International Journal of Medical Science and Clinical Invention 7(11): 5103-5110 2020
DOI:10.18535/ijmsci/v7i11.07 ICV: 77.2
e-ISSN:2348-991X, p-ISSN: 2454-9576
© 2020, IJMSCI

Research Article,

Molecular Gene Expression of Toll-Like Receptors 4 & 10 in Cellular Subsets of Human Peripheral Blood among Patients with Prostatitis: Conventional, Real Time Pcr and DNA Sequencing Techniques

Ihsan E. AlSaimary[1], Hussein N. AlDhaheri[2], Murtadha M. ALMusafer[3]

[1]Microbiology Department, College of Medicine, University of Basrah, Basrah, Iraq
[2,3]Department of Surgery College of Medicine, University of Basrah, Basrah, Iraq
Email Address : ihsanalsaimary@gmail.com

Abstract:

Summary:

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 , 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). 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 227bp for TLR4 on agarose gel (1%) , (50voltage for 1hour) with a presence 100% , (PCR product) was 279bp for TLR10 on agarose gel(1%) , (50volt/1hour) with a presence 80%. The results of the present study indicate that the Toll like receptor alleles associated with risk of prostatitis.

Key words: prostatitis, TLR4, TLR10, PCR

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 and Akira. 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, *et al*., 2004

and Poltoraka, *et al*., 1998), TLR10 is so far an orphan receptor and highly expressed in the human spleen and B cells. (Hasan, *et al*., 2005).

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).

Materials and methods:

Sampling

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.

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.

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. 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).

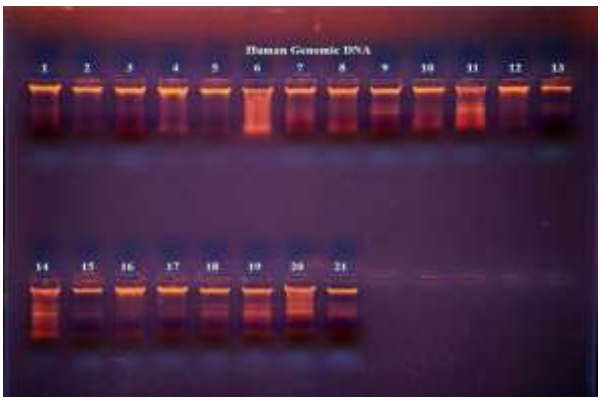

Figure (1) Show Agarose 1% gel electrophoresis image (100vlotage for 30min). That show quality and purity of DNA products extracted from blood samples by commercial DNA extraction Kit.

Polymerase chain reaction technique

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/ µl) for each primer.

Table-1 PCR Master 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) Oligonucleotide sequence and Amplicon Size for each gene used in this study

Gene	Oligonucleotide Sequence (5'-3')	Amplicon Size, bp	Reference
TLR4		227bp	Haidar ., *et al* .2015
Forward	TGGGATCCCTC CCCTGTACCCTTC		
Reverse	CTGGATCCGTT TCTGAGGAGGCTGGATG		
TLR10		279bp	Huang , *et al.*, 2012
Forward	AGAAGAAAG GGAACTGATGAC		
Reverse	CCTGCCAGT AAATACCAAGT		

Table (3) the thermal cycler programs used in this study

Gene	Temperature (°C) /Time					Cycle No.
	Initial Denaturation	Cycling condition			Final extension	
		Denaturation	Annealing	Extension		
TLR4	94/3 min	94/30 sec	73/60 sec	68/1 min	68/5min	30
TLR10	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.

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 4

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 227bp (TLR4 - specific primer) were observed. As seen in figure (2). 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 700 - 1100ΔR. As seen in the figure (3).

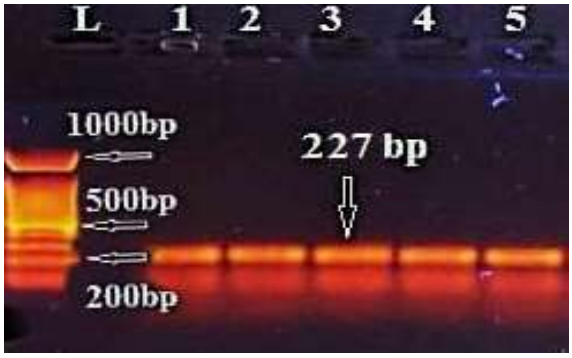

Figure (2) Positive and Negative results of PCR amplification; Lane (1) ladder marker; Lane (1,2,3,4.5) positive TLR4- specific gene (227bp); on 1% agarose, (50voltage for 1hour).

