ORIGINAL ARTICLE

Polymorphism in Toll-like Receptor 10 and Tuberculosis Susceptibility in Egyptian Population

1AL-Shaimaa M. AL-Tabbakh*, 2Rehab S. El Sawy, 1Marwa S. EL-Melouk
1Lecturer of Medical Microbiology and Immunology, Faculty of Medicine, Benha University, Egypt
2Lecturer of Chest Diseases and Tuberculosis, Faculty of Medicine, Benha University, Egypt

ABSTRACT

Background: One-third of the world’s population is infected with Mycobacterium tuberculosis. Difference in clinical outcome of infection implies that host genetics may be implicated in such variability. Investigations of Toll-like receptors (TLRs) revealed new information regarding the immunopathogenesis of tuberculosis. Toll-like receptor 2 (TLR2) mediates crucial immune response against Mycobacterium tuberculosis. There is argument that Toll-like receptor (TLR10) participate in tuberculosis susceptibility by acting as a signaling modulator for TLR2. Objectives: The aim of this study was investigating the relationship between TLR 10 SNP 720A/C (rs11096957) and increase susceptibility to tuberculosis. Methodology: Eighty patients with radiological, microbiological and clinical proven active pulmonary tuberculosis (T.B) were included in this study. (TLR10) polymorphisms and allele distributions were compared between these 80 patients and 70 healthy control subjects. Peripheral blood samples were taken from all patients and controls. Genotyping was accomplished by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Results: When we compare T.B cases with controls, a statistically significant association was observed between T.B susceptibility and SNP 720A/C (rs11096957) in (TLR10). Allele (A) was more frequent in tuberculous cases while allele (C) was more common in controls. It was reported that the AA genotype of (TLR10) SNP rs11096957 was considerably related to the increased risk of developing pulmonary T.B. Homozygosity (AA) has been associated with predisposition to disease by comparing cases to controls (P = 0.045; OR = 2.0; 95% C.I. = 1.0- 4.0), A/C heterozygosity was considerably different in tuberculous cases than in healthy controls with lower risk of developing tuberculosis (P = 0.044: OR = 0.5; 95% C.I. = 0.26–0.98). Conclusion: TLR10 SNP rs11096957 polymorphism is a risk factor for tuberculosis infection.

INTRODUCTION

Tuberculosis (T.B) is a leading cause of mortality caused by a single infectious agent Mycobacterium tuberculosis (M. tuberculosis), it is considered as one of the top 10 causes of death. The global T.B. statistics show that about 10 million new cases of T.B were diagnosed in 2019. T.B infection is a result of a complex interaction between the host and the pathogen. It is governed by a variety of factors, including the opportunity of exposure to pathogens, pathogen virulence factors, the hosts’ behavior, life style, immunity, genetic, environmental and socioeconomic factors.

Innate immunity plays a major role in body defense against T.B infection. Its cells uptake the causal organism after recognition of a highly preserved molecular structures by an expressed set of pattern recognition receptors (PRRs). A variety of innate immunity defense-associated cellular functions were initiated such as phagocytosis, inflammasome activation, autophagy and apoptosis.

TLRs are the most authenticated family among pattern recognition receptors (PRRs), capable of recognizing the conserved structures of microorganisms designed as pathogen-associated molecular patterns (PAMPs) and danger associated molecular patterns (DAMPs). In mammals, about 13 TLRs were discovered, only ten of them (TLRs 1-10) present in humans. They are grouped according to cellular location into TLRs on plasma membrane (TLR1, TLR2, TLR4, TLR5, TLR6 and TLR10) and others on the membrane of endoplasmic reticulum or on the endosomal/lysosomal membrane (TLR3, TLR7, TLR8 and TLR9). TLRs are considered type 1 transmembrane receptors consist mainly of 3 domains; extracellular domain (microbial pattern recognition), transmembrane domain and cytoplasmic domain (for signaling). Upon pathogen infection, TLRs specific ligation results in pro-inflammatory mediators inducement, such as cytokines and chemokines.
TLR10 was first discovered in 2001 by Chuang et al. Its gene on chromosome 14, encodes about (811) amino acids, forms a cluster with genes coding for TLR1 and TLR6 and shows a homogeneity about 50 and 49% respectively. But although this structure homology, there are differences in pattern of expression along with signal transduction paths in TLR10.

TLR10 is expressed predominantly in lymphoid tissues including thymus, tonsils, spleen, lymph nodes and lung with strong expression on cells of immune system such as B cells, dendritic cells, neutrophils and eosinophils. However, it can also be detected on non-immune cells, as trophoblasts. TLR10 forms homodimers and shows heterodimers with TLR1, TLR2 and TLR6 but the assignment of each TLR in the resulting complex needs to be clarified. It is still an orphan receptor has no assured ligand, signaling passage or function.

