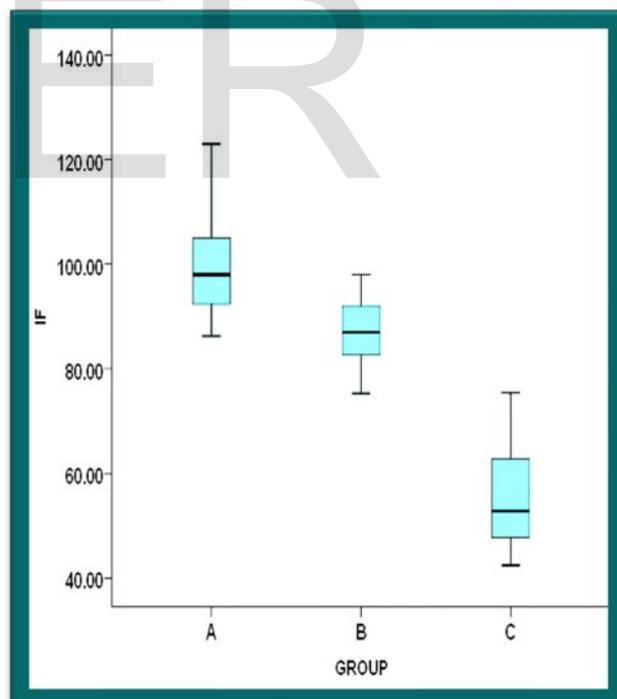
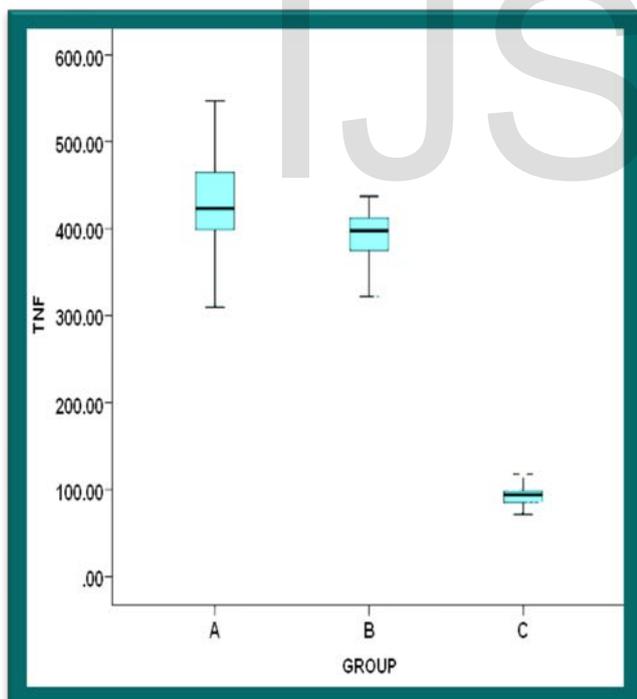
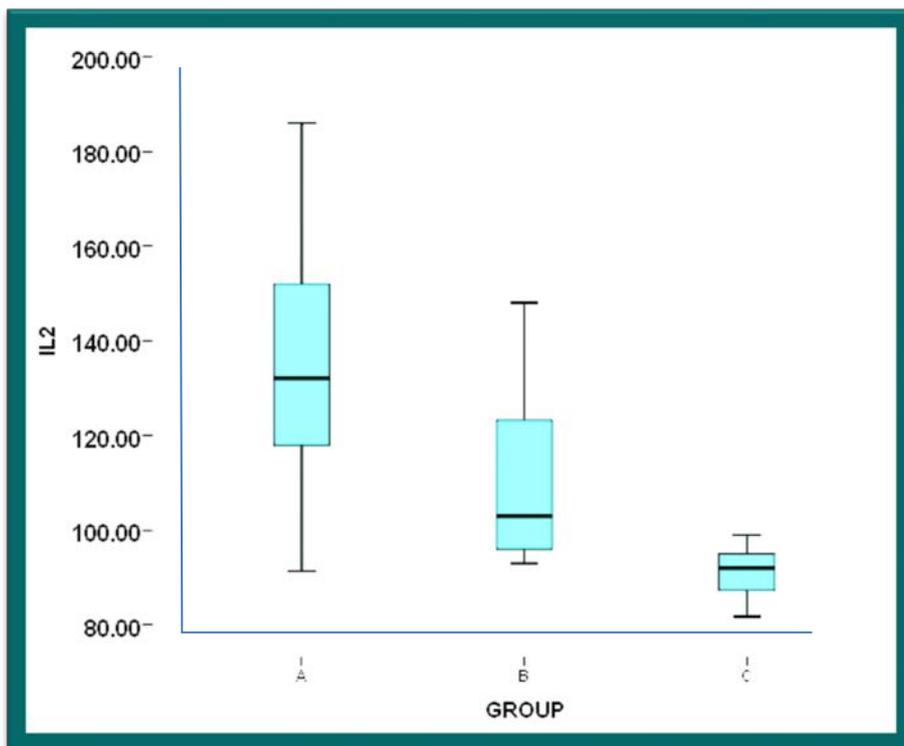


Figure 5: Levels of $IFN-\gamma$ in the three groups of cases (A, B and C).