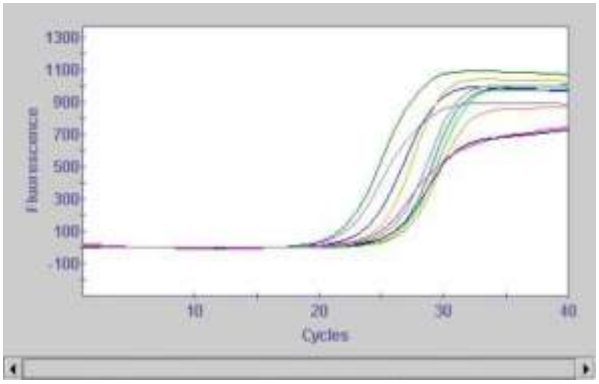

Figure (3) Real-Time PCR amplification plot of target gene for TLR4.

DNA Sequence of FTLR4 gene

Figure (4) showed a DNA sequence of FTLR4

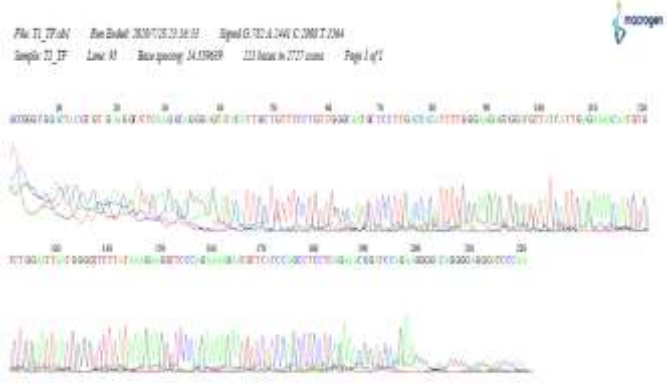

Figure (4) DNA sequence of FTLR4

Alignment of FTLR4 gene

Homo sapiens toll like receptor 4 forward (F TLR4), transcript variant 3, mRNA Sequence ID: NM_003266.4 Length: 12797Number of Matches: 1, Range 1: 3825 to 4014.

Score	Expect	Identities	Gaps	Strand
335 bits(181)	6e-88	187/190(98%)	0/190(0%)	Plus/Plus

```
Query                                                 1
GCCGGGCGGACTACGTGTGAAGGTATTCAAGGCAGGGAGTATACA
TTGCTGTTTCCTGTT  60
                |||               ||              |
||||||||||||||||||||||||||||||||||||||||||||||||
||||||
Sbjct                                              3825
GCCAGGAGAACTACGTGTGAAGGTATTCAAGGCAGGGAGTATACA
TTGCTGTTTCCTGTT  3884

Query                                                61
GGGCAATGCTCCTTGACCACATTTTGGGAAGAGTGGATGTTATCA
TTGAGAAAACAATGT  120

||||||||||||||||||||||||||||||||||||||||||||||||
||||||||||||||||
Sbjct                                              3885
GGGCAATGCTCCTTGACCACATTTTGGGAAGAGTGGATGTTATCA
TTGAGAAAACAATGT  3944

Query                                               121
GTCTGGAATTAATGGGGTTCTTATAAAGAAGGTTCCCAGAAAAGA
ATGTTCATCCAGCCT  180

||||||||||||||||||||||||||||||||||||||||||||||||
||||||||||||||||
Sbjct                                              3945
GTCTGGAATTAATGGGGTTCTTATAAAGAAGGTTCCCAGAAAAGA
ATGTTCATCCAGCCT  4004

Query  181   CCTCAGAAAC  190
              ||||||||||
Sbjct  4005  CCTCAGAAAC  4014
```

