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ORIGINAL RESEARCH

Association of Toll-like Receptors 1, 2, 4, 6, 8, 9 and 10 Genes Polymorphisms and Susceptibility to Pulmonary Tuberculosis in Sudanese Patients

Najwa A Mhmoud

Faculty of Medical Laboratory Sciences, Department of Medical Microbiology and Immunology, University of Khartoum, Khartoum, Sudan

Correspondence: Najwa A Mhmoud, Faculty of Medical Laboratory Sciences, Department of Medical Microbiology and Immunology University of Khartoum, P.O. Box 102, Khartoum, Sudan, Fax +249-83-383590, Email Infkogodaster@gmail.com

Background: Genetic factors are important contributors to the development of a wide range of complex disease. Polymorphisms in genes encoding for toll-like receptors (TLRs) usually influence the efficiency of the immune response to infection and are associated with disease susceptibility and progression. Therefore, we aim to describe the first association between TLR1, TLR2, TLR4 TLR6, TLR8, TLR9 and TLR10 genes polymorphisms and susceptibility to pulmonary tuberculosis (PTB) in Sudanese patients.

Methodology: Here we performed a case study which included 160 tuberculosis patients and 220 healthy matched controls from Sudan. In the study population, we evaluated the possible association between 86 markers in TLR1, TLR2, TLR4 TLR6, TLR8, TLR9 and TLR10 genes polymorphisms and susceptibility to PTB disease in Sudanese population using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP).

Results: From our results it appeared that in the PTB population the TLR1 (rs5743557, rs4833095, rs5743596), TLR2 (rs5743704, rs5743708, rs3804099), TLR4 (rs4986790, rs4986791), TLR6 (rs5743810), TLR8 (rs3764879, rs3764880), TLR9 (rs352165, rs352167, rs187084) and TLR10 (rs4129009) were significantly more often encountered ($p<0.0001$) than in the control population and were associated with PTB in the Sudanese population. For the other polymorphisms tested, no association with PTB was found in the population tested.

Conclusion: The work describes novel mutations in TLR1, TLR2, TLR4, TLR6, TLR8, TLR9 and TLR10 genes and their association with PTB infection in Sudanese population. These results will enhance our ability to determine the risk of developing the disease by targeting specific TLR pathways to reduce the severity of the disease. Future studies are needed in a larger sample to replicate our findings and understand the mechanism of association of TLR polymorphism in PTB.

Keywords: tuberculosis, toll-like receptors, PCR-RFLP

Introduction

Tuberculosis (TB) has been declared a major public health problem worldwide, due to the emergence of almost untreatable strains of *Mycobacterium tuberculosis* (*M. tuberculosis*). In addition to that, the only existing vaccine against TB, the BCG vaccine, fails to protect against pulmonary TB, the most important form of disease manifestation.[1] It kills approximately 10 million people and nine million new TB cases are reported worldwide annually.[1,2] Moreover, in the last two decades, three major coronavirus epidemics have been reported worldwide.[3] The burden of COVID-19, HIV, and TB is one of the major and persistent global health challenges of the twenty-first century.[4–10]

Genetic factors are important contributors to the development of a wide range of complex disease.[11–21] A person who is susceptible to a particular infectious disease, such as TB, the risk of developing the disease is higher than one who has not inherited the genetic risk factor.[11–21] These evidences on the influence of host genetic factors in TB susceptibility led to the development of strategies to identify candidate genes or susceptibility loci in the human genome. Identification of polymorphisms in genes has enabled linkage and association studies to be used in explaining individual variation in susceptibility to and severity of TB in humans.

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In this context, toll-like receptors (TLRs) are a family of proteins that are expressed either on the extra cellular cell surface (TLR1, 2, 4, 5, 6) or in the cytosol or on endosomal membranes (TLR3, 7, 8, 9) of macrophages and dendritic cells. TLRs are essential for recognition of a broad repertoire of pathogen-associated molecular patterns (PAMPs) on macrophages and dendritic cells and play an important role in the innate responses against *M. tuberculosis*.[22–26] Genetic variations of TLR1, TLR2, TLR4, TLR6, TLR8, TLR9 and TLR10 have been associated with the susceptibility to TB in different ethnic groups.[27–33] In contrast, other studies have failed to demonstrate significant associations of TLRs polymorphisms with TB.[34–37]

However, to date, no TLR mutations or single-nucleotide polymorphisms (SNPs) have been established as accepted risk factors for PTB among different ethnic populations. Besides, only one study have been performed on Sudanese patients to understand the role of TLR2 polymorphism in PTB.[31] Therefore, to confirm the role of TLR variants in the increased risk for PTB we conducted the candidate gene association study by investigating 86 SNPs present in TLR1, TLR2, TLR4 TLR6, TLR8, TLR9 and TLR10 genes in a sub-Saharan Sudanese pulmonary tuberculosis patients.

Materials and Methods

Study Population

A prospective, cross sectional, case-control study was carried out during the period between 2019 and 2020 at Abu-Angah Hospital, Khartoum, Sudan. 160 patients with active pulmonary TB and 220 healthy controls were included. EDTA blood samples were taken from all patients and healthy controls. All tuberculosis patients had microbiological (by culture and/or smear) or radiological evidence of *M. tuberculosis* disease (Table 1).

