Original Article
Gynecology

THE ROLE OF CYTOKINES AMONG WOMEN WITH SPONTANEOUS MISCARRIAGE

GHAIDAA A. ABDULLAH*
NADHAM K. MAHDI*

SUMMARY: To evaluate cytokine production in women with unexplained pregnancy loss.

The study enrolled 84 women age 15-41 years with a mean age of 25.07 ± 6.6, during the period from September 2011 till March 2012 from Basrah Hospitals, Iraq. The patients group comprised 46 women with first trimester miscarriage. The control group included 38 women with first trimester successful pregnancy with no history of miscarriage. Cytokines were measured by ELISA.

There were significantly higher levels of IFN-γ, TNF-α, IL-2 and IL-10 in the aborted women as compared to the controls. In relation to IL-4 and IL-6 non-significant difference was detected between women with miscarriage and control groups. Significant effect of age on serum IL-2 and IL-10 in women with first trimester miscarriage was recorded while no effect observed in the levels of IFN-γ, TNF-α, IL-4 and IL-6. Furthermore, significant higher level of all Th-1 cytokines for miscarriage group than control group for women aged 25-34 years was noticed. In contrast, non-significant differences were found between estimated level for miscarriage and control groups of all Th-1 cytokines for age group >34 years.

This study supports the hypothesis of role of Th-1 produced cytokines in the pathogenesis of miscarriage.

Key wors: Cytokines, miscarriage, pregnant women.

INTRODUCTION

Spontaneous miscarriage is the loss of an intrauterine pregnancy without outside intervention before 20 weeks of gestation. Recurrent miscarriage is generally considered to be the loss of 3 or more pregnancies (1).

Cytokines, as extremely potent, versatile, potent mediators of an immense array of reactions ranging from induction of normal immune responses, rejection of allografts, autoimmune diseases, and hypersensitivity, have received much attention from reproductive immunologists. These cytokine patterns also contribute to cross regulation between immune responses and other systems, as observed during the interplay between infection and pregnancy (2,3).

Th-1 cells secrete the cytokines, interferon (IFN)-gamma, tumor necrosis factor (TNF)-beta, interleukin (IL)-2, and TNF-alpha; these so called Th-1 type cytokines activate macrophages and cell-mediated reactions that play critical roles in resistance to infection by intracellular pathogens and in cytotoxic and delayed-type hypersensitivity reactions.

*From Department of Microbiology, College of Medicine, University of Basrah, Basrah, Iraq.

Th-2 cells secrete the cytokines IL-4, IL-5, IL-6, IL-10, and IL-13 which induce antibody production and are commonly found in association with strong humoral immunity. Some of these Th-2 cytokines are anti-inflammatory (2).

Furthermore, Th-1 and Th-2 cells are mutually antagonistic to each other; thus, an individual who produces a strong Th-1 response usually tends to have a low Th-2 response and vice versa. Clinical evidence and experimental studies suggest that Th-1 type (inflammatory) responses are weakened during pregnancy, while Th-2 responses are augmented (4).

Greater understanding of the effects of cytokines will give insight into normal regulatory processes in tissues and may lead to therapeutic advances (5). Therefore, the objective of this study is to assess serum cytokines concentrations in maternal serum of first trimester pregnancies complicated by miscarriage in comparison with normal pregnancy at same time intervals providing thus a diagnostic advantage in pregnancy cases where the mother cannot immunologically support the fetus.

MATERIALS AND METHODS

Study enrolled 84 females aged 15-41 years with mean ages (25.07 ± 6.6) during the period from September 2011 till March 2012 (from Basrah Maternity and Childhood Hospital, Basra General Hospital, Alseef for primary health care center, Gynecology and Obstetrics specialty clinic). Out of them 46 women were diagnosed to have first trimester complete miscarriage (including 24 first miscarriage, 15 second miscarriage, 7 more than 3 miscarriages) depending on ultrasound imaging after exclusion.

Exclusion criteria were as follows:

1) Remarkable previous medical (hypertension, diabetes, asthma) history.

2) Remarkable previous gynecological history (other abortion causes).

3) Medication intake.

Thirty eight women were diagnosed to have normal pregnancy (with no history of miscarriage) at first trimester depending on pregnancy test in urine and ultrasound imaging as control group.

