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Association of Interleukin 6 (IL6) and 8(IL8) among Women with Spontaneous Abortion in Khartoum State 2018

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Research Article

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Abstract

Background: Interleukin 6 and Inetrleukin 8 are secreted by macrophage and have been shown to exert deleterious effects on pregnancy, inhibiting fetal growth and development. Possible immunologic causes of spontaneous miscarriages have been extensively researched. The change in the cytokines balance synthesis in favor of those synthesized by macrophage cells with an increase of interleukin 6 and interleukin8 secretion is considered essential for maintaining a normal pregnancy.

Aim: To determine the level of cytokines (interleukin6 and interleukin8) in pregnant and aborted women to assess the role of cytokines (IL 6 and IL8) as a factor to predict the outcome of pregnancy and To compare between serum interleukin 6 and 8 in women with spontaneous abortion and pregnant women.

Method: This across sectional study tested using Five ml of venus blood were collected from patients with abortion and healthy pregnant and non-pregnant control in plain container. All samples were centrifugated at 3000 rpm for 5 minutes and seperated sera were collected in sterile tubes. Serum was treated according to uniform standards and stored at – 20 refrigerators until analysed. Serum IL 6 and IL8 were determined by Enzyme Linked Immunosorbent Assay (ELISA) .All samples were run according to manufacturer instructions (Human IL, Bio legend, USA) with linearity range 0.94- 60.0 pg/ml.

Results: There is a highly significant increase of control of pregnant women ($P<0.01$) in concentration of IL6 (6.8 ±5.1)pg/ml in compare to aborted women in different trimesters of pregnancy. Whereas healthy control non pregnant women show highly significant decrease in concentration (1.6 ±0.65)pg/ml compared to abortion patients. The results of this study showed there was a highly significant increased ($P<0.01$) concentration of IL-8 (26.5 ±13.1) pg/ml in serum of aborted women in trimesters of pregnancy,compared with control group of non-pregnant women which were(6.9 ±0.93) while control group of pregnant women show slightly significance increased($P<0.01$) concentration (31.1 ±14.1)pg/ml compared with aborted women.

Conclusion: The serum level of interleukin 6 and 8 was significantly higher in pregnant women as compared to aborted women .The serum level of interleukin6 and 8 in aborted women was significantly higher as compared to non-pregnant women. The results indicated there is no clarify association between cytokines (IL6 and IL8) and abortion.

Keywords: Gestational Diabetes; Diabetes Mellitus; Hypertension; Proteinuria; Renal Disorder

Introduction

Pregnant women can miscarry the fetus spontaneously in many cases, Miscarriage also known as spontaneous abortion and pregnancy loss is the natural death of an embryo or fetus before it is able to survive independently. Some use the cut-off of 20 weeks of gestation, after which fetal death is known as a stillbirth. Miscarriage is a common

gynaecological problem with huge financial and personal implications. Eleven to twenty per cent of all clinically recognized pregnancies are lost before the 20th week of gestation, with miscarriages often being divided into early (≤12 completed weeks from last menstrual period) and late (≥13 weeks). Spiral artery remodelling is a key feature of early pregnancy; failure of this process has been implicated in sporadic miscarriage. The molecular triggers that initiate spiral artery remodelling are not clear, although cytokines such as IL-6 and IL -8 may play role Cellular immune effector mechanisms have been proposed as being responsible for at least proportion of "unexplained recurrent spontaneous abortion"(RSA). Unexplained RSA accounts for about 40-60% of all cases of RSA [1]. Recent attention has focused on elucidating the immunobiological roles of cytokines in normal human pregnancy is following the accumulated reports of complex cytokine activity within uteroplacental tissue [2]. Cytokines are important mediators in the bi-directional interaction between the maternal immune system and the reproductive system during pregnancy [3]. Interleukin-6 (IL-6) and interleukin-8 (IL-8) are pro-inflammatory cytokines produced by several tissues upon the stimulus of a number of factors , among which are membrane LPS from gram negative bacteria , Viruses and several cytokines Their action is directed towards either myeloid or non-myeloid cellular targets [4]. IL-6 produced by murine fetoplacental tissues and this cytokine comprise the profile of cytokines produced by the T-helper (Th-2) subset of T-helper cells During pregnancy these cytokines are involved in different ways in the regulation of the mechanisms of implantation and placentation Fetal maturation and uterine contraction[5]. These cytokines help to maintain the trophoblast in early pregnancy [6]. They also play a major role in intrauterine infection especially after premature rupture of membranes and in preterm and term labor irrespective of infection [7,8]. Successful pregnancy may depend, at least in part on the bias of the maternal immune response shifting away from Th-1 type response towards a Th-2 phenotype both in murine model and human [9,10]. IL-6 might have both beneficial effects and detrimental effects on the events of early pregnancy also implicated in the pathophysiology of abnormal pregnancies and other disease such as Rheumatoid Arthritis, Autoimmune disease, preeclampsia and obesity [11]. IL-8 production has been reported in cervical stromal fibroblasts glandular epithelial cells and leucocytes and levels of IL-8 in human cervical tissue increase during pregnancy [12-14]. There is increasing evidence that this cytokine is involved in the cervical changes during labour and that IL-1β may regulate the cervical secretion of IL-8 [1 5]. IL6 is a multifunctional cytokine with pivotal roles in the inflammatory response and in directing T cell differentiation in adaptive immunity. IL6 is widely expressed in the female reproductive tract and gestational tissues, and exerts regulatory functions in embryo implantation and placental development, as well as the immune adaptations required to tolerate pregnancy. Here, we summarise the current understanding of how membrane-bound and soluble receptors mediate IL6 signalling to regulate leukocytes and non-haemopoietic cells. We review the published literature regarding the expression and actions of IL6 in the uterus, decidua and placenta, and studies implicating this cytokine in pregnancy disorders. Elevated IL6 is frequently evident in the altered cytokine profiles characteristic of unexplained infertility, recurrent miscarriage, preeclampsia and preterm delivery. Notably, there is compelling evidence indicating altered systemic IL6 trans-signalling in women prone to recurrent miscarriage, with excessive IL6 bioavailability potentially inhibiting generation of CD4+ T regulatory cells required for pregnancy tolerance [16].