The healthy controls had no evidence of tuberculosis disease by clinical examination, and were matched on age, gender and BCG status (Table 1).

Table 1 Characteristics of the Study Population

		Patients	Controls	p-value
Total number		160	220	
Mean age /yrs (range)		26 (11–70)	30 (11–70)	1.0*
Gender (male/female)		111/49	65/155	0.0041**
Occupation	Governmental employee	22 (13.8)	21 (9.55%)	0.05**
	Workers	63 (39.4%)	44 (20%)	<0.0001**
	Other job	40 (25%)	40 (18.3%)	0.58**
	Jobless	3 (1.8%)	13 (5.9%)	<0.0001**
	Housewife	13 (8.1%)	17 (7.7%)	0.03**
	Student	19 (11.9%)	85 (38.6%)	0.15**
BCG vaccination		112 (70.6%)	212 (96.8%)	0.63**
Definite tuberculosis	Presence of MTB in sputum based on both smear and culture	92 (57.5%)	0 (0%)	
	Presence of MTB in sputum specimen only by smear	109 (68.1%)	0 (0%)	
	Presence of MTB in sputum specimen only by culture	47 (29.4%)	0 (0%)	
Hepatitis C test		Negative	Negative	
Hepatitis B Ag test		Negative	Negative	
HIV1, 2 test		Negative	Negative	
Nasopharyngeal swab for (COVID-19)		Negative	Negative	

Notes: *Unpaired *t*-test, **Fisher exact test.

The collected blood samples were tested for other infectious diseases and that included hepatitis B (HBsAg, InTec products, INC, China), hepatitis C (Rapid Anti-HCV Test, InTec products, INC, China), syphilis (RAPIDAN TESTER, product code: RTTP01, Turkey), and HIV (HIV1, 2 Cassette test, Clinotech Diagnostics & Pharmaceuticals, Canada). Blood samples were stored at −20 ° C until use. Nasopharyngeal swab for was used to define Corona Virus Disease-2019 (COVID-19).

Ethics Statement

The present study was approved by the Ethics Committee of University of Khartoum, Khartoum, Sudan (5/2018). This study adheres to the Declaration of Helsinki (1964). Written informed consents were obtained from all participants in the study or legally responsible guardians for participants less than 18 years old.

DNA Isolation

Genomic DNA was isolated from blood samples with the large volume kit for the MagNA Pure system (Roche, Almere, The Netherlands) according to the manufacturer's descriptions. The isolated DNA was stored at −20°C.

Genotyping

Genomic variants of TLR1 (rs5743604, rs5743611, rs5743618, rs76600635, rs5743551, rs5743557, rs5743565, rs5743566, rs5743580, rs5743594 rs4833095, rs5743595, rs5743596); TLR2 (rs1816702, rs5743704 rs5743708, rs7656411, rs11938228, rs893629, rs1898830, rs121917864, rs4696480, rs3804099, rs5743699 rs3804100); TLR4 (rs7869402, rs1927907, rs1927911, rs1927914, rs6478317, rs55912718, rs5030719, rs10759931, rs10759933, rs2770150, rs1554973, rs11536878, rs11536879, rs7873784, rs11536889, rs4986790, rs4986791, rs11536897, rs11536898); TLR6 (rs3796508, rs5743810, rs5743831, rs1039559, rs6531670, rs5743788, rs5743794); TLR8 (rs4830805, rs4830808, rs3747414, rs3761624, rs1548731, rs2109134, rs3788935, rs1013150, rs5744068, rs3764879, rs5744080, rs3764880, rs17256081, rs5741883, rs2407992, rs178998); TLR9 (rs5743836, rs164637, rs352139, rs352140, rs352143, rs352162, rs352165, rs352167, rs187084); and TLR10 (rs4129009, rs7694115, rs10856839, rs11466645, rs4274855, rs11096955, rs11096956, rs11096957, rs7698870, and rs10776483) genes were detected by PCR followed by restriction enzyme fragment analysis (PCR-RFLP) (Figures 1 and 2). All PCR primers are stated in Table 2. Each of the PCRs consisted of a pre-denaturation step of 4 minutes at 94°C and 40 cycles each of 30 seconds denaturation at 94°C, 30 seconds annealing at 55°C and 30 seconds elongation at 72°C. This was followed by a post-elongation step of 7 minutes at 72°C. Restriction

