Determination Levels of Tumor Necrosis Factor Receptor and Tolk like Receptor -4 and Bacterial Vaginosis in Women with Recurrent Spontaneous Abortion

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Abstract

The presents study included Determination levels of Tumor necrosis factor and Tolk like receptor -4 in women with recurrent spontaneous abortion in province of Babylon, 100 samples were collected, whereas 50 samples with recurrent abortion women and 50 healthy women as control groups **,**the results of presents study shown that the concentration of tumor necrosis factor receptor in serum of women with recurrent abortion and control was showed significantly differences between control and cases (P≤ 0.044), the Mean of tumor necrosis factor receptor (TNFR) in serum of patients women with recurrent abortion was 52.08 pg/ml,Whereas in control, the concentration of tumor necrosis factor receptor in serum of healthy women was 42.77 pg/ml.

Tolk like receptor 4 concentration in serum of women with recurrent abortion and control was significantly differences between cases and control (P ≤ 0.001), the mean of Tolk like receptor 4 concentration in serum of women with recurrent abortion was 3.95 pg/ml While In serum of women without abortion (control), the mean of Tolk like receptors 4 was 2.84 pg/ml.

The results of statistical analysis shown that the tolk like receptor-4 marker that is significantly differences at (p ≤ 0.05) positive correlation with age (r = .698**) , and its positively correlation with abortion (r= .505*) , the tumor necrosis factor receptor was significantly differences at (p ≤ 0.05) and positively correlation with age and the value of r was reached to .539* and its positive correlation with abortion (r =.854**)

Keyword: *Recurrent abortion, Tumor necrosis factor receptor and Tolk-like receptor 4*

Introduction

Recurrent spontaneous abortion is defined according to the American society for reproductive medicine " two or more consecutive pregnancy losses before 24 weeks of gestation''[1, 2] the gross risk of abortion is 15% after one abortion , 17-31%after two successive abortion , and 25-46% after three or more repeated abortion [3, 4].

Many epidemiological that are causes recurrent spontaneous abortion such as infection (0.5-5%), genetic (2-4%), anatomic (10-15%), thrombotic (≥15%), endocrine (17-20%), immunologic (20%) and environmental (40-50%), in women with recurrent abortion that causes rising NK cell count and autoantibody levels that lead to decreased blood flow in uterine during early pregnancy, Hence, autoimmunity play pathological role in causing recurrent abortion in pregnant women [5, 6].

Cytokines influence all steps of reproduction, including the risk of miscarriage, specifically, Th2 cytokines such as interleukin (IL)-4, IL-10 and IL-13] are associated with pregnancy success, whereas Th1 cytokine responses such as interferon (IFN)-γ, and tumour necrosis factor (TNF)-α predominate in spontaneous miscarriage. Although the complexity of the cytokine network at the feto-maternal interface has increased with the discovery of newer cytokines and improved understanding of the role of specific cellular subtypes (e.g. natural killer cells, dendritic cells and regulatory T cells), the evidence that a Th1 response in

the decidua may lead to miscarriage remains substantial.

Toll-like receptors (TLRs) are a recently identified group of vertebrate receptors that play a central role in determining the Th1/Th2 balance of immune responses. The human TLR family consists of 10 receptors that orchestrate the innate immune response by linking pathogen recognition with immune cell activation. Individual TLRs recognize a distinct, but limited, repertoire of conserved microbial products, and the best-characterized receptor-ligand pair is TLR4 and lipopolysaccharide (LPS or endotoxin). In most situations, TLR activation promotes the generation of a Th1-dominated immune response and inhibits Th2 cytokine production [7].

Material and Methods

Patients and control groups

The study was done on women that are attended to departments of obstetrics and gynecology at maternity and child teaching hospital, and some private clinics in Babylon governorate that are suffering from recurrent spontaneous abortion from 1/12/2019 to 1/6/2020. This included 50 patients and 50 pregnant women that are apparent healthy that are selected as a control groups

Blood Samples

Five milliliter of venous blood that are collected from women with recurrent spontaneous abortion and healthy women , the samples were placed in tubes containing a gel and then the blood was separated by centrifuge at 3000 rpm for 15 minutes, the serum was putted in Eppendrof tubes and then stored in a deep freeze at -20C˚ until used [8].