Figure 6: Levels of $TNF-\alpha$ in the three groups of cases (A, B and C)

The ratios of mean Th1 to Th2 cytokines concentrations in the RSM (group A) were consistently higher than the ratios in the non-RSM (group B) and control group (group C) Table (3). In all combinations evaluated, the RSM group showed highly significant differences (p<0.001) when compared with control group. While

in compared between RSM group with non –RSM that show no significant differences between IFN- γ :IL-4 ,TNF- α :IL-4 and TNF- α :IL-10 and significant differences (p<0.05) in case of IL2:IL4 and IL-2:IL-10 . Figure (7 ,a,b,c)

Table 3 Ratio of Th1- to Th2-from fetoplacental tissues in three study groups

Th1:Th2 pg/ml	RSM(A) group N=65	Non-RSM(B) group N=36	CONTROL (C)groups N=71	p- value
IL-2:IL4	3.09±0.83	2.69±0.69	1.91±0.39	(A/B*) (A/C**)
IL-2:IL6	1.37±0.27	1.03±0.24	0.52±0.083	(A/B**) (A/C**)
IL2:IL-10	3.36±1.15	2.92±0.95	0.99±0.13	(A/B*) (A/C**)
IFN- γ :IL-4	2.29±0.38	2.09±0.44	1.14± 0.27	(A/B) (A/C**)
IFN- γ :IL-6	1.02±0.13	0.79±0.12	0.31±0.05	(A/B**) (A/C**)
IFN- γ :IL-10	2.49±0.62	2.26±0.58	0.59±0.11	(A/B*) (A/C**)
TNF- α :IL-4	9.91±1.99	9.35±1.97	1.95±0.48	(A/B) (A/C**)
TNF- α :IL-6	4.39±0.61	3.57±0.49	0.53±0.093	(A/B**) (A/C**)
TNF- α :IL10	10.69±2.68	10.12±2.73	1.01± 0.18	(A/B) (A/C**)