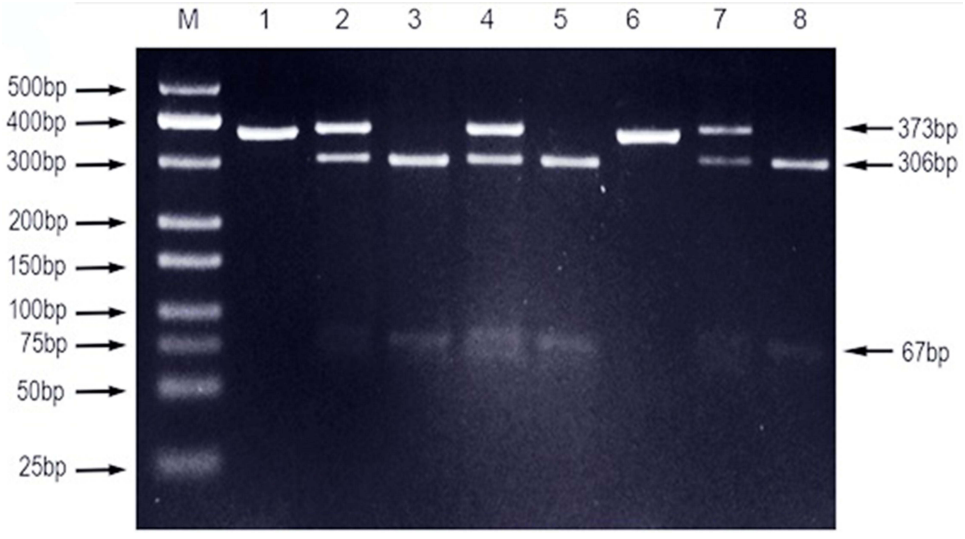

Figure 1 PCR-RFLP assay for analyzing the TLR4-rs4986790 A/G polymorphisms in Sudanese pulmonary Tuberculosis patients.
Notes: Lane M shows DNA marker; lanes 1 and 6 show AA genotype; lanes 2, 4, and 7 show GA genotype; lanes 3, 5, and 8 show GG genotype.

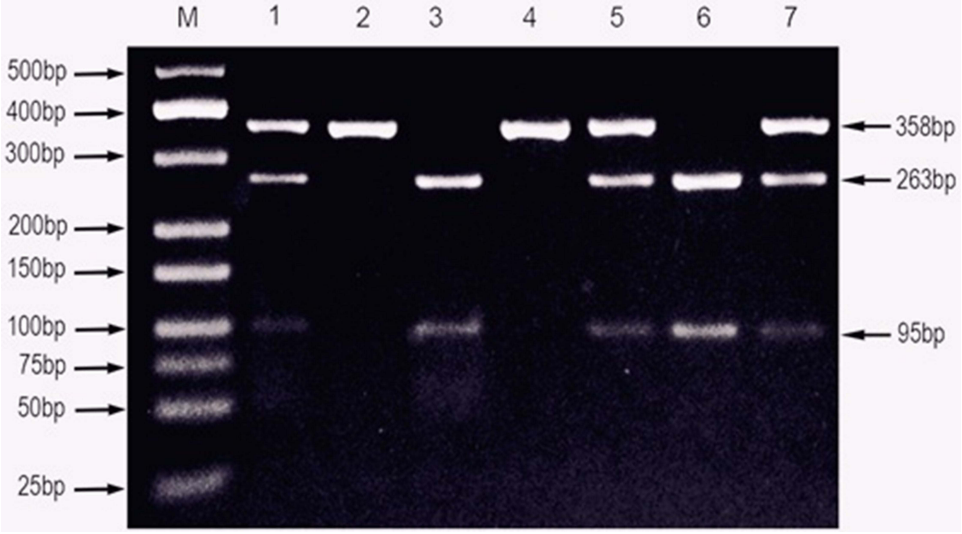

Figure 2 PCR-RFLP assay for analyzing theTLR8-rs3764879 G/C polymorphisms in Sudanese pulmonary Tuberculosis patients.
Notes: Lane M shows DNA marker; lanes 2 and 4 show GG genotype; lanes 3 and 6 show CC genotype; lanes 1, 5, and 7 show CG genotype.

endonucleases were selected using the NEBcutter software (http://nc2.neb.com/NEBcutter2/). Restriction endonucleases were obtained from Fermentas (st. Leon-rot, Germany), and Roche (Penzberg, Germany) and were used as described by the manufacturer. Restriction fragments were visualized by electrophoresis on 2% agarose gels (Hispanagar, Sphaero Q, Leiden, The Netherlands).

The criteria for the selection of SNPs for this study were based on their previously reported to change the level or function of corresponding gene products and influence susceptibility/resistance to infections. Also, the selection was based on the publically available information on the polymorphisms of TLR genes available in the 1000 Genomes project for the South Asian population (http://www.internationalgenome.org).[38]

Statistical Analysis

The mean age of the patient population and the control population were compared by the unpaired *t*-test. Gender, occupation and BCG-vaccination status between the patient and control population were compared with the Fisher exact test. Verification of Hardy-Weinberg equilibrium (HWE) was performed with Pearson's χ^2 test. The effect of the TLR1; TLR2; TLR4; TLR6; TLR8; TLR9; and TLR10 polymorphisms on susceptibility to tuberculosis were assessed with the Fisher exact test. P-value of <0.05 was deemed statistically significant. All statistical analyses were performed using SPSS for Windows v11.0 statistical analysis software. This study was in accordance with the principles of the Helsinki Declaration (1964). This study was in accordance with the principles of the Helsinki Declaration (1964) This study was in accordance with the principles of the Helsinki Declaration (1964)

Results

Characteristics of Tuberculosis Patients and Healthy Control Subjects

One hundred and sixty Sudanese tuberculosis patients were included into the study. The diagnosis of tuberculosis was based on the presence of *MTB* in a positive Ziehl-Nielson (ZN) smear of a sputum specimen and/or by positive culture with tuberculosis and radiological evidence (chest X-ray) (Table 1). The control population comprised 220 healthy unrelated people from the same endemic area in Sudan, they were matched on gender and BCG-status (Table 1) and showed no signs of any lung disease. Unfortunately, the occupation of the control population differed from that of the patient population.