Immunological Markers

Tumor Necrosis Factor Receptors

Tumor necrosis factor receptor of human is measured by Boster's human TNFsR I ELISA kit was depend on standard sandwich enzyme-linked immune-sorbent assay, A monoclonal from mouse specific for TNFsR I has been precoated onto 96 well plates , the standard and sample are added to the wells, a biotinylated goat TNFsR lis added subsequently and then followed by washing with PBS buffer , Avidin-Biotin-peroxidase complex was added and unbounded conjugates were washed away with PBS buffer , HRP substrate TMB was used to visualize HRP enzyme reaction, TMB was catalyzed by HRP to produce a blue color product that change into yellow after adding acidic stop solution , the density of yellow color is a proportion to the human TNFsR l amount of sample captured in plate and absorbance is measured at 450 nm. .

· Tolk Like Receptors-4

Tolk like receptor 4 is detection by an enzyme-linked immunosorbent assay kit, the plate has been precoated with human TLR4 antibody,40µl from sample is added and binds to antibodies coated on the well, and then 10 µl biotinylated human TLR4 antibody is added and binds to TLR4 in the sample , then 50 µl streptavidin-HRP is added and binds to the biotinylated TLR4 antibody, after incubation unbound streptavidin-HRP is washed away during a washing step, 50 µl from substrate solution is then added an color develops in in proportion to the amount of human TLR4, the reaction is determined by addition 50 µl from acidic stop solution and absorbance is measured at 450 nm.

Statistical Analysis

Statistical analysis was carried out using statistical package for social science SPSS statistical software for windows version 24 to found means, Standard deviation, Least Significant differences by Duncan and Correlation by ANOVA

Results

Tumor necrosis factor receptors TNFR

The concentration of tumor necrosis factor receptor in serum of women with recurrent abortion and control was showed significant differences between control and cases ($P \leq 0.044$), the Mean of tumor necrosis factor receptor (TNFR) in serum of patients women with recurrent abortion was 61.91 pg/ml While the highest concentration in serum of women with recurrent abortion was 94.57 pg/ml and the lowest concentration of in serum of women with recurrent abortion was 15.55 pg/ml. Whereas in control, the concentration of tumor necrosis factor receptor in serum of women was 38.50 pg/ml, While the highest concentration of tumor necrosis factor receptor (TNFR) was 76.19 pg/ml and the lowest concentration of tumor necrosis factor receptor (TNFR)

was 14.43 pg/ml Table (1) and figure (1-1).

Tolk like receptors 4 TLR4

Tolk like receptor 4 concentration in serum of women with recurrent abortion and control was significant differences between cases and control ($P \leq 0.001$), the mean of Tolk like receptor 4 concentration in serum of women with recurrent abortion was 4.42 pg/ml While the highest concentration of Tolk like receptors 4 was 7.30 pg/ml and the lowest concentration of Tolk like receptors 4 was 2.42 pg/ml ,

In serum of women without abortion (control), the mean of Tolk like receptors 4 was 2.33 pg/ml ,Whereas the highest concentration of Tolk like receptors 4 was 4.59 pg/ml and lowest concentration of Tolk like receptor 4 was 1.29 pg/ml Table (1) and figure (1-2).

Table. (1). the variation in TNF-α level expressed as mean ± SD in patients and control groups.

Marker	Group	N	Mean	Lower-upper value	Std. Deviation	Std. Error Mean	t.test	P.value
TNFR	Control	45	38.5080	42.77-76.19	14.28940	2.13014	-6.402	0.000
	Patient	49	61.9129	15.55-94.57	20.79679	2.97097		
TLR4	Control	44	2.3317	1.29-4.59	0.96700	0.14600	-8.265	0.000
	Patient	48	4.4250	2.42-7.30	1.43500	0.21000		

