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Polymorphism in promoters of *TLR-2* gene in Iraqi patients with tuberculosis

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Abstract: Despite the existence of Tuberculosis control programs globally, TB remains one of the major public health problems worldwide. It is one of the greatest challenges, which face health systems in the 21st century. A defect in the genes of the immune response is the most plausible explanation for susceptibility of some individuals and resistance of others to TB. TLR2(CD282) is one of the toll-like receptors and plays a role in the immune system. TLR2 is present on the surface of certain cells and it distinguishes foreign bodies, also it have role in passing of adequate signals to the cells of the immune system. The main goal of this study is to produce molecular analysis for promoter of TLR-2 genes in Iraqi patients with TB. Seventy-four blood specimens were collected from 74 patients in the Institute of the Tuberculosis and Chest Disease–Basra city's, blood specimens also collected from 74 healthy individuals as control. Extracted DNA was amplified using set of specific primers for promoter regions TLR-2 gene; amplified amplicons were sequenced and analyzed. Genotyping of TLR-2 gene promoter revealed that out of 74 found with TB, 38 (51.3%) patients found to possess C allele (T→C), while 36 (48.7%) possessed T allele. C allele presents only in heterozygous state in Iraqi society, and closely correlated with increasing susceptibility of some individuals to TB.

Key words: *Mycobacterium tuberculosis*, TLR- 2 gene .

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Introduction:

Mycobacterium tuberculosis is the main causative agent of TB in human. A cough for three weeks or longer , loss of weight , appetite loss, fever, drenching night sweats and tiredness or lack of energy are the most common symptoms of TB (1). Statistics indicate that one-third of the world's population is harboring a latent TB infection, approximately 1.5million deaths occurring annually from the active disease coming in second after human immunodeficiency virus(HIV) as a cause of death worldwide (2). In Iraq TB is serious problem, it threats all society sectors. Issa *et al*. (3) studied the reality of TB in Basra province (second largest city after the capital) from 2010 to 2014 and found that the total number of patient is 3218, including 1595(49.6%) male and 1623(50.4%) female. Issa *et al*. (3) also found that TB targeted all age groups even those who defiantly vaccinated. Genetic factors of individuals produce satisfied explain, at least in part why some people resist infection more successfully than others do (4). Rare gene disruptions cause fatal vulnerability to certain pathogens, but more subtle differences are common and arise from minor variations in many genes. Cytokines and receptors play

critical role in interactions and integration between the cells of immune system, which leads to effective defense against TB (1;5). TLRs are a family of single membrane-spanning receptors of which have been characterized in man and mouse. TLRs play a critical role in both innate resistance and the initiation of adaptive immunity to infectious agents. They act by recognizing pathogen-associated molecular patterns (PAMPs) or endogenous inflammation associated molecules (6). These are distinct molecular structures on microbes and different sets of TLRs have been associated with the response to different classes of microorganisms e.g. recognition of viruses by TLR3, TLR7, TLR8 and TLR9. The TLRs known to be involved in recognition of MTB are TLR2, TLR4, TLR9, and possibly TLR8 (7). TLR2 can form heterodimers with both TLR1 and TLR6.These heterodimers have been implicated in the recognition of mycobacterial cell wall glycolipids (8 and 9). A polymorphism in human TLR2 may be has role in increased susceptibility tuberculosis because any change in expression can lead to weakened immune responses, and thus increase the susceptibility to infections and inflammation-related disease (10). Two mutations was found in *TLR2* gene which *R753Q* and *R677W* were found to be associated with an increased risk of TB development in Korea, Tunisia and Turkey (11 and 9). The main goal of this study is to produce molecular analysis for promoters of TLR-2 genes in Iraqi patients with TB.

