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RESEARCH ARTICLE

Assessment of Immunomolecular expression and prognostic role of TLR7 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. 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 , The molecular expression study include extracting DNA from blood of Prostatitis patients , Prostitis patients and Control group by using specific primers for conventional PCR and Real Time PCR , the amplification of all extracted DNA from blood samples was preform and confirm by using electrophoresis with (100volt/30min). PCR product was 149bp for TLR7 on agarose gel (1%), (50voltage for 1hour) with a presence 100%. The results of the present study indicate that the Toll like receptor alleles associated with risk of prostatitis.

Keywords: prostatitis, TLR7, PCR

1 | INTRODUCTION

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 in 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)**. Role of TLRs in the defense against prostate infections is the most evolutionarily conserved role of TLRs in host defense is the regulation of antimicrobial responses by epithelial cells, the first line of defense at mucosal sites such as the respiratory, gastrointestinal and genitourinary tracts and the skin. Nevertheless, the widely accepted hypothesis is that non-sterile sites (i.e. Mouth, colon, or vagina) would require a response system different from that of sterile sites (bladder, kidney, prostate and testis). It is conceivable that the pattern of expression of TLRs would then differ at sterile versus non-sterile sites and that at non-sterile sites epithelial cells might be less efficient reactive than at sterile sites where even a low load of deleterious microorganisms should be rapidly detected and eliminated. **Quayle (2002) .**

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. Affect (1781)

Molecular Study

The molecular expression includes extracting DNA from blood cells of prostatitis patients and the control group by using specific primers for Conventional PCR and Real Time PCR. Duque and Descoteaux (2014)

Genomic DNA Extraction

The quality and purity of extracting DNA from blood samples of prostatitis patients by using a Favogren DNA Extraction kit was high and every DNA for each sample was separately extracted and the results confirm throughout gel electrophoresis, DNA-based techniques have been further simplified benefiting from the introduction of PCR. Brede and Shoskes (2011) Various strategies have been adopted in attempting to use PCR for genome analysis and specification purposes**. (Fairbrother *et al.*, 1998)** as seen in the figure (1).

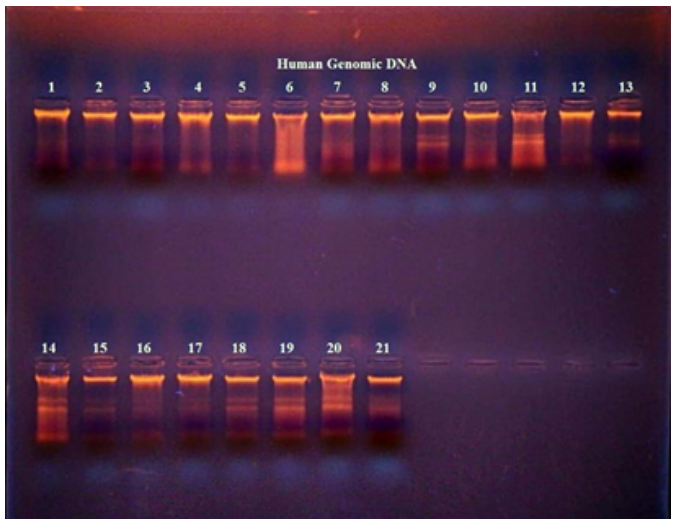

FIGURE 1: Show Agarose 1% gel electrophoresis image (100vlotage for 30min). That showquality and purity of DNA products extracted from blood samples by commercialDNA extraction Kit.

Polymerase chain reaction technique

Supplementary information The online version of this article (https://doi.org/10.15520/jmrhs.v3i11.278) contains supplementary material, which is available to authorized users.

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PCR is a very effective method to amplify a particular DNA as many copies of a specific DNA (Bartlett 2003), all samples were assayed for the presence of the *TLR1 and TLR2* genes by PCR using previously described primers, for PCR used diluted forward and reverse primers and the primers working solution were prepared by diluting the stock solution with TE buffer to get final working solution (10 pmole/Ml) for each primer.

TABLE 1: PCRMaster mix Volume:

PCR mix		*Volume*
Bio-Lab Master Mix		12. 5µL
DNA template		1. 5µL
Primer	Forward Primer	0. 5µL
	Reverse Primer	0. 5µL
Nuclease free water		10 µL
Total		25µL

TABLE 2: Oligonucleotidesequence and Amplicon Size gene used in this study

Gene	*Oligonucleotide Sequence (5'-3')*	*Amplicon Size, bp*	*Reference*
TLR7		149bp	Valente, *et al.*, 2012
Forward	AAACTCCTTGGGGCTAGATG		
Reverse	AGGGTGAGGTTCGTGGTGTT		

TABLE 3: thethermal cycler programs used in this study

Gene	*Temperature (°C) /Time*					*Cycle No.*
	Initial Denaturation	*Cycling condition*			*Final extension*	
		Denaturation	*Annealing*	*Extension*		
TLR7	94/3 min	94/30 sec	55/60 sec	68/1 min	68/5min	30

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.

3 | RESULTS:

The results of amplification of extracted DNA from blood samples was preform and confirm by using electrophoresis , in this analysis the resulted DNA bands that came from a successful binding between the extracted DNA and the target specific primers for each one of toll like receptors , and the bands will appear under UV light as a compact band by using ethidium bromide stain as indicator DNA stain ,also electrophoresis allow to estimate the size of molecular DNA by using (100-1500bp DNA ladder) and (100- 1000bp DNA ladder) as DNA marker , the results will show the amplified DNA (PCR products) for each TLRs.