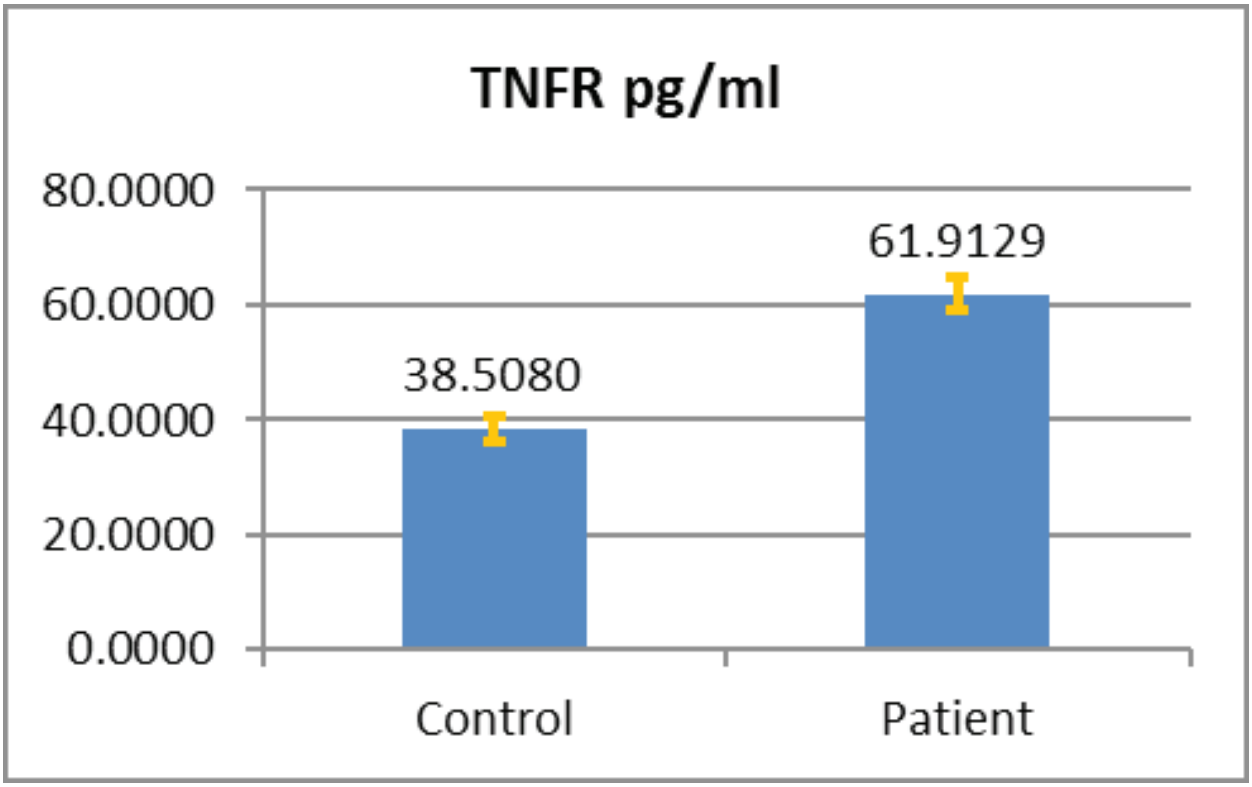

Figure (1-1): The concentration of tumor necrosis factor receptor in serum of control and women with recurrent abortion

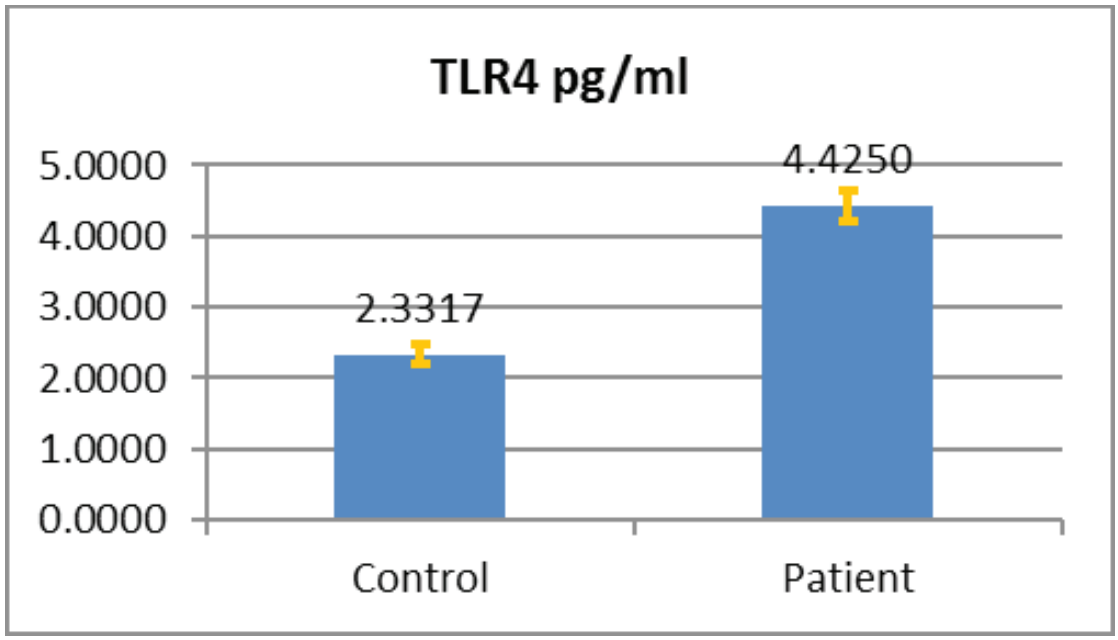

Figure (1-2) The concentration of Tolk like receptor 4 in serum of control and women with recurrent abortion