Table 2 PCR Primers and Restriction Enzymes for genotyping the Different Single Nucleotide Polymorphisms

Gene(s)	Rs Number	Primer Sequence(s) (5'→-3')	Chromosome Position	Gene Location	Restriction Enzymes	Reference
TLR 1						
T/C	rs5743604	F: GAAGAGCAGTCCCAATACCACCAT	chr4:38801285	Intron 3	Bsl I	[53,54,58,67]
		R: GGCCCCAAGCTTCCCAAAATTA				
C/G	rs5743611	F: GCATCTTCCATTTTGCCATTATCTTC	chr4:38798593	Exon 4	*Alu I*	[53,54,58,67]
		R: CCAATTCCTGGTTGAATTTGAAAACA				
G/T	rs5743618	F: TCCTCATGACTCTTTTCTGC	chr4:38797027	Promoter	Hind III	[53,54,58,67]
		R: GAGGCTTGTCCCTTGCTCCAC				
A/G	rs76600635	F: GCATCTTCCATTTTGCCATTATCTTC	chr4: 38800323	Intron	Bsl I	[53,54,58,67]
		R: CCAATTCCTGGTTGAATTTGAAAACA				
A/G	rs5743551	F: CCGAGATGTTCCCAGCACAG	chr4: 38806033	Promoter	Hind III	[53,54,58,67]
		R :CTGCTTTGCTTGTGCCTCTT				
C/T	rs5743557	F:ACGTTGGATGGAGTGTGCTTCAGCAAAAC	chr4:38806827	Near-gene-5	Bsl I	[53,54,58,67]
		R:ACGTTGGATGTCTTGCCTTTTCATCCATCC				
A/G	rs5743565	F: ACGTTGGATGAGCTGAACAGCAGCATTGCC	chr4:38805983	Exon 2	Hph I	[53,54,58,67]
		R: ACGTTGGATGTTCTCTTCACCTAATCCCGC				
C/G	rs5743566	F: ACGTTGGATGAAGGACTAGCTAGTGGGAAG	chr4:38805942	Exon 2	Mbo II	[53,54,58,67]
		R: ACGTTGGATGAGTTACTCCCGGAGGCAATG				
A/G	rs5743580	F:ACGTTGGATGAACATCCAGAGTGACTCAGC	chr4:38804405	Intron	Hpy188 III	[53,54,58,67]
		R:ACGTTGGATGTGGCTATGGTAAGCTTCTC				
C/T	rs5743594	F:ACGTTGGATGGCTACTCACACAAGGAGCAA	chr4:38802751	Intron	Bsl I	[53,54,58,67]
		R:ACGTTGGATGGGGTTAGGATTTCAACATAAG				
G/A	rs4833095	F:ACGTTGGATGCTGGAGGATCCTAATGAAAG	chr4:38799710	Exon 4	Bc II	[53,54,58,67]
		R:ACGTTGGATGCCTAAGTATTCTGGCGAAAC				
T/C	rs5743595	F:ACGTTGGATGGGAAATTTCCGGGTCTTTCA	chr4:38802644	Intron	Mly I	[53,54,58,67]
		R:ACGTTGGATGGCAAGGAAGATGATGCAGAC				
C/T	rs5743596	F:ACGTTGGATGTGCTCAGGGTCTTCATGAAC	chr4:38802528	Exon 3	Hph I	[53,54,58,67]
		R:ACGTTGGATGGGTGCCCAATATGCCTTTGT				
TLR 2						
C/T	rs1816702	F: ACGTTGGATGGTGTGAGCCTTACTAAAGGT	chr4:154609523	Intron	BseL I	[53,54,58,67]
		R: ACGTTGGATGCTTTGATTCCTCTAGCGCTG				
C/A	rs5743704	F: ACGTTGGATGTGCATCATAGCAGATGTTCC	chr4:154625951	Exon 3	Aci I	[53,54,58,67]
		R: ACGTTGGATGTGAAAATGATGTGGGCCTG				
G/A	rs5743708	F:ACGTTGGATGCCAGGTAGGTCTTGGTGTTC	chr4:154626317	Exon 3	Msp I	[53,54,58,67]
		R:ACGTTGGATGTTCTTCTGGAGCCCATTGAG				

(*Continued*)

Table 2 (Continued).

