Pulmonary tuberculosis susceptibility and association with single nucleotide polymorphisms in toll-like receptors (TLR-2 and TLR-9)

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ABSTRACT

This study examines the potential association between single nucleotide polymorphisms (SNPs) in toll-like receptor (TLRs) genes (TLR-2Arg753Gln (rs5743708, G2258A), TLR-9-1237T/C (rs5743836) and TLR-9 +1174G/A (rs352139)) and tuberculosis (TB) infection. We enrolled 238 Egyptians that included 140 patients newly diagnosed with pulmonary TB and 98 healthy control subjects. All subjects were genotyped for TLR-2 Arg753Gln and TLR-9-1237T/C using restriction fragment length polymorphism polymerase chain reaction (RFLP-PCR). Genotyping of TLR-9 +1174G/A was performed using amplification refractory mutation system-polymerase chain reaction (ARMS–PCR). TLR-2 Arg753Gln and TLR-9 -1237T/C using restriction fragment length polymorphism polymerase chain reaction (RFLP-PCR). Genotyping of TLR-9 +1174G/A was performed using amplification refractory mutation system-polymerase chain reaction (ARMS–PCR). TLR-2 Arg753Gln and TLR-9 -1237T/C showed no significant change in genotype/allele distribution, indicating no link between these two SNPs and TB susceptibility. On the other hand, a significant increase in TLR-9 -1237TC genotype (P = 0.012) and C allele (P = 0.009) was found in TB-infected patients compared to normal controls. Haplotype analysis showed a significant decrease in GCG and ATG haplotypes in TB patients (P = 0.004 and P = 0.001, respectively). TLR-2 Arg753Gln and TLR-9 -1237T/C genotype frequencies appeared to be in linkage disequilibrium (LD) (D’ = 0.9856, r2 = 0.0675). TLR-9 -1237 T/C (5743708) might be associated with increased TB risk in Egyptians. Additional larger-scale epidemiological studies are recommended to validate our results.

KEYWORDS: SNP, TB, TLR-2, TLR-9, Egyptians.

1. Introduction

According to the World Health Organization report, tuberculosis is the world’s deadliest infectious disease [1]. About one-quarter of the world’s population has latent tuberculosis, which means people have been infected by *Mycobacterium tuberculosis* but are not yet sick and cannot transmit the disease. People infected with *M. tuberculosis* (M. tb) have a 5-15% lifetime risk of falling sick with TB. However, immune-compromised persons, such as people living with human immunodeficiency virus (HIV), malnutrition or diabetes, or people who use tobacco, have a much higher risk of developing TB disease [2, 3]. The largest number of new TB cases in the WHO Asian region accounted for 44%, followed by the WHO African region, which accounted for 25%, and the WHO Western Pacific accounting for 18%. In 2020, the 30 nations...
with the highest TB burden accounted for 86% of new TB cases. India, China, Indonesia, the Philippines, Pakistan, Nigeria, Bangladesh, and South Africa account for two-thirds of the total, with India leading the pack, followed by China, Indonesia, the Philippines, Pakistan, Nigeria, Bangladesh, and South Africa [1].

Genetic variations in the host affect M. tb immunity and are crucial for determining disease resistance or susceptibility [4]. Toll-like receptors (TLR) act as pattern recognition receptors (PRR), which identify specific molecules expressed by microbes like viruses and bacteria, so it is called pathogen-associated molecular patterns (PAMPs) [5]. They are in the frontline against invading pathogens during the initiation of the innate immune response. They are important in both innate and adaptive immune responses, and they identify distinct molecular patterns associated with certain infections [6].

TLR-2, in association with TLR-1 or TLR-6, is essential for recognizing bacterial lipoproteins and lipopeptides [7]. TLR-2 also recognizes glycolipids such as lipoteichoic acid from Gram-positive bacteria [8, 9]. Many studies have shown that TLR-2 is the main receptor recognizing lipoproteins and lipopeptides. Triacylated lipoproteins are recognized by the TLR-1-TLR-2 complex [10, 11]. The 37.4 kb long TLR-2 gene is found on chromosome 4 [12].