Correlations among TLR-4, TNFR, Age and Abortion

Table (2) correlation among TLR-4, TNFR, Age and abortion , the results of statistical analysis shown that the tolk like receptor-4 marker that is significantly differences at ($p \leq 0.05$) positive correlation with age ($r = .698^{**}$) , and its positively correlation with abortion ($r= .505^{*}$) , the tumor necrosis factor receptor was significantly differences at ($p \leq 0.05$) and positively correlation with age and the value of r was reached to $.539^{*}$ and its positive correlation with abortion ($r =.854^{**}$)

Table (2) correlation among TLR-4, TNFR, Age and abortion

Correlations					
		TLR-4	**TNF**	**Age**	**Abortion**
TLR-4	Pearson Correlation	1	-.019	. 698**	.505*
	Sig. (2-tailed)		.892	.005	.030
	N	56	56	48	56
TNFR	Pearson Correlation	-.019	1	.539*	.854**
	Sig. (2-tailed)	.892		.040	.021
	N	56	57	49	57
Age	Pearson Correlation	.698**	.539*	1	.702**
	Sig. (2-tailed)	.005	.040		.000
	N	48	49	50	50
Abortions	Pearson Correlation	-.205	.854**	.702**	1
	Sig. (2-tailed)	.130	.021	.000	
	N	56	57	50	58
**. Correlation is significant at the 0.01 level (2-tailed).					

Discussion

One of a pro-inflammatory cytokine is a tumor necrosis factor receptor (TNFR) that excreted by Th1 cells and placenta , it links with inflammatory mechanisms that regarding with an implantation , placentation and pregnancy loss.

The results of present study shown significantly differences increased in the level of tumor necrosis factor receptor in women with recurrent abortion which reached to 61.91±20.79pg/ml, While it was 38.50±14.28 in healthy without bacterial vaginosis, the current results was agreement with most other studies about tumor necrosis factor receptor concentration like the study of Hamad (2018) that shown significant rise in the level of tumor necrosis factor in women with recurrent abortion (78.5±6.61) While in healthy women, its concentration (59.5 ±6.35) Whereas Azizieh and Raghupathy, (2015) that had been shown the level of TNFR was significantly increased at 1st trimester (4159.8 pg/ml); 2nd trimester (3489.5 pg/ml) and the 3rd trimester (4149.2 pg/ml) in women with recurrent abortion as compared with 1st trimester (1176.4 pg/ml); 2nd trimester (4320.9 pg/ml) ; and the 3rd trimester (7307.4 pg/ml) respectively in the normal healthy women, these increased in level of tumor necrosis factor receptor in women with recurrent as compared with the healthy women may be related to the presence of the auto antigen that induces human maternal peripheral blood mononuclear cells ,and thus lead to the production of a large amount of TNFR in sera of women with recurrent abortion [9].

The results of present study shown significantly differences at ($P \leq 0.001$),increasing in the level of Tolk like receptor-4 in women with recurrent abortion which reached to 4.42 ± 1.43 pg/ml, While it was 2.33±0.96 in healthy without bacterial vaginitis.

Toll-like receptor 4 (TLR4) belongs to the pattern recognition receptor family, which plays a key role in the human defense mechanism and responds to invading pathogens with high selectivity and sensitivity (Gay et al.,2014 and Chen et al.,2018). TLR4 is sensitive to pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS) and lipo-oligosaccharide. Moreover, TLR4 recognizes PAMPs from fungi, viruses, mycoplasmas, and bacterial vaginosis, in addition to PAMPs, TLR4 can be activated by certain endogenous ligands produced due to tissue injury and/or inflammation [10, 11]. This receptor–ligand interaction initiates an intracellular signaling cascade that leads to the subsequent proinflammatory response, thus, due to the involvement of TLR4 in various pathological conditions [12, 13].

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Ethical Approve: We declare that the study does not need ethical approval.

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