Gene(s)	Rs Number	Primer Sequence(s) (5'→-3')	Chromosome Position	Gene Location	Restriction Enzymes	Reference
T/C	rs7656411	F:ACGTTGGATGCCTTTAAATTACTGTGTATC	chr4:154627655	Near gene-3	BspL I	[53,54,58,67]
		R:ACGTTGGATGGTACATGTGAGCTAAATAG				
C/T	rs11938228	F:ACGTTGGATGTAGGGACATGCCCATATAGG	chr4:154621946	Near-gene-5	BsaA I	[53,54,58,67]
		R:ACGTTGGATGTAGTGTGTTGCTGGTATAGG				
G/A	rs893629	F:ACGTTGGATGACATCACCTGAAACACACCG	chr4:154604968	Near-gene-5	Pst I	[53,54,58,67]
		R:ACGTTGGATGGATTCATGAGCCTGAGTTCG				
A/G	rs1898830	F:ACGTTGGATGGATCCCCTATTTTCTAGCAC	chr4:154608453	Intron	EcoRV	[53,54,58,67]
		R: ACGTTGGATGAAAACTGGAAAAGGAATAG				
C/T	rs121917864	F:CACGTGAGTCTGAGTTTC	chr4:154626088	Exon 3	Msp I	[53,54,58,67]
		R:GTCTGGTTCAAGAAGTCATACCCCAACCCA AGA GAG'				
T/A	rs4696480	F : ACGTTGGATGCTCACCATGTGATGCTTTCC	chr4:154607126	Intron	*Mwo I*	[53,54,58,67]
		R : ACGTTGGATGGGGAAGTCCAAGATTGAAGG				
T/C	rs3804099	F:ACGTTGGATGCTGCTTCATATGAAGGATCAG	chr4:154624656	Exon 3	Tai I	[53,54,58,67]
		R : ACGTTGGATGGATCTACAGAGCTATGAGCC				
T/C	rs5743699	F :'ATTTGAACTTATCCA GCACACGAAT	chr4:154625291	Exon 3	Msp I	[53,54,58,67]
		R :TCCAGTGTCTTGGGAATGCA				
T/C	rs3804100	F : ACGTTGGATGTTGAACTTATCCAGCACACG	chr4:154625409	Exon 3	*Neo I*	[53,54,58,67]
		R : ACGTTGGATGTTCCAGTGTCTTGGGAATGC				
TLR 4						
C/T	rs7869402	F : ACGTTGGATGTTTAGGGAGACACAGATGGC	chr9:120478032	Exon 3	Bsl I	[53,54,58,67]
		R : ACGTTGGATGACCTTCACACGTAGTTCTCC				
G/A	rs1927907	F: ACGTTGGATGTTTTTCAAACAAGAAGTAG	chr9:120472764	Intron	*Not I*	[53,54,58,67]
		R: ACGTTGGATGGGGTATCCAGTGGATTGAAG				
C/T	rs1927911	F: ACGTTGGATGAGACCTTCCTTAGTCATGGC	chr9:120470054	Intron	Sty I	[53,54,58,67]
		R: ACGTTGGATGCATCACTTTGCTCAAGGGTC				
T/C	rs1927914	F : ACGTTGGATGGTGCTTGGAGGATATTACAG	chr9:120464725	Near-gene-5	Sph I	[53,54,58,67]
		R : ACGTTGGATGGAACCTGATTTAAAACAGGA				
A/G	rs6478317	F:ACGTTGGATGTCTATCCCAAGATCGGTTCC	chr9:120480210	Near-gene-5	Bsl I	[53,54,58,67]
		R:ACGTTGGATGAAGAGGCTAGAAGAAGATAG				
A/G	rs55912718	F: AATTCACCAAGCCCAGGCAGAG	chr9:120466480	Near-gene-5	BstU I	[53,54,58,67]
		R: GAGGAAGTGAAAGCGGCAACCT				
G/A	rs5030719	F: GCTGAGAAACTTGACCTTCCTGGAC	chr9:120475936	Near-gene-5	*Not I*	[53,54,58,67]
		R: AGAACCTGGAGGGAGTTCAGACAC				

(*Continued*)

Table 2 (Continued).