One of the most important receptors for innate defense against intracellular pathogens is TLR-9, which activates an intracellular receptor signaling cascade [13]. Unmethylated cytosine–phosphate–guanine [CpG] dinucleotide patterns in bacterial, viral, and fungal DNA are recognized by this receptor [14]. TLR-9-encoding gene is located on chromosome 3p21.3 and spans approximately 5 kb. It consists of 2 exons and encodes 1032 amino acids [15].

Host genetic polymorphisms have been proposed to play a vital role in disease susceptibility [16-18], resulting in the either insufficient or excessive acute inflammatory response. This might be controlled by single nucleotide polymorphisms (SNPs) in genes [19, 20]. TLR polymorphisms have been linked to TLR expression regulation, active tuberculosis development, and an altered immunological response to the disease. We examined whether SNPs in TLR-2 [rs5743708, G2258A] and TLR-9 [rs5743836 and rs352139] are associated with TB susceptibility in the Egyptian population.

2. Materials and Methods

2.1. Study subjects

This study was conducted on 238 Egyptians, of which 140 patients were newly diagnosed with pulmonary TB at the Abbassia Chest Hospital, Cairo, Egypt. The diagnosis of pulmonary TB was determined via clinical, bacteriological, and radiological investigations based on the WHO criteria [21]. All experiments were done following the Human Ethical Clearance Committee guidelines for Clinical Researches according to Helsinki Declaration (1964) and performed on the human subjects. The protocol was IRB-approved at VACSER-EGYPT Institutional Bioethics Review Board (BERD-VACSERA, EGYPT). Informed consent was obtained from each participant. All subjects filled out a questionnaire. Data about age, gender, residence, past medical history, and current symptoms were collected to obtain TB patients’ clinical and demographic characteristics and healthy controls. Sterile vacutainer tubes containing Na-EDTA as an anticoagulant were used to collect approximately 3 to 5 ml of blood samples. All blood samples had been aliquoted and stored at -20°C until use.

2.2. DNA extraction

Genetic DNA was isolated from EDTA-anticoagulated peripheral blood and stored at -20°C according to the manufacturer’s instructions using the TIAN amp genomic DNA extraction kit (Qiagen, Korea, Cat#DP304-02). All extracted DNA was run in electrophoresis on 1% agarose gel stained with ethidium bromide to confirm its quality. DNA concentration was measured using a NanoDrop™ 2000/2000c Spectrophotometer (Thermo Scientific Inc., USA).

2.3. Genotyping for SNPs in TLR-9-1237T/C (rs5743836) and TLR-2Arg753Gln (rs5743708) by restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR)

Alleles of TLR-9-1237T/C gene were amplified from genome by using specific primers; F1
[5'-GCTGGATGCGCCCTGTGTA-3'] and R1 [5'-GCCTCAGGGGCTTTGGGAT-3'] [22]. Taq PCR Master [2x] (Mardin Life Science Corporation, USA), 10 pmol of each primer, and 100 ng of template DNA were included in each reaction mixture. The PCR amplifications were performed in the Thermal cycler PCR (HVD, Germany). The cycling condition consisted of a hot start for one cycle only, at 95°C for 6 min followed by 35 cycles of three temperatures (denaturation at 95°C for 45 sec, primer annealing at 56°C for 30 sec, and extension at 72°C for 30 sec); then a final extension step at 72°C for 5 min. In accordance with the manufacturer’s instructions, the BstNI restriction enzyme digested the TLR-9 -1237T/C PCR product, which was 123 bp in size (New England Biolabs, UK). After digestion, the TLR-9 CC genotype was identified by 87 bp and 36 bp bands. A single band at 123 bp corresponds to the homozygous undigested (TT) genotype, while bands of 123 bp, 87 bp, and 36 bp correspond to the heterozygous (CT) genotype. Electrophoresis used 1% agarose gel to analyze the resulting DNA fragments stained with ethidium bromide (3UVTM Transilluminator-UVP). A 100-bp DNA ladder (Fermentas, Thermo Fisher Scientific) was used to measure the size of PCR products.