Figure (5) shows Alignment of FTLR4 gene

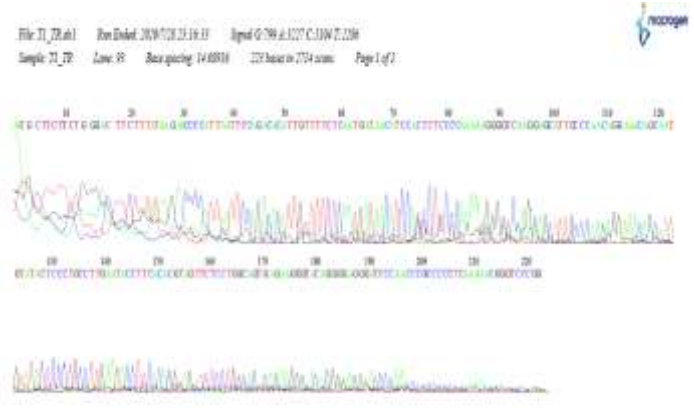

Figure (6) showed a DNA sequence of RTLR4

Alignment of RTLR4 gene

Homo sapiens toll like receptor 4 reverse (R TLR4), transcript variant 3, mRNA Sequence ID: NM_003266.4, Length: 12797Number of Matches: 1, Range 1: 3793 to 3988

Score	Expect	Identities	Gaps	Strand
322 bits (174)	5e-84	190/197(96%)	3/197(1%)	Plus/Minus

```
Query  5     CTTCTTCTGGG-A-
CTTCTTTATAAGAACCCCATTAATTCCAGACACATTGTTTTCTCA
A  62
             ||| ||||||| |
||||||||||||||||||||||||||||||||||||||||||||||||
|
Sbjct  3988  CTT-
TTCTGGGAACCTTCTTTATAAGAACCCCATTAATTCCAGACACAT
TGTTTTCTCAA  3930

Query  63
TGATAACATCCACTCTCCCCAAAAAGGGGTCAAGGAGCATTGCCC
AACAGGAAACAGCAA  122
             ||||||||||||||| ||||||| |
||||||||||||||||||||||||||||||||||
Sbjct  3929
TGATAACATCCACTCTTCCCAAAATGTGGTCAAGGAGCATTGCCC
AACAGGAAACAGCAA  3870

Query  123
TGTATACTCCCTGCCTTGAATACCTTCACACGTAGTTCTCCTGGC
AGTGAGAAGGGTACA  182

||||||||||||||||||||||||||||||||||||||||||||||||
||||||||||||||||
Sbjct  3869
TGTATACTCCCTGCCTTGAATACCTTCACACGTAGTTCTCCTGGC
AGTGAGAAGGGTACA  3810

Query  183   GGGGAGGGATCCCAACC  199
              |||||||||||||| ||
Sbjct  3809  GGGGAGGGATCCCAGCC  3793
```

Figure (7) shows *Alignment of RTLR4 gene*

Phylogenetic tree analysis for TLR4

Phylogenetic analysis of the TLR4 isolates were analyzed by macrogen and compared with sequences of different TLR4 available in a Gen Bank database, show a clear convergence between our TLR4isolates and that of the Gen Bank database. As seen in the figure (8).

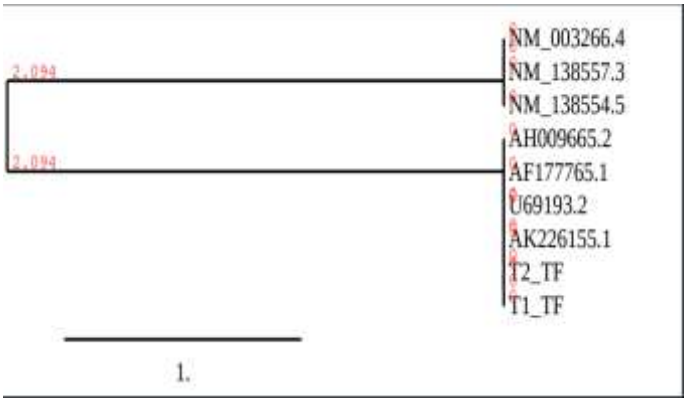

Figure (8) Phylogenetic tree analysis for TLR4

Toll like Receptor 10

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 279bp (TLR10 - specific primer) were observed. Figure (9).

Figure (9) Positive and Negative results of PCR amplification; Lane (1) ladder marker; Lane (1,2,3,4,6,7,9,10,11,12,13,14,15,16,17,18,20) positive TLR10- specific gene (279bp); Lane (5,8,19) Negative TLR7- specific primer on 1% agarose, (50voltage for 1hour).

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 - 2500ΔR. As seen in the figure (10).