Gene(s)	Rs Number	Primer Sequence(s) (5'→-3')	Chromosome Position	Gene Location	Restriction Enzymes	Reference
C/T	rs10759931	F: ACCTCAGTGGGCTCTGGGGTAG	chr9:120464147	Near-gene-5	Kpn I	[53,54,58,67]
		R: CCCTTCCTCTGAACCACCTCCT:				
A/C	rs10759933	F :ACGTTGGATGGGTAATAAAATATCCAATATCG	chr9:120470372	Intron	Hind III	[53,54,58,67]
		R : ACGTTGGATGTGCTCATCTTCTCTGTATCC				
T/C	rs2770150	F: ACGTTGGATGCACTCAATCATTTACTGACC	chr9:120463139	Near-gene-5	*Not I*	[53,54,58,67]
		R: ACGTTGGATGACACATGGTCTGCCTTCTGG				
C/T	rs1554973	F: TGAAGCCATGAATCATTAAGCCAATA	chr9:120480812	Intron	Hae III	[53,54,58,67]
		R: TCCAAAAGCCACGCTACTCAAA				
C/A	rs11536878	F:ACGTTGGATGTTCTTGACTACCCACCACAG	chr9:120471553	Intron	ApeK I	[53,54,58,67]
		R:ACGTTGGATGGCGACATATAACAGTAGGTG				
A/C	rs11536879	F: CCTGTTGGGGTCAGAAGACCTG	chr9:120472211	Intron	*Hinf I*	[53,54,58,67]
		R:CATTTAATCCAGCAGAATAAGTTTCATCATTT				
G/C	rs7873784	F: ACGTTGGATGGCTCTAAAGATCAGCTGTAT	chr9:120478936	Exon 4	Bsl I	[53,54,58,67]
		R: ACGTTGGATGGGTACCCTCTTAACAAAATG				
G/C	rs11536889	F :GTTTCCTGTTGGGCAATG CT	chr9:120478131	Near-gene-3	Bcc I	[53,54,58,67]
		R :CATTAATTCCAGCACATTGTTTTCTC				
A/G	rs4986790	F: TCTTGCTGGAGTATTCCCTATGA	chr9:120475302	exon 4	Nco1	[53,54,58,67]
		R: GCCACAACCTGCTCTTGC				
C/T	rs4986791	F:GGTTGCTGTTCTCAAAGTGATTTTGGGA**G**AA	chr9:120475602	Exon 4	Hinf I	[53,54,58,67]
		R:ACCTGAAGACTGGAGAGTGAGTTAAATGCT				
G/A	rs11536897	F: TTCTGCATAAGTGTGTTTATTCAAGCAAA	chr9:120480010	Exon 4	*Xho I*	[53,54,58,67]
		R: TCCAGAGGATTTAAAGAGCTAAAAAGAGG				
C/A	rs11536898	F: TTCTGCATAAGTGTGTTTATTCAAGCAAA	chr9:120480210	Exon 4	*Not I*	[53,54,58,67]
		R: TCCAGAGGATTTAAAGAGCTAAAAAGAGG				
TLR 6						
T/C	rs3796508	F: ACGTTGGATGGTTGCAAAAGATATCCTGCC	chr4:38830736	Exon 1	Nla III	[53,54,58,67]
		R: ACGTTGGATGGCAGGGCCTTGAAATCATTG				
C/T	rs5743810	F: ACGTTGGATGATTTTTATCAGAACTCACC	chr4:38830350	Exon 1	Ava II	[53,54,58,67]
		R: ACGTTGGATGAGGCATTTCCAAGTCGTTTC				
T/C	rs5743831	F: GTTGGCTTGAGCACCCAAAATG	chr4:38827414	Intron	*BstN I*	[53,54,58,67]
		R: CTTCTGCCTAACCCCGTGTCCT				
T/C	rs1039559	F: ACGTTGGATGGCAAGCAGCAGACACATCAA	chr4:38831596	Near-gene-5	Mbo II	[53,54,58,67]
		R: ACGTTGGATGCTCAGCCTTTTTTCTCCCAC				

(*Continued*)

Table 2 (Continued).

Gene(s)	Rs Number	Primer Sequence(s) (5'→-3')	Chromosome Position	Gene Location	Restriction Enzymes	Reference
T/C	rs6531670	F: CCACGAGTGCCCACATATTTCAC	chr4:38835022	Intron	*Bfal*	[53,54,58,67]
		R: CAGTGTCAGTGGCATTTGGGTTC				
C/G	rs5743788	F:ACGTTGGATGGACACTGCAAGAGAGTGAAA	chr4:38833207	Promoter	Nco1	[53,54,58,67]
		R:ACGTTGGATGCAAGTCCCTATCATATATGG				
C/T	rs5743794	F: TGAGTTGCCTTTGCTCGTTTGTC	chr4:38832727	Intron	Hind III	[53,54,58,67]
		R: AGCCAGATAACTGACACCACCTAACA				
TLR 8						
G/A	rs4830805	F: ACGTTGGATGAAGGGAACGTGGAAAATCCG	chrX:12927759	Intron	*Kpn I*	[53,54,58,67]
		R: ACGTTGGATGATCAGCAGAGACCTGATAGC				
C/T	rs4830808	F: ACGTTGGATGGGTAAATGGGTCTCCATTTAG	chrX:12932334	Intron	Apo I	[53,54,58,67]
		R: ACGTTGGATGTCCCACTGGAATTGTTTAGG				
C/A	rs3747414	F: ACGTTGGATGGGTCTTAGTTTCAAGTGCGG	chrX:12939412	Exon 2	Hind III	[53,54,58,67]
		R: ACGTTGGATGAGTCAGTAGTCTGAAGCACC				
A/G	rs3761624	F: ACGTTGGATGTTGGTTTTCTCCCACTCCTG	chrX:12923681	Near-gene-5	Nco1	[53,54,58,67]
		R: ACGTTGGATGCCCTGGCCACAAGAATAAAG				
C/T	rs1548731	F: ACGTTGGATGACCCAAAGAAGTCCATGAGG	chrX:12927947	Intron	Bst7 II	[53,54,58,67]
		R: ACGTTGGATGGAGTGACCTTGAAAACATTC				
A/T	rs2109134	F: ACGTTGGATGCCCAGATGAACTCAGAGATG	chrX:12927186	Intron	Mae I	[53,54,58,67]
		R: ACGTTGGATGAAGTGCATCATAGTGTCGCC				
A/G	rs3788935	F: ACGTTGGATGGTCTAGTATCTATGTCAAGC	chrX:12922659	Near-gene-5	Msp I	[53,53,58,67]
		R: ACGTTGGATGGTGTGGAGAAAACTGAAGGC				
G/A	rs1013150	F: ACGTTGGATGATCACTCAGGGCAGTGTAAG	chrX:12932441	Intron	Nla III	[53,54,58,67]
		R: ACGTTGGATGTTACTAGGAAAAAGATGAG				
C/T	rs5744068	F: ACGTTGGATGTCATTTCCTTGCTAGCTGCC	chrX:12935058	Intron	Mwa I	[53,54,58,67]
		R: ACGTTGGATGCCCACAACAAGGAATGCAAG				
G/C	rs3764879	F: GTGTGTGTCTGATTTGGGTTG	chrX:12924697	Intron	*Bfal*	[53,54,58,67]
		R: TTTCTAGGCTCACACCATTTG				
C/T	rs5744080	F: ACGTTGGATGGCTGACAAATTTGGAGTTGC	chrX:12937804	Exon 2	Hind III	[53,54,58,67]
		R: ACGTTGGATGGTAGGGAGCTTGGCAGTTTG				
A/G	rs3764880	F: ACGTTGGATGGCTAAAGAAATAGAAGTGGC	chrX:12924826	Exon 1	Hpa I	[53,54,58,67]
		R: ACGTTGGATGCTGCTGCAAGTTACGGAATG				
C/T	rs17256081	F: ACGTTGGATGGCACTAAAATTTTACAATGC	chrX:12926045	Intron	Alu I	[53,54,58,67]
		R: ACGTTGGATGATGTGTCATTGGCCCAGTTG				