Toll Like Receptor 7

The result of PCR amplification which was performed on the extracted DNA was confirmed by electrophoresis as the strands of the DNA, which result from the successful binding appear as a single band under U.V illuminator using ethidium bromide as a specific DNA stain. Only the bands with expected size 149bp (TLR7-specific primer) were observed. As seen in figure (2).

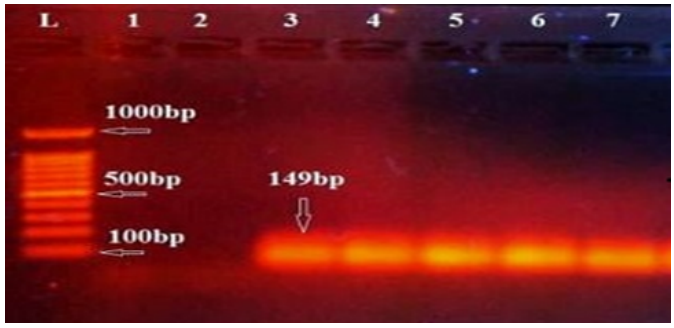

FIGURE 2: Positive andNegative results of PCR amplification; Lane (1) ladder marker; Lane(3,4,5,6,7) positive TLR7- specific gene(227bp); Lane (1,2) negative TLR7-specific gene on 1% agarose, (50voltage for1hour).

In real time polymerase chain reaction for toll like receptor 1 for 20 samples the starting time of amplification was begun after 20 cycles / min, and the quantitative account of the amplification line was between 1200 - 1900ΔR. As seen in the figure (3).

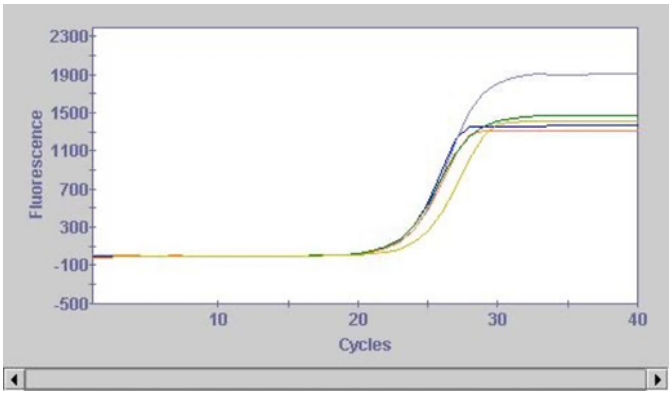

FIGURE 3: Real-Time PCR amplification plot of target gene for TLR7.

4 | DISCUSSION

Findings obtained by this study showed that after the amplification of all extracted DNA from blood samples was preform and confirm by using electrophoresis with (100volt/30min) , the result of this estimation revealed that the amplified DNA(PCR product) was 135bp for TLR1(50voltage for 1hour) and the presence of TLR1 was 75% from total samples , (PCR product) was 125bp for TLR2 on agarose gel(1%) ,(50volt/1hour) with a presence of 95% , there are few reports concerning TLR expression by the prostate Konig et al. (2004) **,** which is an organ usually neglected by immunologists, in spite of the many pathologies that affect it and the tremendous health impact that.

They have on human population. Although only a small percentage of men suffer of infectious chronic or acute prostatitis [National Institutes of Health (NIH) Categories I and II], chronic, noninfectious prostatitis (NIH Category III) is a highly prevalent disease among young adults. Chronic, nonbacterial prostatitis is an inflammatory state of the prostate with direct impact on the quality of life of the patients. Yet, its etiology is unknown. Furthermore, prostate cancer is one of the major causes of death in Western population, being the most commonly diagnosed cancer in men in industrialized countries. There is an expanding body of literature suggesting a link between chronic inflammation and cancer Gatti et al. (2006) **, Fan et al. (2019)**agree with our results in expression of TLR2and TLR10 his study found that prostate tissues expressed TLR2 and TLR10 in both epithelial and stromal cells and that TLR2 and TLR10 were co expressed on plasma membrane and in cytosol of prostate epithelial cells (RWPE-1). Almahfoud et al. (2019) **.** In addition, TLR10 expressed in the RWPE-1 cells consisted predominantly of its isoform type.

In the research of Nagashima et al. (2015) microarray analysis of gastric biopsy specimens from *Helicobacter pylori* (*H. Pylori*) -positive and uninfected subjects showed that TLR2, TLR4, and TLR6–10 were upregulated >2-fold in infected subjects. They then used *H. Pylori* to infect NCI-87 gastric epithelial cells for 24 h and found increases in TLR1, TLR2, TLR6, and TLR10 mRNA levels. Al-Hamdani et al. (2019) As far as their studies and our findings are concerned, the expression of all TLRs should undoubtedly increase with inflammatory grades**. Tajalli-Nezhad et al. (2019)** their review focused on aspects of TLR signaling during acute neuropathological challenges in the brain and highlighted the potential neuroprotective capacity of estrogen, progesterone and vitamin D3 and their putative interactions with TLR signaling pathways after cerebral ischemia and TBI. In particular, TLR2 and TLR4 signaling appeared to be pivotal for controlling pathogenic immune responses following stroke. All three hormones were able to modulate TLR2 and TLR4 signal transduction. Thus, these steroids and the vitamin can be considered as therapeutic options for stroke therapy.

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