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CASE STUDY

Flow Cytometric detection of Toll Like receptors on blood cells among patients with prostatitis

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Abstract

The Aim of this study was to determine Immunogenetic expression of Toll-like receptor gene clusters related to prostatitis, to give acknowledge about Role of TLR in prostatitis immunity in men from Basrah and Maysan provinces. A case–control study included 135 confirmed prostatitis patients And 50 persons as a control group. Data about age, marital status, working, infertility, family history and personal information like (Infection, Allergy, Steroid therapy, Residency, Smoking, Alcohol Drinking, Blood group, Body max index (BMI) and the clinical finding for all patients of Prostatitis were collected , This study was conducted to detect the various TLRs isolated from Prostatitis patients by phenotypic and molecular methods with a study of titer of prostate specific antigen and detection of TLRs by flow cytometry.In flow cytometry results the present study found that TLR2 in Prostatitis patients was 94.6%, and Prostitis (prostate cancer) was 1.16% and in the control group was 48%, and found TLR4 was 98.5% in prostatitis patients, and Prostitis was 85.1% and in the control group was 97% by total monocytes of total white blood cells.

Keywords: flow cytometry, prostatitis, blood cells

1 | INTRODUCTION

TLR was first discovered in drosophila in the early 19 century and later in mammals other than humans and later in humans in 1992. O'neill (2008) and **Medzhitov et al. (1997) .** In 1997, TLRs was first shown to have clinical importance of recognizing and eliminating pathogens and harmful particles in humans. Garantziotis et al. (2008) and **Sánchez et al. (2004) .** Toll like receptors a well known group of pattern recognizing receptors in the innate immunity. Toll-like receptors are a group of transmembrane receptors act as a key role in the innate immunity. TLRs block the invasion of the pathogens by recognizing the pathogen-associated molecular patterns (PAMPs), which are they highly preserved components derived from bacteria, viruses, fungi, and parasites. It can also recognize endogenous damage-associated molecular patterns (DAMPs) in several disorders and diseases such as cancer. Takeda et al. (2003)**.** At present, there are 13 types of toll like receptors in the nature, 10 are present in human and other 3 in animals TLR1s, TLR2, TLR4, TLR5,

and TLR6 are expressed on the cell surface, TLR3, TLR7, TLR8, and TLR9 are found exclusively inside endosomes. Various types of TLRs show specifically for ligand recognition like TLR2 recognizes bacterial lipoproteins, TLR3 recognizes double-stranded RNA/polyinosinic polycytidylic acid, TLR4 recognizes lipopolysaccharides (LPS), TLR5 recognizes flagellin, TLR7 recognizes single-stranded RNA, and TLR9 recognizes CpG-containing DNA (CpG-ODN). Heil (2004) and **Poltorak et al. (1998) ,** TLR10 is so far an orphan receptor and highly expressed in the human spleen and B cells **. Hasan et al. (1950)**

2 | MATERIALS AND METHODS

Sampling

This case control study was conducted between October 2019 to July 2020 in Basrah and Missan province. During collection process data about each patient were reported in the paper questionnaire for each one, which included age, marital status, infertility, family history, personal information and clinical finding of the diseases. Blood samples were collected from peoples that are symptomatic and asymptomatic patient in various hospitals of Basrah and Missan province. From a total number of (135) patients with prostatitis were taken from two provinces from the Basrah teaching hospital and Missan teaching hospital that included in the present study and the age of patients was between 40 - >70 years and (50) individuals regarded as a control group without any urological problems were also studied.

Flow cytometry instruments and kits

Table (1) show the instrument and Kits of flow cytometry.

TABLE 1: Flow cytometry instrument and kits

Item	Model	Company	Country
Flow cytometry	Bricyte E6	Mindary	China
Anti-h TLR2	ABCW021805	Minneapolis	USA
Anti-h TLR4	ABOG021708	Minneapolis	USA

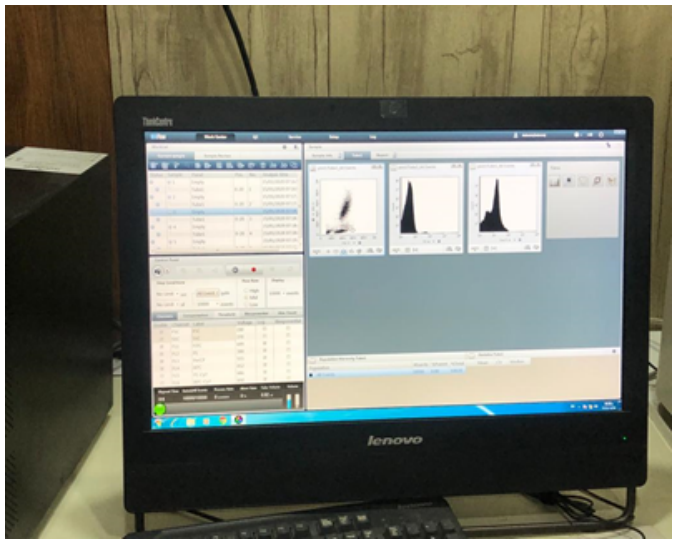

FIGURE 1: Flow cytometry analyzer. Bricyte E6 , Mindary ,China , Karbala city , Al jawadian laboratory

FIGURE 2: Flow cytometry kits for TLR2 and TLR4.