Immunity versus M. tuberculosis is cell mediated initiated by numerous cytokines and chemokines produced by macrophage as well as dendritic cells (DCs) that express an assorted array of TLRs; among them is TLR2. They play a pivotal role in defense against M. tuberculosis infection through interaction with their ligands present in M. tuberculosis, activating the Myeloid Differentiation Primary Response 88 (MyD88) which binds initial complex of subsequent molecules. MyD88 mediates translocation of NF-κ B into the nucleus which induces the transcription of inflammatory mediators, expression of adhesion molecules, recruitment and invigoration of macrophages, DCs and polymorpho-nuclear cells (PMNs) in the M. tuberculosis inclosing area. Differences in the expression and/or activation of TLRs can affect the immunological status of T.B patients.

TLR10 has a modulatory effect which is chiefly inhibitory. When it forms heterodimers with TLR2, it inhibits the TLR2-mediated immune responses thus enhancing T.B advancement.

TLR10 single nucleotide polymorphisms (SNPs) is shown to be linked with several diseases like, bacterial infection-associated gastritis, tuberculosis, autoimmune thyroiditis, etc. Nevertheless, no evidence available on how these TLR10 polymorphisms aggravate susceptibility to different diseases. They may impact the expression of TLR10 gene, affect its structure and/or function, involve the ligands recognition, signaling, NF-κ B activation and subsequent cytokines production.

The current study, investigate the relation between (TLR10) SNP 720A/C (rs11096957) and T.B infection susceptibility by analyzing cases and controls genotype distributions.

METHODOLOGY

The Ethical Committee of Benha Faculty of Medicine approved the study and a written assent was obtained from whole cases and control subjects participated in this study.

Subjects:
This case control study was executed in the period from January 2019 to January 2020, applied on 150 individuals (80 pulmonary T.B patients admitted at Chest Department, Benha University Hospital and 70 healthy controls).

Inclusion criteria: active pulmonary tuberculosis cases with their diagnosis based upon clinical complaint (fever, cough, hemoptysis, sweating and weight loss) and chest X-ray. Diagnosis was confirmed by examination of sputum samples collected early in the morning for 3 successive days for detection of acid-fast bacilli (AFB) in a Ziehl–Neelsen-stained sputum smears and isolation of M. tuberculosis on suitable media.

Exclusion criteria: individuals taking steroids for treatment of inflammatory conditions or patients with co-infection with chronic chest diseases, diabetes, immunological diseases or cancer.

The control group was selected from healthy individuals who have negative T.B history, some were blood donors at the department of blood transfusion and some were healthy employees at different hospital departments.

DNA extraction and genotyping:
SNP region of (TLR 10) was examined using PCR-RFLP method. Using G-spin™ Total DNA Extraction Mini Kit (iNtRON Biotechnology, Korea), genomic DNA was extracted from venous blood sample of the patient and control groups following the manufacture instructions. In a PCR tube, a PCR amplification reaction of a total volume 50μl containing 5μl of the extracted DNA template, 25μl of 2×EasyTaq® PCR SuperMix (TransGen Biotech Co, China), 1μl of the forward primer [5’AGAAGGTAGCCTGCCCCATC3’], 1μl of the reverse primer [5’TCGGATCTGAAAGTGTTCCA3’] and 18μl of nuclease free water.

The amplification reaction in the thermal cycler followed these steps: initial denaturation (1 cycle) for 5 min at 94°C, denaturation (35 cycles) for 30 s at 94°C, annealing (35 cycles) for 30 s at 59°C, extension (35 cycles) for 1 min at 72°C then final extension step (1 cycle) for 10 min at 72°C. PCR products (10μL) were separated in 2% agarose gel after being treated with HinI restriction enzyme (Thermoscientific, USA) at 37°C for about 6 hours. AA genotype represents the undigested PCR product (a single band of 81bp). The CC genotype represents the digested products (two bands of 73bp and 9bp) and the AC genotype represents the three bands.

Figure 1
Fig. 1: PCR-RFLP analysis of TLR10 polymorphism shows, 720A/C genotype after digestion with Hin1II. Lanes 2, 6 and 8 show the AA genotype (290 bp), lane 3 shows the CC genotypes (175 bp, 115 bp) and lanes 1, 4, 5 and 7 show the AC genotype (290, 175, 115 bp). M is the molecular weight marker (100 bp Fermentas).

Statistical analysis:
Data were analyzed using SPSS statistical software (IBM SPSS: version 21). Association analysis between TLR10 SNP 720A/C (rs11096957) and T.B was performed by Chi square test. A P-value of less than 0.05 was considered to be statistically significant. Odds ratio (OR) with 95% confidence interval (CI) were calculated for each SNP for evaluating the relative risk.

RESULTS

The allele and genotype frequencies of (TLR10) SNP 720A/C (rs11096957) were calculated in tuberculous (N = 80) and non-tuberculous (N = 70) Egyptian population.