Materials and methods:

The specimens were collected at the institute of the tuberculosis and chest disease–of Basra province from February to September 2015. Seventy-four patients (33) male and (41) female with pulmonary TB, with average age between (38.73±23 years) were included in present study, while 74 volunteers, (36) male and (38) female, age mean between (35.19±7.86 years) were accredited in the current study as healthy control (HC). Blood samples were collected from each patient and control by vein puncture using disposable syringes. For DNA extraction, 2 ml of collected blood was put in EDTA tubes, Reliaprep blood DNA Miniprep kit (Promega, USA) was used For DNA extraction, the extraction steps were done according to company instructions. The extraction process was verified by characterization of genomic DNA bands in agarose gel electrophoresis by loading of 6µl DNA mixed with 3µl of bromophenol blue in the wells of the 1% agarose gel. Set of primers for PCR amplification of TLR-2 promoter was used as shown in table (1). For PCR reaction, the following reaction mixture was used: 1.5µl of genomic DNA, 12.5 µl of Premix Taq v.2 plus dye (Takara Biomedical Technology. China), 0.5 µl $MgCl_2$, 0.5 µl of each primer (GeneScript Make Research Easy, China) and 9.5 µl of nuclease free water. The PCR conditions for amplifying TLR-2 promoter gene were initial denaturation at 95°C for 5 min., followed by 30 cycles consist of 30 Sec. at 95°C, 35 Sec. at 53°C and 35 Sec. at 72°C with a final extension at 72°C for 10 min. The amplified products were determined by electrophoresis on agarose gel containing 0.5 µg/ml Ethidium bromide. Before sequencing, PCR products were purified by Gel / PCR Extraction Kit (BIOMIGA Ezgene, China) according to the manufacturer′s recommendations.

All samples were sent to GeneScript company (GeneScript Make Research Easy, China) for sequencing. Two types of file came back from the company ABI and text file, DNA Dynamo software was used to analyse the data results, multiple alignment for high-quality sequences were done for each other plus reference sequence at GenBank performed to find DNA polymorphism within sequences.

Table (1): Primers using in PCR amplification of TLR-2.

Primers		Sequence	product size	Reference
TLR-2	sense	5'- ATTGCAAATCCTGAGAGTGGGAA -3'	306bp	(14)
TLR-2	atisense	5'- CAAACTTTCATCGGTGATTTTCACA -3'		

Results:

Promoters of TLR-2 were successfully amplified. The amplicons of the each promoter were visualized by agarose gel electrophoresis, whilst PCR product sizes was determined by comparison with marker as in figure (1).

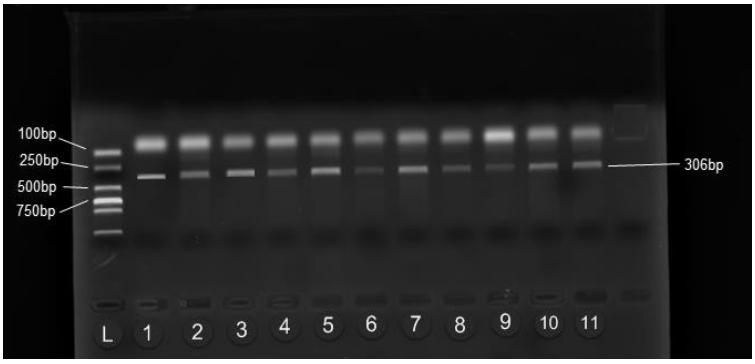

Figure (1): PCR products of the DNA amplicons of TLR-2visualized by 2% agarose gel electrophoresis, for 1 hour in (50 V), product sizes were determined by comparison with 2000bp marker. Lane L: 2000bp DNA marker, lanes 1-11: TLR-2 bands.

Table (2) shows that out of 74 patients with TB 38 (51.3%) patients found to possess C allele (T→C), while 36 (48.7%) possessed T allele (figure 2), so according HWE 7% of population tend to have T allele with homozygous pattern. Only one SNP found in TLR-2 promoter.

Table (2): The genotype and allele frequencies of the identified SNPs in the TLR-2 completely studied population (148samples).