Supplementary information The online version of this article (https://doi.org/10.15520/jmrhs.v3i11.279) contains supplementary material, which is available to authorized users.

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Detection of TLRs by Flow cytometry

The blood specimens of patients tested to estimate the numbers of TLRs on the monocytes cells by using flow cytometry techniques, and the work done according to manufacturer's instruction as the following steps.

1. A 50μL of anti-coagulated (EDTA, ACD) whole blood was added to the bottom of 12 × 75mm polystyrene tube, along with the conjugated antibody's, vortex and incubate the tube in the dark at room temperature for 10 minutes.
2. A 100μL of reagent A (RBCs lysis) was added to each sample and vortex followed by an incubation for 10 minutes at room temperature in the dark.
3. A 1μL of reagent B (platelet lysis) was added to each sample and vortex followed by incubation for 20 minutes in the dark.
4. The mixture was centrifuged at 300 x g for 5 minutes . Then poured off the supernatant and re suspend in 1ml of PBS (phosphate buffer saline).
5. The mixture was centrifuged at 300 x g for 5 minutes. And again poured off the supernatant in 300ml of PBS.
6. In the final step, the mixture was analyzed the sample on the flow cytometry.

Statistical analysis

Statistical analysis is performed with SAS JMP Pro statistical program version 13.2.1 and Microsoft Excel 2013. Numerical data were described as mean, standard deviation of the mean. Logistic regression was used for comparison between various groups. The lowest level of accepted statistical significant difference is below or equal to 0.0001.

Flow cytometry plots for patients with prostatitis and control group.

Figure (3) shows the getting area of flow cytometry after lysis process of blood cells for control sample , flow cytometry divide the cells according to size and shape , and the shape includes the cytoplasmic contents of cell and their granules and also shape of nucleus therefore , because the sample was a blood samples after passing the lysing steps by breakdown of RBCs and Platelets and get only WBCs.as seen in the following figure.

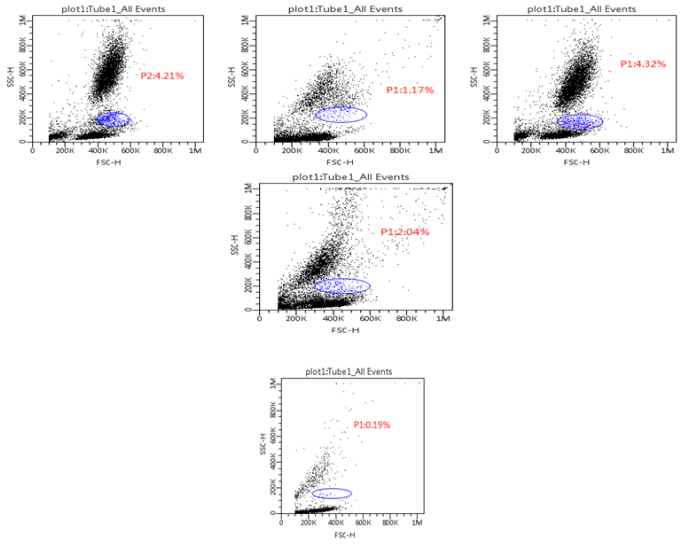

FIGURE 3: show Getting area of flow cytometry after lysisprocess.

Flow cytometry plots for TLR2 in patients with prostatitis and control group.

Figure (4) shows histogram of flow cytometry that explains the availability of TLR4 CD marker for various samples with percentage of TLR2 for patients with prostatitis and control group.