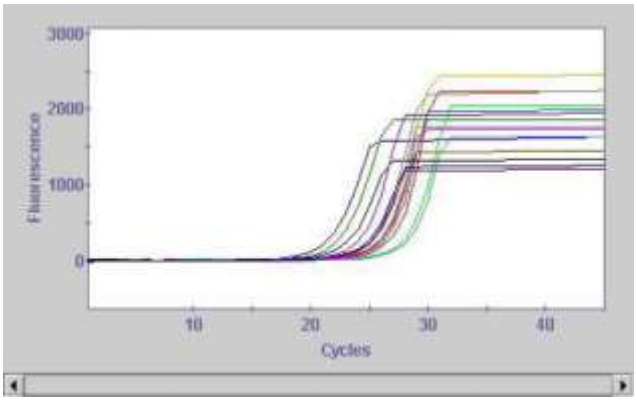

Figure (10) Real-Time PCR amplification plot of target gene for TLR10.

DNA sequencing for (FTLR10)

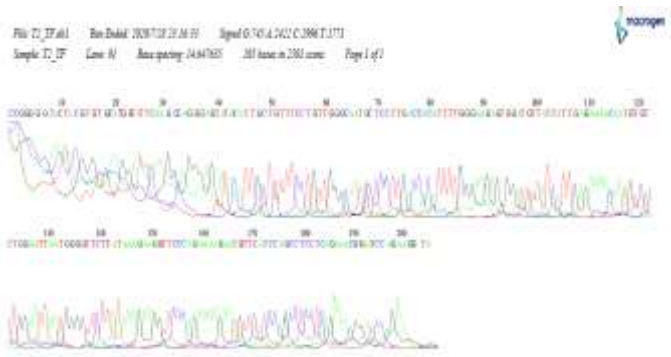

Figure (11) DNA sequence of FTLR10

Alignment of FTLR10 gene

Homo sapiens toll like receptor 10 forward (F TLR10), mRNA, Sequence ID: NM_001195108.2, Length: 3705Number of Matches: 1, Range 1: 473 to 705

Score	Expect	Identities	Gaps	Strand
431 bits (233)	9e-117	233/233(100%)	0/233(0%)	Plus/Plus

```
Query  16
AAGGTTCCCGCAGACTTGACCCCAGCCACAACGACACTGGATTTA
TCCTATAACCTCCTT  75

||||||||||||||||||||||||||||||||||||||||||||||
|||||||||||||||
Sbjct  473
AAGGTTCCCGCAGACTTGACCCCAGCCACAACGACACTGGATTTA
TCCTATAACCTCCTT  532

Query  76
TTTCAACTCCAGAGTTCAGATTTTCATTCTGTCTCCAAACTGAGA
GTTTTGATTCTATGC  135
```

```
|||||||||||||||||||||||||||||||||||||||||||||||||
|||||||||||||||
Sbjct  533
TTTCAACTCCAGAGTTCAGATTTTCATTCTGTCTCCAAACTGAGA
GTTTTGATTCTATGC  592

Query  136
CATAACAGAATTCAACAGCTGGATCTCAAAACCTTTGAATTCAAC
AAGGAGTTAAGATAT  195

|||||||||||||||||||||||||||||||||||||||||||||||||
|||||||||||||||
Sbjct  593
CATAACAGAATTCAACAGCTGGATCTCAAAACCTTTGAATTCAAC
AAGGAGTTAAGATAT  652

Query  196
TTAGATTTGTCTAATAACAGACTGAAGAGTGTAACTTGGTATTTA
CTGGCAGG  248

|||||||||||||||||||||||||||||||||||||||||||||||||
||||||||
Sbjct                                          653
TTAGATTTGTCTAATAACAGACTGAAGAGTGTAACTTGGTATTTA
CTGGCAGG  705
```

Figure (12) shows Alignment of FTLR4 gene

DNA sequencing for (R TLR10)

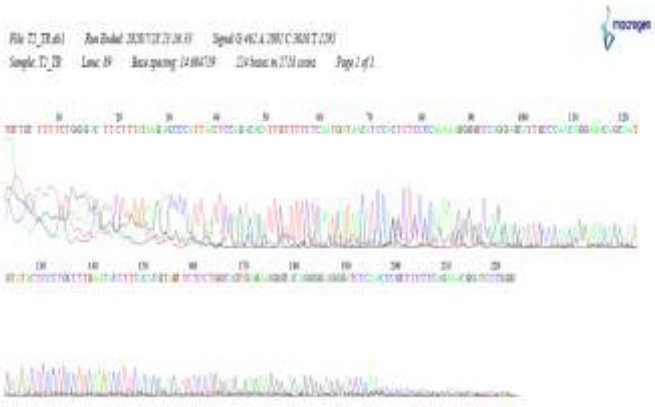

Figure (13) DNA sequence of RTLR10

Alignment of RTLR4 gene

Homo sapiens toll like receptor 10 (TLR10), transcript variant 1, mRNA, Sequence ID: NM_030956.4, Length: 3991Number of Matches: 1

Range 1: 712 to 944.