Material and Methods

Study Design

Descriptive cross- sectional study conducted during the period of September to November 2018.

Study Area

This study carried out at different maternity hospitals in Khartoum state (Omdurman Maternity Hospital and Bahry Teaching Hospital).

Study Population

Women with spontaneous abortion, pregnant women and apparently healthy subjects as a control group were enrolled in this study.

Inclusion Criteria

Women with spontaneous abortion. Pregnant healthy women.

Exclusion Criteria

All women with infections, gestational diabetes, diabetes mellitus, history of smoking, hypertension, proteinuria, renal disorder, cardiovascular problem, hepatic or endocrine disease, metabolic disorders and current infection were excluded from the study.

Collection of Samples

Five ml of Venus blood were collected from patients and healthy control in plain container. Then the samples were centrifugated at 3000 rpm for 5 minutes and sera were separated and collected in sterile tubes .All serum were treated according to uniform standards and stored at–20

refrigerator until samples analysed. serum IL 6 and IL8 were determined by Enzyme Linked Immunosorbent Assays (ELISA) according to manufacturer instructions (Human IL ELISA MAX Deluxe Set , BioLegend , USA).

Data Collection

The study include fourty five (45) women who had spontaneous abortion including all trimesters of pregnancy of age 20-35 years and 45 apparently healthy control (pregnant women) and 6 healthy control (non pregnant women) groups. The level of IL-6 and IL-8 was measured in serum samples (patient and control groups) by using Enzyme linked immunosorbent assay (ELISA) technique. This was performed as described by the techniques provided by the manufacturer (leaflet kits).

Quality Control

The standard product was manufactured under stringed process to ensure consistency and complete traceability and standard curve was run with each assay.

Measurement of Interleukin 6

The plate has been washed 4 times and blocked by added 200µl assay diluents to 100 µl diluted captured antibody. The plate sealed and incubated at room temperature for 1 hour in plate shaker (500 rpm with 0.3 cm circular orbit). 100µl diluent standard and sample were added to appropriate well. Sealed well incubated at room temperature for 2 hours.100µl diluted detection antibody was added to already washed plate and incubated at room temperature in shaker .100µl diluted Avidin –HRP solution was added to well and incubated for 30 minutes at room temperature in shaker .Then soaked for 30 seconds to 1 minute in 100µl freshly mixed TMB substrate to each well in the dark for 15minutes.Stop solution was added to each well and read the absorbance at 570 nm within 15minutes or in 540 nm.

Quality Control

Standard curve was run with each assay.

Measurement of Interleukin 8

The plate has been washed 4 times and blocked by added 200µl assay diluents to 100 µl diluted captured antibody. The plate sealed and incubated at room temperature for 1 hour in plate shaker (500 rpm with 0.3 cm circular orbit).

100µl diluent standard and sample were added to appropriate well. Sealed well was incubated at room temperature for 2 hours. 100µl diluted detection antibody was add to already washed plate and incubated at room temperature in shaker.

100µl diluted Avidin –HRP solution was added to well and incubated for 30 minutes at room temperature in shaker. Then soaked for 30 seconds to 1 minute in 100µl freshly mixed TMB substrate to each well in the dark for 15minutes. Stop solution was added to each well and read the absorbance at 570 nm within 15minutes or in 540nm.

Quality Control

Standard curve was run with each assay.

Reading of Results

Reading of results by using microplates reader which detect biological and chemical data using absorbance in UV-VIS spectrum from 220 to 1000 nm.