Alleles of the TLR-2 gene were amplified from the genome by using specific primers; F1 [5'-CATTTCCCCAGGGCTTTCTGCAAGCTCC-3'] and R1 [5'-GGAACCTAGGACTTTATCGCAGCTC-3'] [22]. Taq PCR Master [2x] (Mardin Life science corporation, USA), 10 pmol of each primer, and 100 ng of template DNA were included in each reaction mixture. The cycling protocol consisted of a hot start for one cycle only, at 95°C for 6 min followed by 35 cycles of three temperatures (denaturation at 95°C for 45 sec, primer annealing at 56°C for 30 sec, and extension at 72°C for 30 sec); then a final extension step at 72°C for 5 min. According to the manufacturer’s instructions, the PstI-HF restriction enzyme digested the 254 bp TLR-2 PCR product (New England Biolabs, UK). Upon digestion, bands of 214 bp and 40 bp corresponded to the TLR-2 AA genotype, while 254 bp, 214 bp, and 40 bp were designated as heterozygous (GA). Electrophoresis stained with ethidium bromide was used to visualize (3UVTM Transilluminator-UVP) the resultant DNA fragments on a 3% agarose gel. A 100-bp DNA ladder (Fermentas, Thermo Fisher Scientific) was used to measure the size of PCR products.

2.4. Genotyping for TLR-9 +1174G/A [rs352139] using amplification refractory mutation system-polymerase chain reaction [ARMS-PCR]

Alleles of TLR-9 [+1174G/A] gene was amplified from genome by using primers; F1 [5'-GCTGGATGG CCC TGT TGA-3'], F2 [5'-GCTGGATGG CCC TGT TGG-3'] and R1 [5'-GCCTCAGGGCTTTGGGAT-3']. In addition to internal control primers (forward and reverse), ARMS-PCR was performed in two tubes, each of which included a forward primer unique to one allele and a common reverse primer and internal control primers. Taq PCR Master [2x] (Mardin Life Science Corporation, USA), 10 pmol of each primer, and 100 ng of template DNA were included in each reaction mixture. The cycling protocol consisted of a hot start for one cycle only, at 95°C for 6 min, followed by 35 cycles of three temperatures [denaturation at 95°C for 45 sec, primer annealing at 56°C for 30 sec, and extension at 72°C for 30 sec], then a final extension step at 72°C for 5 min. To detect the amplified gene product of the PCR reactions, 3.0% agarose gel electrophoresis was used to separate the 260 bp PCR product.

2.5. Statistical analysis

Statistical analyses were performed by a statistical computer program, SPSS (Statistical Package for the Social Science, IBM Corporation, USA, version 19). Comparisons between M.tb patients and controls were made using an independent t-test, while pre- and post-treatment were made using paired t-test. The results were presented as mean ± SE. Chi-square tests were used to compare the allele frequency and genotype distribution of different groups. The relative risk was measured using odds ratios (with a 95 percent confidence interval (CI)). Haplotype frequencies and Linkage Disequilibrium (LD) parameters (D/ and r²) were calculated using the SNPstats online tool (https://www.snpstats.net/start.htm). All values were two-tailed, and P<0.05 was statistically significant.
3. Results

3.1. Demographic and clinical characteristics of the study population

In this cross-sectional study, samples from 238 unrelated individuals were obtained. They were divided into 140 patients diagnosed with pulmonary TB and 98 healthy subjects as controls. Clinical symptoms, identification of acid-fast bacilli in sputum smear samples, Mycobacterium tuberculosis-positive cultures in Löwenstein-Jensen medium, and X-ray evidence of cavitary lesions in the lung were used to make the diagnosis of pulmonary tuberculosis. The absence of clinical symptoms of active pulmonary tuberculosis, no medical history of TB, and no other infectious or autoimmune diseases, cancer, or other diseases affecting host immunity was found in the control subjects.

Table 1 shows the demographic and clinical features of 140 TB patients and 90 healthy controls. The participants in this study ranged from 18 to 65 years old. Sputum positivity was found in 83.6% of TB patients, while diabetes mellitus was found in 4.35%. An X-ray examination was used to confirm the presence of tuberculosis. At the sampling time, none of the patients had reported any other clinical complaints.