(*Continued*)

Table 2 (Continued).

Gene(s)	Rs Number	Primer Sequence(s) (5'→-3')	Chromosome Position	Gene Location	Restriction Enzymes	Reference
C/T	rs5741883	F: ACGTTGGATGAAGCGAGCATCTTTCTCCTG	chrX:12924221	Near-gene-5	Hae III	[53,54,58,67]
		R: ACGTTGGATGACAATGAACACTCATTGAGC				
G/C	rs2407992	F: ACGTTGGATGGACTCGCTGGCAAATTAAGG	chrX:12939112	Exon 2	Apo I	[53,54,58,67]
		R: ACGTTGGATGGACACGTCTGGATTTATCCC				
G/A	rs178998	F: ACGTTGGATGCCTAAACTGTATGCATTGCC	chrX:12917787	Near-gene-5	Hinc II	[53,54,58,67]
		R" ACGTTGGATGTGGGCCTTCCTGTAGAAGAC				
TLR 9						
A/G	rs5743836	F: GAGGGTGACATGGGAGCAGAGA	chr3:52260782	Promoter	Mva I	[53,54,58,67]
		R:CTGTGTAGCCCCTGGGCATTCT				
G/A	rs164637	F:CCCTGCAGGATGACCTCTCTTT	chr3:52265215	Exon 2	*BstNI*	[53,54,58,67]
		R:AGGCACCCACCATCTGGATGTA				
T/C	rs352139	F:CTGTGGACATCGATATCGGTGT	chr3:52258372	Intron	Hea II	[53,54,58,67]
		R:AAGCTTCGCTGCGGCAGAAACCCTGT				
C/T	rs352140	F:TCTAGACATCATGCTGGCCATGACC	chr3:52256697	Intron	BstU I	[53,54,58,67]
		R: CAGAGCCACTCAACAGTGGACT				
T/C	rs352143	F: CCCTGCAGGATGACCTCTCTTT	chr3:52264907	Exon 2	Nco1	[53,54,58,67]
		R: AGGCACCCACCATCTGGATGTA				
T/C	rs352162	F: CCCTGCAGGATGACCTCTCTTT	chr3:52252969	Intron	Bgl II	[53,54,58,67]
		R: AGGCACCCACCATCTGGATGTA				
A/G	rs352165	F: CCCTGCAGGATGACCTCTCTTT	chr3:52242902	Intron	*Afl II*	[53,54,58,67]
		R: AGGCACCCACCATCTGGATGTA				
T/C	rs352167	F: CCCTGCAGGATGACCTCTCTTT	chr3:52238656	Intron	BspTI	[53,54,58,67]
		R: AGGCACCCACCATCTGGATGTA				
A/G	rs187084	F:CTGTGGACATCGATATCGGTGT	chr3:52261031	Promoter	Afl II	[53,54,58,67]
		R:AAGCTTCGCTGCGGCAGAAACCCTGT				
TLR 10						
A/G	rs4129009	F:CCAGAATGAGTGGTGCCATT	chr4:38773268	Exon 4	Vsp I	[53,54,58,67]
		R: GTATGTGGTCCCCAACTTCCC				
A/G	rs7694115	F: ACGTTGGATGCTATAGGTTGCCTCAAACAG	chr4:38779094	intron	Ava I	[53,54,58,67]
		R:ACGTTGGATGCAGCAGAAGATTTAGAGTCC				
A/C	rs10856839	F: ACGTTGGATGGTTTCTGATGAGTCTCATTG	chr4:38777236	Exon 4	Acc I	[53,54,58,67]
		R: ACGTTGGATGGGGTTTTGAGCTCATCTTC				
T/A	rs11466645	F: GATGAGGAAATTGAAGGATCT3′	chr4:38778203	Exon 4	Xba I	[53,54,58,67]
		R: CACTGCTACTTCCCCAGTGC3′				

(*Continued*)

Table 2 (Continued).