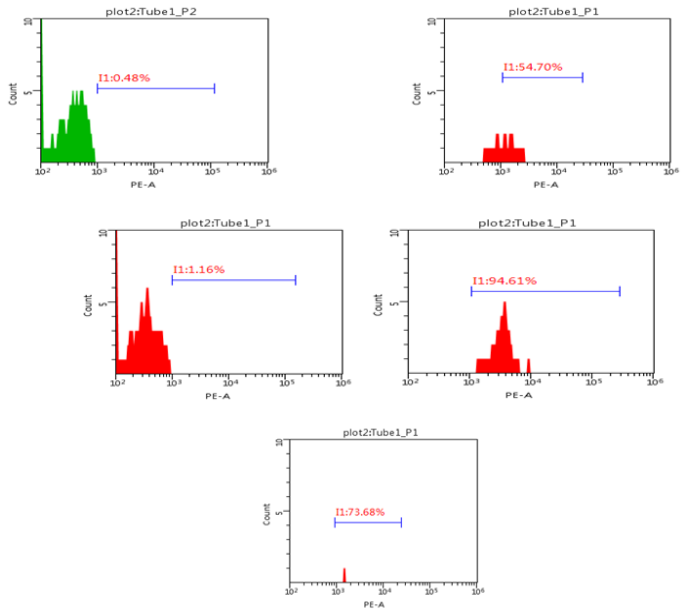

FIGURE 4: show the availability ofcells for certain CD marker for various samples with percentage of TLR2 forpatients with prostatitis and control group.

Flow cytometry plots for TLR4 in patients with prostatitis and control group.

Figure (5) shows histogram of flow cytometry that explains the availability of TLR4 CD marker for various samples with percentage of TLR4 for patients with prostatitis and control group.

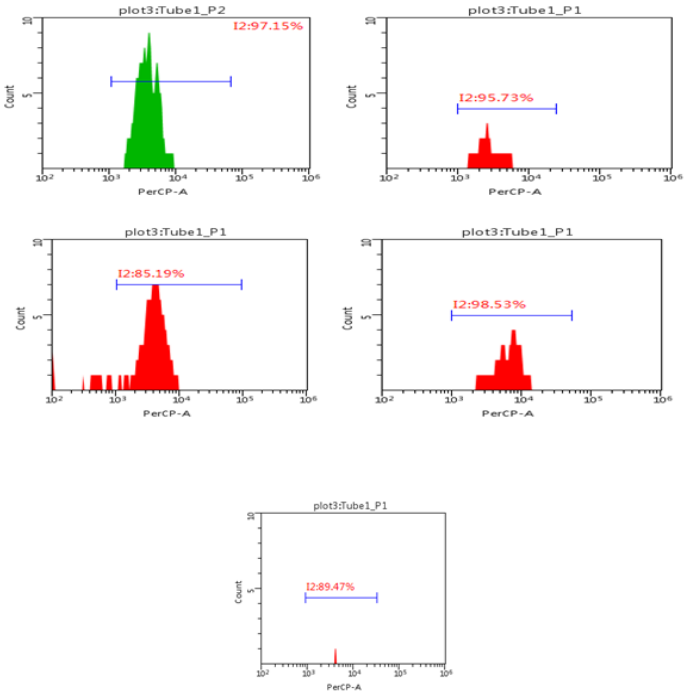

FIGURE 5: show the availability ofcells for certain CD marker for various samples with percentage of TLR4 forpatients with prostatitis and control group.

Number of TLR2 and TLR4 on Monocytes

Table (2) show the number and percentage of TLR2 and TLR4 among patients with prostatitis and control group that found TLR2 in Prostatitis patients was94.6% , with Prostitis(prostate cancer) was 1.16%and in control group was 48% , and found TLR4 was 98.5% with prostatitis patients , with Prostitis was 85.1% and in control group was 97% by total monocytes from total white blood cells.

Table (2) illustrate the number and percentage of TLR2 and TLR4 on Monocytes in blood component of patients with prostatitis and Prostitis (Prostate cancer) in comparison with control. P<0.05

TABLE 2: illustrate the number and percentage of TLR2 andTLR4 on Monocytes in blood component of patients with prostatitis and Prostitis(Prostate cancer) in comparison with control. P<0.05

TLRs on Monocytes	Prostatitis		Prostitis		Control	
	%	No. $\times 10^9$ monocyteS from total monocytes	%	No. $\times 10^9$ monocyteS from total monocytes	%	No. $\times 10^9$ monocytes from total monocytes
TLR2	94.6	0.283	1.16	0.00348	48	0.00144
TLR4	98.5	0.295	85.1	0.255	97	0.291
Total Monocyte From total WBCs	2.04	0.142	4.32	0.518	4.21	0.265

Number of W.B.Cs among patients with prostatitis and control group.

Table (3) show number of W.B.Cs & R.B.Cs among patients with prostatitis and control group. That found the number of W.B.Cs in prostatitis was 12.9×10^9 /L with Monocytes ratio 16.8%, Lymphocytes ratio 62.2% and Granulocytes ratio 21%.

TABLE 3: illustrate the number and percentage of white bloodcells.