3.2. Distribution of TLR-2 and TLR-9 SNPs among Egyptian patients with pulmonary tuberculosis

Table 2 shows the distribution of TLR-2 Arg753Gln genotypes and alleles among Egyptian patients with pulmonary tuberculosis and healthy controls. The GG genotype and G allele are the most common genotype/allele in both groups, according to TLR-2 Arg753Gln SNP analysis, whereas the AA genotype and A allele are the least common. There was a complete disappearance of the AA genotype in the control group. In the TLR-2 Arg753Gln SNP, there was no statistically significant difference in the distribution of all genotypes/alleles between the two groups.

Concerning TLR-9 -1237T/C SNP, the TT genotype and T allele are the most frequent, while the CC genotype and G allele was found to be least frequent in both groups. A total loss of TT genotype in the control group was noticeable in TLR-9 -1237. TLR-9 -1237T/C SNP analysis showed a statistically significant increase in TC genotype \((P = 0.012)\) and C allele \((P = 0.009)\) in TB patients compared with controls. On the other hand, TT genotype and T allele were significantly elevated in the control group \((P = 0.02\) and \(P = 0.007\), respectively). Accordingly, TC genotype \((OR = 3.282; CI: 1.193-9.033)\) and C allele \((OR: 2.253; CI: 1.301-3.921)\) could be considered as risk factors for TB infection while TT genotype \((OR = 0.247; CI: 0.091-0.671)\) and T allele \((OR: 0.416; CI: 0.239-0.724)\) could be considered as a protective genotype and allele, respectively.

In Egyptian patients with pulmonary tuberculosis, the GA genotype and G allele were the most common genotype/allele at the TLR-9 +174G/A polymorphic location. The AA genotype is the least common and may be associated with tuberculosis \((OR = 2.252; CI: 0.514-12.429)\). Despite this, the genotypic/allelic distributions between TB patients and controls were insignificant.

Table 1. Demographic and clinical characteristics of the recruited subjects (patients and controls).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls ((N = 98))</th>
<th>TB patients ((N = 140))</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years ± SD)</td>
<td>35.35 ± 8.38</td>
<td>38.74 ± 13.06</td>
<td>NS</td>
</tr>
<tr>
<td>Positive TB culture (N, %)</td>
<td>0 (0%)</td>
<td>117 (83.6%)</td>
<td>0.000</td>
</tr>
<tr>
<td>X-ray examination</td>
<td>0 (0%)</td>
<td>140 (100.0%)</td>
<td>0.000</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1 (1.02%)</td>
<td>6 (4.3%)</td>
<td>NS</td>
</tr>
<tr>
<td>Autoimmune disease</td>
<td>1 (1.02%)</td>
<td>0 (0%)</td>
<td>NS</td>
</tr>
</tbody>
</table>
r2 value -0.0273. Our data showed that GTG and GTA haplotypes have the highest frequency in both groups, while ATG was the least frequent haplotype. The ACA and ACG haplotypes were not found. Interestingly, GCG haplotype and ATG showed a significant decrease in TB patients (P = 0.004 and P = 0.001, respectively).

4. Discussion
TLRs have a significant role in recognizing pathogens, activating innate host defense, and acting in multiple cellular processes [23]. TLR-2 is a well-known molecule intracellularly expressed

### Table 2. Genotype distribution and allelic frequency of the TLR2 Arg753Gln, TLR9 (-1237T/C), and TLR9 (+1174G/A) SNPs in controls and pulmonary tuberculosis patients.