Locus TLR-2	Genotype	Patients (%) (n=74)	Healthy control (n=74)	Absolute Frequency	Allele frequency	p-value
				P.	P	P
-597	TT CT CC	36(48.7%) 38(51.3%) 38	69 5 5	0.55 0.38	0.07	0.003

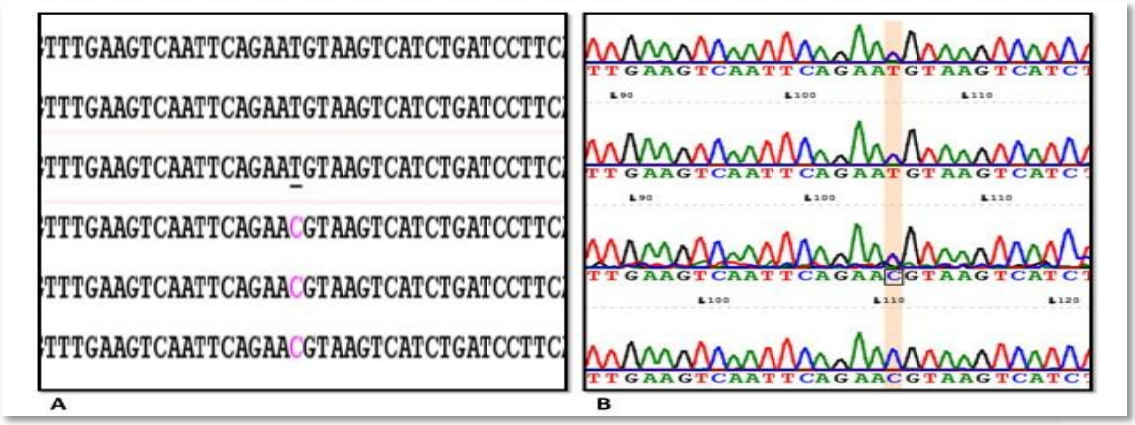

Figure (2): (A) Alignment of sequences (T/C) allele appear with red letter at position -312. (B) Alignment of chromatograms appear its peaks quality.

Discussion:

In spite of the availability of effective chemotherapy and vaccine, tuberculosis is a global health concern for both developing and developed countries (12) .Recently tuberculosis become more complex due to persistence in aging populations and the rise of drug-resistant strains (13). The infection rate has continued growing, particularly in developing countries. There are many factors significantly contributed in the re-outbreak and increase of the number of infections, among the most important of these factors are genetic makeup of individuals. Only one SNP found in TLR-2 promoter, the number of patients possessed heterozygote C allele is 38(51.3%) and this number is about seven times the number of healthy individuals who carry this allele. Our results widely match with what Naderi *et al*., (14) and Zhao *et al*., (15) recorded only one main SNPs in TLR-2 promoter region, which is (T→C) ,it found at position -597(rs3804099). TLR-2 gene found in position 4q31.3, It mediates responses of both innate and adaptive immunity to microbial pathogen. TLR-2 are important mediators of the inflammatory response in the first line of host defense by recognition of MTB and binding pathogen associated molecular patterns (PAMPs), which are conserved structures in the cell wall or genetic components of the invading pathogen (16). The causal polymorphism may lie in the promoter region, a regulatory region, may be associated with an impaired immune response to *M. tuberculosis* and lead to more aggressive disease, Caws *et al*., (17) have found that individuals with the 597C allele of TLR-2 were more likely to have tuberculosis caused by the East-Asian/Beijing genotype than other individuals. Concerning Arab populations, no further studies in Arab countries discussed the effect of TLR-2 gene polymorphism except one Ben-Ali *et al*., (18) when he study Tunisian population, but he studied SNPs in different positions. Our results matched with what Naderi *et al*., (14) were found when they study Iranian population.

Conclusion:

Iraqi society tends to share two alleles in the subject of TLR-2, T allele and C allele; C allele only presents in

heterozygous state in Iraqi society. C allele is also closely correlated with increasing susceptibility of some individuals to TB.

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