Score	Expect	Identities	Gaps	Strand
425 bits(230)	4e-115	233/234(99%)	1/234(0%)	Plus/Minus

```
Query  17
ATCTAAATATCTTAACTCCTTGTTGAATTCAAAGGTTTTGAGATC
CAGCTGTTGAATTCT  76

|||||||||||||||||||||||||||||||||||||||||||||||||
|||||||||||||||
Sbjct  944
ATCTAAATATCTTAACTCCTTGTTGAATTCAAAGGTTTTGAGATC
CAGCTGTTGAATTCT  885

Query  77
GTTATGGCATAGAATCAAAACTCTCAGTTTGGAGACAGAATGAAA
ATCTGAACTCTGGAG  136

|||||||||||||||||||||||||||||||||||||||||||||||||
|||||||||||||||
Sbjct  884
GTTATGGCATAGAATCAAAACTCTCAGTTTGGAGACAGAATGAAA
ATCTGAACTCTGGAG  825

Query  137
TTGAAAAAGGAGGTTATAGGATAAATCCAGTGTCGTTGTGGCTGG
GGTCAAGTCTGCGGG  196

|||||||||||||||||||||||||||||||||||||||||||||||||
|||||||||||||||
Sbjct  824
TTGAAAAAGGAGGTTATAGGATAAATCCAGTGTCGTTGTGGCTGG
GGTCAAGTCTGCGGG  765

Query  197
AACCTTTCTTAGAGACATGTTGGAGCAGTTGGTCATCAGTTCCCC
TTTCTTCTG  250

|||||||||||||||||||||||||||||||||||||||||||||||||
|||||||||
Sbjct                                          764
AACCTTTCTTAGAGACATGTTGGAGCAGTTGGTCATCAGTTCCC-
TTTCTTCTG  712
```

Figure (14) shows *Alignment of RTLR4 gene*

Phylogenetic tree analysis for TLR10

Phylogenetic analysis of the TLR10 isolates were analyzed by macrogen and compared with sequences of different TLR10 available in a Gen Bank database, there is no convergence between our TLR10 isolates and that of the Gen Bank database. As seen in figure (15).

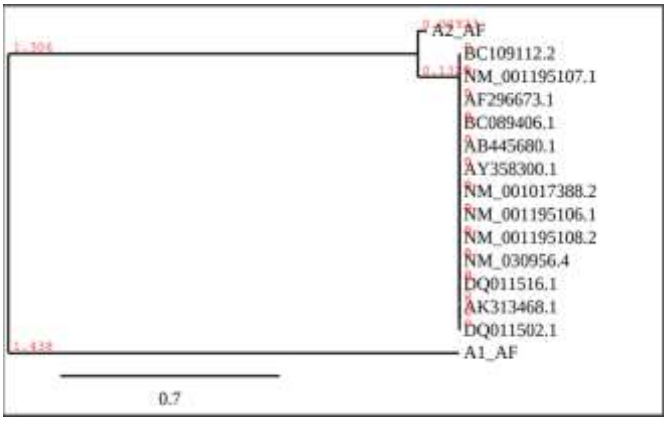

Figure (15) Phylogenetic tree analysis for TLR10.

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 a*l.,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). (Alsaimary, 2014). 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.(Amer, *et al*.,2014) As far as their studies and our findings are concerned, the expression of all tlrs should undoubtedly increase with inflammatory grades.(Tajalli , *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. The results of our study of sequencing of TLR4 and TLR10 were shown as following there are clear convergence between our TLR4 isolates and that of the Gen Bank database. Where there were single mutation appeared in forward TLR4 as A to G 317 1,2. And for reverse TLR4 showed five mutations when we compared with a Gen Bank database as T to C **288** 1, 2. T to A 296 1,2. T to G 298 1,2. A to C 301 2. G to A 406 1,2. And there is no convergence between our TLR10 isolates and that of the Gen Bank database.