<table>
<thead>
<tr>
<th>Position</th>
<th>Control (n = 98)</th>
<th>TB Patients (n = 140)</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR2 Arg753Gln (rs5743708) (N, %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>93 (94.9%)</td>
<td>131 (93.6%)</td>
<td>0.783 (0.254-2.411)</td>
<td>NS</td>
</tr>
<tr>
<td>GA</td>
<td>5 (5.1%)</td>
<td>8 (5.7%)</td>
<td>1.127 (0.357-3.555)</td>
<td>NS</td>
</tr>
<tr>
<td>AA</td>
<td>0 (0%)</td>
<td>1 (0.7%)</td>
<td>1.007 (0.993-1.021)</td>
<td>NS</td>
</tr>
<tr>
<td>GAAA</td>
<td>5 (5.1%)</td>
<td>9 (6.4%)</td>
<td>1.278 (0.415-3.936)</td>
<td>NS</td>
</tr>
<tr>
<td>G</td>
<td>191 (97%)</td>
<td>270 (96%)</td>
<td>0.706 (0.237-2.101)</td>
<td>NS</td>
</tr>
<tr>
<td>A</td>
<td>5 (3%)</td>
<td>10 (4%)</td>
<td>1.414 (0.476-4.205)</td>
<td>NS</td>
</tr>
<tr>
<td>TLR9 -1237T/C (rs5743836) (N, %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>82 (83.3%)</td>
<td>98 (70.0%)</td>
<td>0.247 (0.091-0.671)</td>
<td>0.002</td>
</tr>
<tr>
<td>TC</td>
<td>16 (16.7%)</td>
<td>38 (27.1%)</td>
<td>3.282 (1.193-9.033)</td>
<td>0.012</td>
</tr>
<tr>
<td>CC</td>
<td>0 (0%)</td>
<td>4 (2.9%)</td>
<td>1.029 (1.001-1.059)</td>
<td>NS</td>
</tr>
<tr>
<td>TCCC</td>
<td>16 (18.7%)</td>
<td>42 (30%)</td>
<td>3.100 (1.122-8.569)</td>
<td>0.017</td>
</tr>
<tr>
<td>T</td>
<td>180 (91.8%)</td>
<td>234 (83.6%)</td>
<td>0.416 (0.239-0.724)</td>
<td>0.007</td>
</tr>
<tr>
<td>C</td>
<td>16 (8.2%)</td>
<td>46 (16.4%)</td>
<td>2.253 (1.301-3.921)</td>
<td>0.009</td>
</tr>
<tr>
<td>TLR9 +174G/A (rs352139) (N, %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>14 (14.3%)</td>
<td>26 (18.6%)</td>
<td>1.368 (0.074-2.778)</td>
<td>NS</td>
</tr>
<tr>
<td>GA</td>
<td>82 (83.7%)</td>
<td>107 (76.4%)</td>
<td>0.633 (0.326-1.227)</td>
<td>NS</td>
</tr>
<tr>
<td>AA</td>
<td>2 (2.0%)</td>
<td>7 (5.0%)</td>
<td>2.252 (0.514-12.429)</td>
<td>NS</td>
</tr>
<tr>
<td>GAAA</td>
<td>84 (85.7%)</td>
<td>113 (80.7%)</td>
<td>0.698 (0.345-1.411)</td>
<td>NS</td>
</tr>
<tr>
<td>G</td>
<td>110 (56%)</td>
<td>159 (57%)</td>
<td>1.027 (0.711-1.430)</td>
<td>NS</td>
</tr>
<tr>
<td>A</td>
<td>86 (44%)</td>
<td>121 (43%)</td>
<td>0.973 (0.673-1.406)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Genotypes/alleles that could be considered as disease risk factors are presented in bold.

3.3. Haplotypes of SNPs in TB patients and normal controls

Estimating haplotypes of the three studied positions (TLR-2 Arg753Gln, TLR-9 [-1237T/C], and TLR-9 [+1174G/A]) produced 8 haplotypes (Table 3). TLR-2 Arg753Gln and TLR-9-1237T/C genotype frequencies in all participants revealed the same genetic variation, implying that TLR-2 Arg753Gln and TLR-9 [-1237T/C] appeared to be in LD in the Egyptian population (D' = 0.9856, r2 = -0.0675). On the other hand, the LD pattern between TLR-9 [-1237T/C and TLR-9 +1174G/A] showed no LD, with a D' value of 0.082 and r2 value -0.0273. Our data showed that GTG and GTA haplotypes have the highest frequency in both groups, while ATG was the least frequent haplotype. The ACA and ACG haplotypes were not found. Interestingly, GCG haplotype and ATG showed a significant decrease in TB patients (P = 0.004 and P = 0.001, respectively).