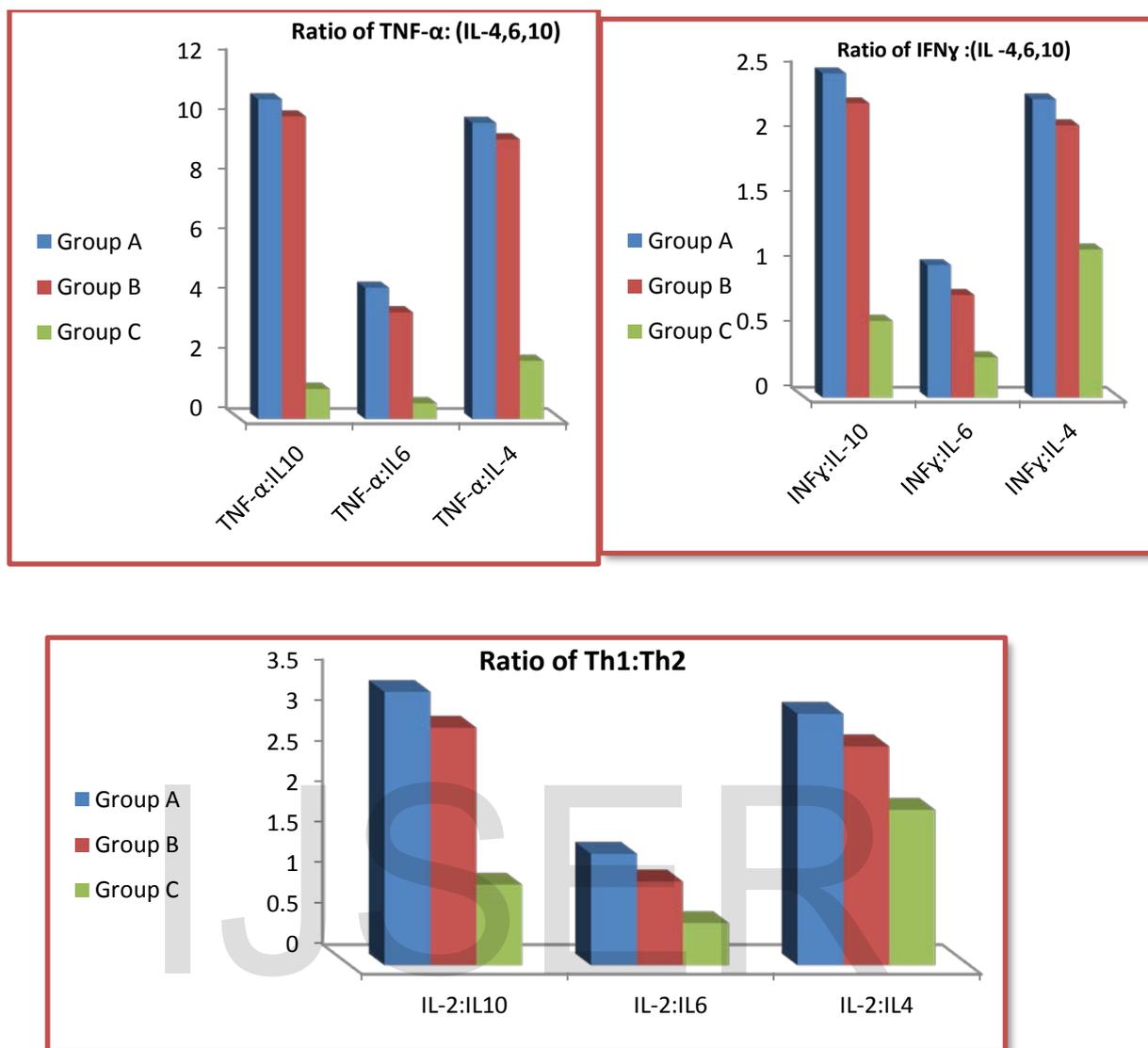


Figure 7(a)IL-2 ratio to (IL-4,IL-6,IL-10)

Discussion

In this study, highly significant increased concentration of Th1cytokines (IL-2 ,TNF- α and IFN- γ) were found in RSM (group A)women as compared with normal pregnant at the time of delivery (group C)and with the non RSM women (group B) . While the concentration of anti-inflammatory (IL-4 ,IL-6 and IL-10) cytokines in normal pregnant women were significant increase than RSM and non RSM women. These results were agreed with Makhseed *et al.*,(2000) and Rezaei and Dabbagh, (2002) who measured the concentration of the cytokines in serum also ,previous studies in

humans (Raghupathy *et al.*, 2000; Raghupathy and Kalinka, 2008) and in mice (Doi *et al.*, 1999; Clark and Croitoru, 2001; Joachim *et al.*, 2003) provide evidence for an increase in Th-1/Th-2 ratio, with a shift towards Th-1 in recurrent miscarriage. In contrast, peripheral blood mononuclear cells (PBMCs) have been shown to secrete higher levels of Th-1 cytokines and lower levels of Th-2 cytokines, in first trimester pregnant women with normal outcome compared with women with spontaneous miscarriage (Zenclussen *et al.*, 2002) or a history of recurrent miscarriage who subsequently miscarried

(Bates *et al.*, 2002). The explanation for these result by possible role of pro-inflammatory cytokines may convert NK cells into lymphokine-activated killer cells that have been shown to lyse trophoblast cells. Direct effects of type 1 cytokines may include the apoptosis of trophoblast cells by TNF- α and IFN- γ , inhibition of secretion of the growth-stimulating cytokines from the uterine epithelium and cytokine induced activation of coagulation mechanisms, which may then lead to vasculitis affecting maternal blood supply to the implanted embryo (Raghupathy, 2003) or it might be due to the progressive increase of progesterone and estrogens which reach high levels during pregnancy, at these high levels, they suppress the Th1- and stimulate Th2-mediated immunological responses (Szekeres-Bartho and Wegmann,1996; Miyazaki *et al.*, 2003).Another

study by Kanai (2001) demonstrated that the HLA-G expressed on the human trophoblasts is one of the key factors in regulating cytokine balance by shirting the Th1/Th2 balance towards Th2 polarization favorable environment for the maintenance of pregnancy. So the successful pregnancy is characterized by a shift toward Th2 type immune response and suppression of adaptive immune responses to ensure acceptance of the semi-allogenic fetal graft; While, susceptibility to recurrent miscarriage is probably mediated by Th1 type immune response with pronounced expression and secretion of pro-inflammatory cytokines like TNF- α and IFN- γ paralleled with decreased production of anti-inflammatory cytokines like IL-10 (Bermas and Hill 1997).

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