There are no studies interested with sequencing of tlrs with the relation of prostatitis so we will compare our results with studies on other diseases. (Kim, *et al*., 2012) suggested in their study that polymorphisms of the TLR4 gene might be associated with the risk of prostate cancer in Korean men because their results showed a significant association with the risk of prostate cancer (P (Corr) = 0.005, OR = 1.81). One common haplotype (ht2) was also significantly associated with the risk of prostate cancer (P (Corr) = 0.009, OR = 1.77). Also (Sun, *et al*., 2005) said The TLR6-TLR1-TLR10 gene cluster may play a role in prostate cancer risk, although further functional studies are needed to pinpoint the disease-associated variants in this gene cluster. (Zhou, *et al.*, 2016) found there were no association between TLR4 and coronary artery disease.

References:

[1.] Arango, g and descoteaux a. (2014). Macrophage cytokines: involvement in immunity and infectious diseases. Front immunol, 5(11):491.

[2.] Kais k.a. Alhadrawi, siham j. M. Al-kaabi, ihsan e.al-saimary. (2015) a study of bacterial types associated with male's infertility in al-najaf al-ashraf governorate. Kufa biology journal.

[3.] Alsaimary ihsan e., Vera h. A. Tossonian, lina m. M. Al-nahi, Mohammad n. A. Al-abass, Hussein a. A. Al-hilfi and ibrahim e. S. Albaldawi. (2014).occurrence of multidrug resistant bacteria (mdrb) among oporative theatres in various hospitals of al-basrah province. Donnish journal of microbiology and biotechnology research. Vol 1(2) pp. 035-041 December.

[4.] Kais k.a. Alhadrawi, siham j. M. Al-kaabi, ihsan e.al-saimary. (2014).evaluationofimmunoglobulins (iga, igg, igm) levels in theseraof patientsoverallandassociatedcases of male infertilityin al-najaf al-ashraf governorate.basrah j o urnal o f veteri nar y r esear ch, vol. 1 1, 4 , pp:67-86.

[5.] Brede, c and shoskes, d. (2011). The etiology and management of acute prostatitis. Nat rev urol 8, 207–212.

[6.] Fan, y; yang, l., Wei, q., ding, y., tang, z., tan, p. And qiu, s. (2019). Toll-like receptor 10 (tlr10) exhibits suppressive effects on inflammation of prostate epithelial cells. Asian journal of andrology, 21 (4), 393.

[7.] Gatti, g; rivero, v., motrich, r. D., & maccioni, m. (2006). Prostate epithelial cells can act as early sensors of infection by up-regulating tlr4 expression and proinflammatory mediators upon lps stimulation. Journal of leukocyte biology, 79(5), 989-998.

[8.] Hasan, u ; chaffois, c., gaillard, c., saulnier, v., merck, e., tancredi, s., guiet, c., brière, f., vlach, j., lebecque, s., trinchieri, g., and bates, e. E. (2005). Human tlr10 is a functional receptor, expressed by b cells and plasmacytoid dendritic cells, which activates gene transcription through myd88. Journal of immunology (Baltimore, md.: 1950), 174(5), 2942–2950.

[9.] Heil, f; hemmi, h., hochrein, h., ampenberger, f., kirschning, c., Akira, s. And bauer, s. (2004). Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. Science, 303(5663), 1526-1529.

[10.] Konig, j. E; senge, t., allhoff, e. P and konig, w. (2004) analysis of the inflammatory network in benign prostate hyperplasia and prostate cancer. Prostate 58, 121–129.

[11.] Nagashima, h; iwatani, s., Cruz, m., jiménez abreu, j. A., uchida, t., mahachai, v. And yamaoka, y. (2015). Toll-like receptor 10 in helicobacter pylori infection. The journal of infectious diseases, 212(10), 1666-1676.

[12.] Poltorak, a; he, x., smirnova, i., liu, m. Y., van huffel, c., du, x., & freudenberg, m. (1998). Defective LPs signaling in c3h/hej and c57bl/10sccr mice: mutations in tlr4 gene. Science, 282 (5396), 2085-2088.

[13.] Quayle, a. J. (2002). The innate and early immune response to pathogen challenge in the female genital tract and the pivotal role of epithelial cells. Journal of reproductive immunology, 57 (1-2), 61-79.

[14.] Takeda. K; kaisho. T and Akira. S. (2003), toll-like receptors. Annurevimmunol21:33576.

[15.] Tajalli-nezhad, s; karimian, m., beyer, c., atlasi, m. A., & tameh, a. A. (2019). The regulatory role of toll-like receptors after ischemic stroke: neurosteroids as tlr modulators with the focus on tlr2/4. Cellular and molecular life sciences, *76*(3), 523-537.