4. Discussion
TLRs have a significant role in recognizing pathogens, activating innate host defense, and acting in multiple cellular processes [23].
by different immune system cells and has a vital role in activating the innate immune system against mycobacterial infection [4]. Genetic polymorphisms may diminish the immune response against TB and may affect the expression of TLRs, splicing RNA, affecting mRNA transcription and protein product [4, 23]. This study examined the association between TLR-9 [rs5743836 and rs352139] and TLR-2 Arg753Gln [rs5743708] polymorphism and susceptibility to tuberculosis infection in the Egyptian population.

One of the most well-studied variations linked to TB susceptibility is the TLR-2 Arg753Gln [rs5743708] gene polymorphism [24]. Dimer generation for TLR-2 homodimers or heterodimers with downstream targets such as Myeloid differentiation primary response 88 (MyD88) is one of the putative activities of the carboxy [C] terminus. Because the TLR-2 gene mutation [Arg753Gln] at the very C terminus impacts the signaling function of the molecule rather than ligand binding, this Arg753Gln polymorphism may reduce macrophages’ responsiveness to bacterial peptides in vitro, eventually leading to a greater susceptibility to TB [25].

The connection between the TLR-2 Arg753Gln polymorphism and the risk of tuberculosis was investigated in this study. In terms of genotype and allele frequencies, there was no significant difference between TB patients and controls. In agreement with our findings, Xue et al. [26] did not find any significant association between TLR-2 Arg753Gln gene polymorphism and TB infection susceptibility in a Chinese population. Similarly, TLR-2 Arg753Gln gene polymorphism was not associated with the increased TB prevalence in an Indian population [27, 28]. Bahrami et al. [29] showed that the TLR-2 Arg753Gln gene was not a risk factor in Iranian asthmatic patients.

In contrast, Ogus et al. [30] found the risk is increased for tuberculosis subjects with heterozygous TLR-2 Arg753Gln in Turkish populations. Furthermore, the association of TLR-2 Arg753Gln gene polymorphism with decreased TB risk was previously reported [31-34]. Soetoro et al. [35] showed an association between Arg753Gln polymorphism of TLR-2 gene and active pulmonary TB disease in the Indonesian population. Hu et al. [24] found that the A allele is associated with increased susceptibility to tuberculosis in Asian and Caucasian groups but not in African or mixed populations.

The SNP -1237T/C is located within the putative promoter region of the TLR-9 gene. This SNP was associated with increases in the gene’s transcriptional activity and function [36]. Several reports found that TLR-9 gene polymorphisms are associated with the development of several

### Table 3. Haplotype frequency of the TLR2 Arg753Gln, TLR9 (-1237T→C), and TLR9 (+1174G/A) SNPs in controls and pulmonary tuberculosis patients.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Control (N = 98)</th>
<th>TB patients (N = 140)</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GTG</td>
<td>0.4137</td>
<td>0.5018</td>
<td>1.00</td>
<td>---</td>
</tr>
<tr>
<td>GTA</td>
<td>0.3771</td>
<td>0.3768</td>
<td>0.73 (0.33-1.61)</td>
<td>NS</td>
</tr>
<tr>
<td>GCG</td>
<td>0.1475</td>
<td>0.0439</td>
<td>0.22 (0.08-0.62)</td>
<td>0.004</td>
</tr>
<tr>
<td>GCA</td>
<td>0.0361</td>
<td>0.0418</td>
<td>1.00 (0.19-5.14)</td>
<td>1</td>
</tr>
<tr>
<td>ATA</td>
<td>0.0022</td>
<td>0.0136</td>
<td>0.26 (0.04-1.80)</td>
<td>NS</td>
</tr>
<tr>
<td>ATG</td>
<td>0.0255</td>
<td>0.0136</td>
<td>1053883136.65 (1053883132.86 - 1053883140.44)</td>
<td>0.0001</td>
</tr>
<tr>
<td>ACA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>ACG</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
inflammatory diseases [37-41]. Hemmi et al. [42] showed that the TLR-9 gene causes an intracellular receptor signal cascade and evokes nuclear transcription factors resulting in pro-and anti-inflammatory cytokine production.