Similar exclusion criteria were applied.

Sampling

Blood samples were collected from miscarriage and control women by vein-puncture and processed generally within one hour. Blood samples were centrifuged by using Hettich EBA20 portable centrifuge, serum then stored in multiple tubes at -20°C.

Cytokines Immunoassay

All procedures and reagent preparation were done according to instruction of manufacturer included with Enyme-Linked Immunosorbent Assay (ELISA) kits. IL-2, IL-4, IL-6 were purchased from Ray Biotech, Inc. USA. IL-10 and TNF-α were purchased from Immunotech Sas – France. While IFN-γ kit was purchased from Cusbio Biotech Co., LTD China.

Statistical analysis

Statistical analysis was performed by SPSS version 15. p results was depicted as independent sample t-test to compare between patients and controls groups, one way ANOVA test was used to determine the difference among the three age groups, p value of >0.05 was considered to indicate statistical significance.

RESULTS

Th-1 cytokines levels in different groups of subject:

The estimated level of serum IL-2 for aborted women was 83.2 ± 81.7 pg/ml (mean ± S.D) while that of healthy pregnant women was 33.2 ±19 pg/ml within first trimester. The difference was statistically significant ($p < 0.05$) (Table 1).

Among the women of patients group, the estimated level of mean serum IFN-γ was 19.25 ± 8.62 pg/ml (mean ± S.D) which was higher than estimated level for control group 14.95 ± 9.28 pg/ml. This difference was found to be statistically significant ($p<0.05$) (Table 1).

The estimated level of mean serum TNF-α for patients group was 356.44 ± 145.89 pg/ml (mean±S.D) which was higher than the estimated level of the control group 196.18 ± 157.34 pg/ml and the relationship is statistically significant ($p < 0.05$) (Table 1).

Th-2 cytokines levels in different groups of subject:

Result of mean serum IL-4 level of patients group was found to be 3.9 ± 2.7 pg/ml (mean±S.D) which was higher than the estimated level for control group 2.8 ± 2 pg/ml but this difference was statistically non significant ($p < 0.05$) (Table 2).

The level of mean serum IL-6 was not significantly different ($p>0.05$) between patients and control groups. The estimated level of mean serum IL-6 for patients group was 79.9 ± 40.2 pg/ml (mean±S.D) which was less than estimated level for control group 80.2 ± 42.6 pg/ml (Table 2).

Table 1: Mean serum level of Th-1 cytokines for patients and control groups.

Cytokine (pg/ml)	Patients (mean ± S.D) N=46	Control (mean ± S.D) N=38	p value
IL-2	83.19 ± 81.70	33.17±19.39	<0.05
IFN-γ	19.25 ± 8.62	14.95 ± 9.28	<0.05
TNF-α	356.44 ± 145.89	196.18 ± 157.34	<0.05

Table 2: Mean serum level of Th-2 cytokines for patients and control groups.

Cytokine (pg/ml)	Patients (mean ± S.D) N=46	Control (mean ± S.D) N=38	p value
IL-4	3.95 ± 2.76	2.89 ± 2.81	>0.05
IL-6	79.96 ± 40.20	80.24 ± 42.62	>0.05
IL-10	20.67 ± 13.82	12.29 ± 9.92	**<0.05**

Mean serum level of IL-10 for patients group (20.67 ± 13.82 pg/ml) which was significantly higher ($p<0.05$) than mean serum level for control group (12.29 ± 9.92 pg/ml) (Table 2).

T helper one (Th1) cytokines results for different age groups:

In this analysis women with miscarriage whom aged 15-24 years old had significantly ($p <0.05$) higher level of mean serum TNF-α pg/ml than mean serum level estimated for control group. In relation to other cytokines (IFN-γ, IL-2) non significant differences were found between their estimated level for patients and control groups of age15-24 years (Table 3).

But significant higher estimated level for patients than control groups of all T helper one cytokines for age group 25-34 yrs old as in (Table 4).

In opposite to age group 25-34 years non significant differences were found between estimated level for patients and control groups of all T helper one cytokines for age group <34 years old (Table 5).

Table 3: Th-1 cytokines results for age group 15-24 years old.