Interpretation of Result

The results were interpretated by comparing them to a standard curve, which allows the concentration of antigens in different samples to be precisely determined.

Ethical Considerations

Study was approved by ethical committee of Alzaiem Alazhari University .Formal consent permission from hospitals and vebral consent was obtained and all subjects under study were informed by the purpose of the study.

Statistical Analysis

All statistical analysis was performed using SPSS software and Microsoft excel 2007 and SPSS and Microsoft excel programmes were used for T-test and correlation coefficient calculations respectively. Data was presented as mean ± S.D a statistical value < 0.05 was considered as significant . The results expressed in the form of tables.

Results

The results of this study showed highest concentration of interleukin 6 which was 24.0 pg/ml and lowest concentration as 0.07 pg/ml with standard deviation (6.4 ± 4.5). At the other hand in interleukin 8 highest concentration was 43.6 pg/ml and lowest concentration was 9.7pg/ml with standard deviation (26.5± 13.1) in aborted group table 1. And showed highest concentration of interleukin 6 which was 40.2 pg/ml and lowest concentration as 2.4 pg/ml with standard deviation (6.8±5.1). At the other hand in interleukin 8 highest concentrations was 76.9 pg/ml and lowest concentration 9.7pg/ml with standard deviation (31.1 ± 14.1) in pregnant group table 2.

	N	Mean	Std Deviation	Range of Il 8		P. Value	One Way Anova
				Minimum	Maximum		
Pregnant control	45	31.1	14.1	9.7	7 6.9		Highly Significant
Nonpregnant control	06	6.9	0.93	5.6	9.7	0.05	(P. Value <0.05)
patients	45	26.5	13.1	6.7	5 4.3		
Total	96						

*ANOVA= Analysis of variations
*N= Sample size
Table 1: (4-4) Mean levels of lL-8 (pg/ml) in serum of women with spontaneous abortion and control groups.

	N	Mean	Std Deviation	Range Of Il 8		P. Value	One Way Anova
				Minimum	Maximum		
Pregnant control	45	6.8	5.1	2.4	40.6		Highly Significant
Nonpregnant control	06	1.6	0.65	0.7	2.6	.00	(P. Value <0.0001)
patients	45	6.4	4.5	0.07	24.0		
Total	96						

*ANOVA= Analysis of variations
*N= Sample size
Table 2: (4-5): Mean levels of lL-6(pg/ml) in serum of women with spontaneous abortion and control groups.

Discussion

In this study 45 pregnant women and 45 abortion patients were enrolled and the serum cytokines was high. Spontaneous abortion is the most common of pregnancy failure, with small numbers of pregnancies can be detectable for spontaneous abortions. The risk of a subsequent miscarriage after one spontaneous loss or after two or three consecutive losses is different [10]. The current study was prompted by an interest in explaining the immunological condition in which regular aborters have a successful pregnancy and in particular to a ascertain whether their cytokine profiles are more conductive to successful pregnancy as opposed to regular aborters who continue to abort. The results of this study were in agreement with other studies on human pregnancy failure. In many studies, There was decreased level of IL-6 in serum of normal pregnant women compared with level of this cytokine in women undergoing recurrent spontaneous abortion [12-14].

Monkeys have shown higher concentration in IL-6 precedes uterine contractions , studies in monkeys suggesting that IL-6 may play a role in physiological mechanisms involved in uterine contractions and the propagation of labour [15]. Thus, increased concentration of IL-6 may reflect a systemic reaction in the mother, leading to labour and delivery and IL-6 found in the serum may originate from the trophoblast [16].

The lower concentration of IL-6 in women with spontaneous abortion consider that IL-6 is a Th-2 type cytokine and that normal pregnancy appears to be a Th-2 biased condition and this might reflect a bias away from Th-2 type reactivity and a shift towards Th-1 dominance [10].

In another study there was a high level of IL-8 in serum of women with at least three spontaneous abortion. The inflammatory cytokines, such as IL-8, may play an important role in the mechanism of protease-induced neurogenic inflammation leading to labor or abortions by recruiting neutrophils and lymphocytes in the endometrium.

Whereas previous study reported that women with spontaneous abortions had significantly decreased plasma level of IL-8, IL-6 and IL-11 compared to those with normal pregnancies [13] and this is agreed with our study.

1. Conclusion

The serum level of interleukin 6 and 8 was significantly higher in pregnant women as compared to aborted women. The serum level of interleukin6 and 8 in aborted women was significantly higher as compared to non-pregnant women. The results indicated there is no clarify association between cytokines (IL6 and IL8) and abortion.

Recommendation

1. Appropriate medical history should be known about the participants
2. other general and immunological examination (CBC, CD4

count, cut of Elisa) should be done
3. Pregnancy trimesters should be enrolled.
4. Further studies and research in pregnancy cytokines must be done.

Acknowledgment

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