The TLR-9-1237C allele is significantly associated with pulmonary TB risk. In line with our data, The TLR-9-1237T/C polymorphism was related to the risk of latent tuberculosis infection [LTBI]. This polymorphism in TLR-9 has been consistently associated with increased transcriptional activity [43, 44], supporting the notion that it enhances TLR-9 function and increases susceptibility to LTBI. Similar to the current results, Berenson et al. [38] showed that TLR-9-1237 was associated with chronic obstructive pulmonary disease development and severity. Our data are analogous to the study of Velez et al. [45], which suggested that the dominant and recessive models of TLR-9-1237T/C polymorphism had a significant association with the TB infection in Caucasian and African-Americans. In contrast, in silico analysis of the TLR-9 promoter activity revealed that the mutated −1237T/C variants provoked higher gene expression than the wild-type promoter [43]. Chen et al. [46] and Mittal et al. [28] reported an insignificant association between TLR-9−1237T/C polymorphism and TB risk.

The SNP rs352139 is found in the TLR-9 gene’s intronic region and is critical for TLR-mediated immunologic response regulation [46]. In the case of the rs352139 polymorphism in TLR-9 genes, we found no evidence of a link between genotype/allele frequencies in TB patients and controls. However, the AA genotype may be a risk factor for tuberculosis. This SNP has been studied in a small number of populations, with mixed results. According to Chen et al. [46], the rs352139 polymorphism is linked to a lower incidence of tuberculosis among Indonesians but a higher risk in Mexicans.

Furthermore, in Indonesian female cohorts, a tangible link between the rs352139 polymorphism and TB susceptibility was discovered [4]. Haiko-Schurz et al. [47] showed that meta-analysis’s TLR-9 rs352139 GA and GG genotypes were associated with an increased risk of TB susceptibility. Additionally, associations between the recessive model and populations from Indonesia and Vietnam have been discovered by meta-analysis; this reflects that a TLR-9 polymorphism could play a vital role in the TB susceptibility in Asian populations [48].

Our results found no linkage disequilibrium between TLR-9.rs5743836 [-1237T→C] and TLR-9.rs352139 [+1174G/A] markers (D'= 0.082 and r2 = -0.0273). This finding agrees with Piotrowski et al. [49], who found that TLR-9 SNPs at rs5743836 and rs352139 in Caucasians are present in the same block. Another study [50] found a modest LD between rs352139 and rs5743836, which we agree with.

5. Conclusion

To the best of our knowledge, this is the first comprehensive analysis of TLR-2 [Arg753Gln], TLR-9 [-1237T/C], and TLR-9 [+1174G/A] SNPs and their role in pulmonary tuberculosis in the Egyptian population. The current findings of our pilot investigation imply that increased TLR-9−1237 TC genotypes and C allele in tuberculosis patients may indicate their function in tuberculosis infection susceptibility in Egyptians. This study’s conclusions are limited due to the relatively small sample size. Another limitation of this work was selection of cases from only one region of Egypt, representing a small part of the country. Larger scale primary studies considering gene-gene and gene-environment interactions are still needed to estimate gene polymorphism’s interaction with TB infection susceptibility.

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ETHICS APPROVAL

All experimental protocols described in this study complied with the rules of Sadat City University Animal Experiments Local Ethics Committee and the Guidelines for the Care and Use of Laboratory Animals. The Sadat City University Ethics Committee granted ethical approval (No. 19 06 2019, Sadat, Egypt).
CONSENT FOR PUBLICATION
All authors approve the publication of this work.

DATA AVAILABILITY
Datasets generated during the current study are not publicly available but could be obtained from the corresponding author on reasonable request.

CONFLICT OF INTEREST STATEMENT
The authors declare that they have no conflict of interest.

REFERENCES