Allele (A) was more common in cases while allele (C) was more frequent in controls. Allelic frequencies showed no statistically significant difference between the two groups (table 1) but statistically significant difference in genotypic frequencies is found between them (table 2). The (AA) homozygotes were significantly overrepresented in patients suffering from T.B showing an association with predisposition to the disease (P = 0.045; OR = 2.0; 95% C.I. = 1.0-4.0).

Our results also revealed that (A/C) heterozygosity shows a significant difference in tuberculous patients than in healthy controls (P = 0.044; OR = 0.5; 95% C.I. = 0.26-0.98) with decreased risk association (protection).

Table 1: Analysis of TLR10 SNP 720A/C (rs11096957) in Egyptian patients with tuberculosis and controls: Allelic frequencies and number

<table>
<thead>
<tr>
<th>Alleles</th>
<th>Case (No.=80)</th>
<th>Control (No.=70)</th>
<th>X²-test</th>
<th>OR&amp;CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of alleles=160</td>
<td>No. of alleles=140</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>102 (63.75%)</td>
<td>79 (56.4%)</td>
<td>1.67</td>
<td>-</td>
<td>0.196</td>
</tr>
<tr>
<td>C</td>
<td>58 (36.25%)</td>
<td>61 (43.6%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Analysis of TLR10 SNP 720A/C (rs11096957) in Egyptian patients with tuberculosis and controls: Genotypic frequencies and number

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Case (No.=80)</th>
<th>Control (No.=70)</th>
<th>X²-test</th>
<th>OR&amp;CI</th>
<th>p-value</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/A</td>
<td>33 (41.3%)</td>
<td>18 (25.7%)</td>
<td>4.01</td>
<td>2.0</td>
<td>0.045*</td>
<td>Predisposition</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(1.0-4.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/C</td>
<td>36 (45.0%)</td>
<td>43 (61.4%)</td>
<td>4.04</td>
<td>0.5</td>
<td>0.044*</td>
<td>Protection</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.26-0.98)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>11 (13.7%)</td>
<td>9 (12.9%)</td>
<td>0.026</td>
<td>-</td>
<td>0.87</td>
<td></td>
</tr>
</tbody>
</table>

*Significant

DISCUSSION

Family members of Toll-like receptors play important roles in protection against M. tuberculosis infection, particularly TLR2 which can initiate anti M. tuberculosis immune response by recognizing its components and stimulating pro-inflammatory signals as MyD88 and TRIF (TIR domain-containing adapter inducing interferon β)-dependent pathways.

Although, very few studies have involved TLR10 in response to infection with M. tuberculosis, it was proven that TLR10 in association with TLR2 has the ability to recruit the proximal adaptor (MyD88) to the complex, but cannot stimulate MyD88 and TRIF-dependent signaling, suggesting that TLR10 acts as a negative regulator which could participate in the progression of T.B.
Other studies suggested that TLR10 had no known ligands. TLR2 can bind with TLR1 and TLR6 forming heterodimers and this was manifested also with TLR10. It is thus assumed that TLR10 could compete with TLR1 and TLR6 out of the TLR2 heterodimeric complexes resulting in suppression of responses via TLR2 ligands.

Single nucleotide polymorphisms (SNPs) in TLRs genes have an important role in predisposition to many diseases including T.B. This DNA variation alters TLRs ability to bind their cell-surface ligands in M. tuberculosis. This allows the bacterium to evade elimination through the immune system with subsequent disease advances. SNPs within TLR genes can cause defects in intracellular signaling of the host defense system making the host susceptible to T.B.

TLR2 is the known receptor for the M. tuberculosis. Studies found that changes in its structure might modify tuberculosis risk. Indeed, TLR2 genes SNPs are associated with disease tendency in Tunisian, Turkish, Croatian, Chinese, Sudanese and Egyptian populations. Multiple studies have found that TLR1, TLR2, TLR4, TLR6, TLR8 and TLR9 genes polymorphisms are related to elevated T.B susceptibility.

In the analysis of the TLR10 inhibitory effect upon the immune response achieved after stimulation with TLR2 ligands is influenced by polymorphisms in TLR10 gene.

In the case of TLR10, the (A) allele might possess a higher affinity for TLR2 than the (C) allele thus effectively preventing associations between TLR2 with neither TLR1 nor TLR6. The result would be inhibition of inflammatory action, and M. tuberculosis might survive, multiplicate and clinical manifestations of T.B appear.

We also diminished possibility of confusing factors by precluding comorbidities as chronic chest diseases, diabetes, cancer, immunological disorders and the use of steroids for treating inflammatory conditions.

CONCLUSION

TLR10 single nucleotide polymorphism A/C (rs11096957) was associated with increased susceptibility to tuberculosis in Egyptian population.

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

REFERENCES

1. GLOBAL TUBERCULOSIS REPORT 2020.