Cytokine (pg/ml)	Patients (mean ± S.D) N=18	Control (mean ± S.D) N=19	p value
IFN-γ	17.53 ± 7.73	15.83 ± 12.59	<0.05
IL-2	18.54 ± 15.78	32.06 ± 23.79	<0.05
TNF-α	342.10 ± 84.19	181.46 ± 170.40	**>0.05**

Table 4: Th-1 cytokines results for age group 25-34 years old.

Cytokine (pg/ml)	Patients (mean ± S.D) N=18	Control (mean ± S.D) N=11	p value
IFN-γ	21.49 ± 10.12	13.33 ± 3.71	>0.05
IL-2	131.62 ± 101.78	40.83 ± 9.69	>0.05
TNF-α	382.48 ± 200.93	199.43 ± 144.14	>0.05

Table 5: Th-1 cytokines results for age group <34 years.

Cytokine (pg/ml)	Patients (mean ± S.D) N=10	Control (mean ± S.D) N=8	p value
IFN-γ	19.33 ± 8.89	14.58 ± 5.39	<0.05
IL-2	112.50 ± 106.73	25.42 ± 16.87	<0.05
TNF-α	335.37 ± 120.45	230.81 ± 179.58	<0.05

Th-2 cytokines results for different age groups:

T helper two cells secret three types of cytokines IL-4, IL-6, IL-10.

In this analysis for women aged 15-24 years old, the estimated levels of mean serum IL-4 pg/ml as well as mean serum IL-10 pg/ml for patients was higher than the estimated for control, and in contrast for the estimated level of mean serum IL-6 pg/ml for controls which was higher than that estimated for patients , all these differences were statistically non significant (Table 6).

While for women aged 25-34 yrs old only mean serum IL-4 pg/ml cytokine of Th-2 cells was detected to have significant higher level for patients group than level estimated for the control groups.

Although estimated level of mean serum IL-6, IL-10 pg/ml for patients was higher than that estimated for controls. But these differences were statistically non significant (Table 7).

For women older than 34 years mean serum level of IL-4 pg/ml cytokine of Th2 cells was detected to have higher level for patients group than level estimated for control group.

While estimated level of mean serum IL-6 pg/ml for control groups was higher than that estimated for patient groups but these differences were statistically non significant.

But mean serum IL-10 pg/ml was detected to have significant higher level for patients group than level estimated for control group (Table 8).

DISCUSSION

The effects of pre-inflammatory and anti-inflammatory cytokines on the concepts and thus on the success or failure of pregnancy are interesting and have been reported in this study (6). Evidence supporting this study showed that the administration of one of the Th-1 cytokines like IL-2 to normal pregnant mice causes

Table 6: Th-2 cytokines results for age group 15-24 years old.

Cytokine (pg/ml)	Patients (mean ± S.D) N=18	Control (mean ± S.D) N=19	p value
IL-4	3.29 ± 1.55	2.82 ± 2.78	<0.05
IL-6	74.96 ± 25.91	83.95 ± 57.53	<0.05
IL-10	18.73 ± 10.05	14.83 ± 11.44	<0.05

Table 7: Th-2 cytokines results for age group 25-34 years old.

Cytokine (pg/ml)	Patients (mean ± S.D) N=18	Control (mean ± S.D) N=11	p value
IL-4	4.96 ± 4.27	1.46 ± 1.25	**>0.05**
IL-6	86.77 ± 53.17	66.56 ± 13.94	<0.05
IL-10	13.98 ± 9.51	10.21 ± 9.98	<0.05

Table 8: Th-2 cytokines results for age group < 34 years.

Cytokine (pg/ml)	Patients (mean ± S.D) N=10	Control (mean ± S.D) N=8	p value
IL-4	5.66 ± 3.93	4.96 ± 3.83	<0.05
IL-6	76.71 ± 36.27	84.92 ± 28.39	<0.05
IL-10	36.21 ± 15.08	8.41 ± 4.43	**>0.05**

abortion by increasing fetal resorption rates (7). In addition, results of this study are harmonized with previous report that Th-1 cytokines levels (IFN-γ, TNF-α) are augmented during abortion and may be accused for abortion, as Th-1 cytokines, IFN-γ inhibited in vitro proliferation of human trophoblast cells (8).