W.B.Cs	Control	Prostatitis	Prostate cancer
Normal value: 3.5- 10×10^9\L	6.3× 10^9/L	12.9×10^9\L	12.0×10^9\L
Monocytes N.V: 1-1.5 × 10^9\L	0.3×10^9\L	1.1×10^9\L	
(N.V: 2-15)%	6.2%	16.8%	
Lymphocytes N.V: 0.5-1×10^9/L	2.1×10^9\L	4.5×10^9\L	4.5×10^9\L
(N.V 15- 50)%	33.0 %	62.2%	37.9%
Granulocytes N. V :1.2-8.0×10^9\L	3.9×10^9\L	1.4×10^9\L	0.6×10^9\L
(N.V: 35-80) %	60.8%	21.0%	5.0%
Total	100%	100%	100%

Normal Values

Table (4) show normal values of blood components

TABLE 4: illustrate Normal values of blood components .

Blood components	Results	Normal value
R.B.Cs	4.78 $\times 10^{12}$ \L	Normal value: 3.5-5.5 $\times 10^{12}$\ L
H.B	13.7 g\dl	Normal value: 11.5-16.5 g\dl
H.C.T	43.5%	Normal value: 35.0-55.0 %
PLT	256 $\times 10^{9}$ \ L	Normal value: 100-400 $\times 10^{9}$ \ L

3.5.7 Blood Components among patients

Table (5) shows the results of blood components among patients with prostatitis and control group.

TABLE 5: illustrates the results of blood component samong patients with prostatitis and control group.

Blood components	Control	Prostatitis	Prostate cancer
R.B.Cs	2.78 $\times$10^12\L	4. 20*10^12 \ L	3.44*10^12 \ L
H.B	13.7 g \ dl	13.1 g\dl	9.8 g \dl
H.C.T	43.5%	44.6%	31.4%
PLT	256$\times$10^12 \L	95 *10^12 \L	502*10^12 \L

3 | DISCUSSION:

The blood samples add to a 50μL of anti-coagulated (EDTA,ACD) along with the conjugated antibody's(anti-hTLR2 and anti-hTLR4) , and vortex with incubation for 10min in the dark followed by added a 100μL of reagent A(RBCs lysis) and incubate for 10 min in the dark at room temperature , third step started with added a 1μL of reagent B (platelets lysis) with incubation for 20min in dark too , after incubation the samples Centrifuge at 300 $\times$ g for 5 minutes and re suspended with 1ml of PBS (phosphate buffer saline) , and centrifuge again with same speed and time of previous step but with re suspended with 300ml of PBS. Finally entered the sample to flow cytometry to detect the conjugation antigen- antibody complex of TLR2 and TLR4 on Monocytes , in the present study found that TLR2 in Prostatitis patients was 94.6% , and Prostitis(prostate cancer) was 1.16% and in control group was 48% , and found TLR4 was 98.5% with prostatitis patients , and Prostitis was 85.1% and in control group was 97% by total monocytes from total white blood cells. The results of present study disagree with Gatti et al. (2006) when he say No surface expression of TLR4, TLR2, or CD14 was detected on the cell line, neither on basal conditions nor upon stimulation with LPS. Although these molecules were easily detected at the surface of rat-activated peritoneal cells (data not shown), expression of TLR4 protein was only detected when MAT-LU cells were permeabilized, indicating an intracellular localization of TLR4. Stimulation of the cells with LPS did not modify its subcellular localization; however, an increase in the percentage of cells expressing TLR4 as well as enhanced levels of expression of this receptor was seen after 24 h of treatment with LPS (basal: 34.3%, mean fluorescence channel: 16.80; 24 h: 46.6%, mean fluorescence channel: 22.44; 48 h: 35.8%, mean fluorescence channel: 18.24). Also, the percentage of cells expressing TLR2 raised intracellularly (basal: 8.2%; LPS 24 h: 23.4%; LPS 48 h: 13.1%), and an intracellular expression of CD14 protein was also detected. To directly demonstrate the intracellular expression of TLR4 by MAT-LU cells, Rezania et al. (2014) also say their results

Showed clearly that, none of the cell lines expressed TLR2 or TLR4 on their surface. Indeed, no detectable levels of surface CD14 were found in prostate cancer lines. The results clearly showed that such treatments could not up regulate surface expression of TLR2 and 4 in prostate cancer cell lines. Based on the results of Western blot and flow cytometric analyses, we were then about to localize TLR2 and TLR4 expression in prostate cancer cells.

Another study demonstrates TLRs in other diseases and their activation or signaling pathway. Alabbasi et al. (2010)

The present study demonstrate the cell surface expression of TLR2 and TLR4 on monocytes in patients with prostatitis , the results show a high percentage of these receptors on the monocytes from the total white blood cells that may indicate the type of infection that cause the disease and the activation of

TLRs according to type of pathogen that involved . The prostatitis causes an excitation of the immune system and in this case the availability of TLR2 is high more than TLR4.

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