Therefore, the present results revealed a significant association between raised serum Th-1 cytokines (IL-2, IFN-γ, TNF-α) and first trimester pregnancy loss, strengthen the relation between these agents and fetal rejection.

In contrast, Th-2 cytokines (IL-4, IL-6, IL-10) are not significantly different during first trimester miscarriage. Th-1 and Th-2 are mutually inhibitory to each other when Th-1 reactivity is high, Th-2 reactivity is usually low and vice versa (9).

The non-significant results obtained for Th-2 cytokines by the present analysis might be due to multiplicity of factors and conditions that influence cytokines production. These issues are fundamental and must be considered in future investigations. Thus, Th-2 cytokines have been associated with successful pregnancy.

Classifying cytokine responses with Th-1 and Th-2 may be misleading since many other cells produce cytokines, including CD8 cells, NK cells, mast cells, B cells, basophils, macrophages and keratinocytes (2).

The present investigation tried to determine whether age has any impact on mean serum levels of cytokines in women with first trimester pregnancy failure.

Statistically significant effect of age on serum IL-2, IL-10 in women with first trimester miscarriage was observed. On the other hand age does not influence the levels of IFN-γ, TNF-α, IL-4, and IL-6 in studied women sera with first trimester pregnancy failure. In study done in Denmark, their results show that the spontaneous abortion rate is steady up to the age of 35 years, but then increases remarkably, reaching 41% when the mother is over 40 years old (10). In addition, ageing is associated with increased inflammatory activity in the blood that elderly cohort had increased circulating levels of TNF-γ (11).

To the best of my knowledge, no published work on the role of age among miscarriaged women due to cytokine imbalance to compare with.

One of the possible important limitations of this study is the determination of underlying infections. All bacterial and viral infections are also potentially effect the cytokine levels of pregnant women. Infection and inflammation have both ability to change the levels of circulating cytokine levels.

In conclusion, differences in cytokines activity have been reported for normal pregnant women and those experiencing pregnancy failure. The hypothesis of Th-1 cytokines involvement in the pathogenesis of miscarriage is indicated.

REFERENCES

1. Griebel CP, Halvorsen J, Golemon TB, et al. Management of spontaneous abortion. Am Fam Physician 2005;72:1243-50.

2. Mossman TR, Sad S. The expanding universe of T-cell subsets. Immunol Today1996; 17:138-46 .

3. Coffman RL, Romagnani S. (eds). Redirecting Th1 and Th2 subsets. Springer-Verlag Berlin (1999) cited by Raghupathy R, Kalinka J. Cytokine imbalance in pregnancy complications and its modulation. Front Biosci 2008;13:985-94.

4. Wang X, Chen C, Wang L, et al. "Conception, early pregnancy loss, and time to clinical pregnancy: a population-based prospective study". Fertil Steril 2003;79: 577-84.

5. Kennedy RL, Jones TH. cytokines in endocrinology :their roles in health and disease. Endocrinol 1991;29:167-178.

6. Raghupathy R, Kalinka J. Cytokine imbalance in pregnancy complications and its modulation. Front Biosci 2008;13:985-94.

7. Chaouat G, Menu E, Clark DA, et al. Control of fetal survival in CBAxDBA/2 mice by lymphokine therapy. J Reprod Fertil 1990;89:447-58.

8. Yui J, Garcia-Lloret M, Wegmann TG, et al. Cytotoxicity of tumor necrosis factor- (TNF-α) and gamma-interferon (IFN-γ) against primary human placental trophoblasts. Placenta 1994;15:819-35.

9. Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol 1989;7:145-73.

10. Knudsen UB, Hansen V, Juul S, et al. Prognosis of a new pregnancy following previous spontaneous abortions. Eur J Obstet Gynecol Reprod Bio1991; 139: 31-6.

11. Bruunsgaard H, Skinhøj P, Pedersen AN, et al. Ageing, tumour necrosis factor-alpha (TNF-alpha) and atherosclerosis. Clin Exp Immunol 2000;121:255-60.

Correspondence:
Nadham K. Mahdi
Central Post Office-42001,
P. O. Box 1565,
Ashar, Basrah, IRAQ.
e-mail: nadhammahdi@yahoo.com