Gene(s)	Rs Number	Primer Sequence(s) (5'→-3')	Chromosome Position	Gene Location	Restriction Enzymes	Reference
G/A	rs4274855	F: ACGTTGGATGTCCTCTCTGAGAATCCTGAC	chr4:38777471	Exon 3	Xho I	[53,54,58,67]
		R: ACGTTGGATGTTGGCTGAGAAGTCTCCAAG				
A/C	rs11096955	F:ACGTTGGATGTGAGAGTTTTCAAGTGAGGC	chr4:38776107	Exon 4	Alu I	[53,54,58,67]
		R:ACGTTGGATGCCAATAATATCTTAACAGACG				
G/T	rs11096956	F:ACGTTGGATGCCTGACAATATCAAATGCAC	chr4:38776180	exon 4	Pst I	[53,54,58,67]
		R:ACGTTGGATGAATATTGGAATTTCGTAGG				
A/C	rs11096957	F: ACGTTGGATGTAAGCAATAGAACCGATGTC	chr4:38776491	Exon 4	Hind III	[53,54,58,67]
		R: ACGTTGGATGGGCAAAAGCCAATTTGTAAG				
G/A	rs7698870	F: ACGTTGGATGTACCACGGGAATGAACAGAG	chr4:38781459	Intron	Nco1	[53,54,58,67]
		R: ACGTTGGATGGATCTCTTCTGAATGACCTC				
T/C	rs10776483	F: ACGTTGGATGATGGAATGGGTTCCAGTAAG	chr4:38775040	Exon 4	Hae III	[53,54,58,67]
		R: ACGTTGGATGTGAATTCTACTTTGCCCACC				

Distribution of TLR1, TLR2, TLR4, TLR6, TLR8, TLR9 and TLR10 Genes Polymorphisms

To detect the possible deficiencies in TLR1; TLR2; TLR4; TLR6; TLR8; TLR9; and TLR10 production among tuberculosis patients, genotype (Table 3) and allele frequencies (Table 4) in the promoters of the genes encoding for TLR1, TLR2, TLR4, and

Table 3 Genotype Distributions and Hardy Weinberg Equilibrium in Sudanese Tuberculosis Patients and Healthy Controls

Genotyp	Tuberculosis Patients N=160(%)	HWE of Patient Population*	Control N=220(%)	HWE of Control Population*
TLR 1 rs5743604				
TT	65(40.62)	<0.01	70(31.82)	<0.01
TC	22(13.75)		52(23.63)	
CC	73(45.63)		98(44.55)	
TLR 1- rs5743611				
CC	122(76.25)	<0.01	193(87.72)	<0.01
CG	26(16.25)		19(8.64)	
GG	12(7.5)		8(3.64)	
TLR 1- rs5743618				
GG	148(92.5)	<0.01	201(91,36)	<0.01
GT	8(5.00)		17(7.73)	
TT	4(2.5)		2(0.91)	
TLR 1 rs76600635				
AA	152(95.00)	<0.01	190(86.36)	<0.01
AG	7(4.38)		30(13.64)	
GG	1(0.62)		0(0.00)	
TLR 1- rs5743551				
AA	130(81.25)	<0.01	197(89.55)	<0.01
AG	15(9.37)		18(8.18)	
GG	15(9.37)		5(2.27)	

(*Continued*)

Table 3 (Continued).

Genotyp	Tuberculosis Patients N=160(%)	HWE of Patient Population*	Control N=220(%)	HWE of Control Population*
TLR 1- rs5743557				
CC	60(37.5)		84(38.18)	
CT	71(44.38)	<0.01	70(31.82)	<0.01
TT	29(18.12)		66(30.00)	
TLR 1- rs5743565				
AA	120(75.00)		116(52.73)	
AG	26(16.25)	<0.01	65(29.54)	<0.01
GG	14(8.75)		39(17.73)	
TLR 1- rs5743566				
CC	135(84.38)	<0.01	218(99.09)	<0.01
CG	8(5.00)		2(0.91)	
GG	17(10.62)		0(0.00)	
TLR 1- rs5743580				
AA	79(49.38)	<0.01	81(36.82)	<0.01
AG	39(24.37)		44(20.00)	
GG	42(26.25)		95(43.18)	
TLR 1- rs5743594				
CC	70(43.75)	<0.01	76(34.55)	<0.01
CT	22(14.37)		47(21.36)	
TT	68(42.5)		97(44.09)	
TLR 1- rs4833095				
GG	128(80.00)	<0.01	198(90.00)	<0.01
GA	22(13.75)		18(8.18)	
AA	10(6.25)		4(1.82)	
TLR 1- rs5743595				
TT	122(76.25)	<0.01	212(96,36)	<0.01
TC	10(6.25)		8(3.64)	
CC	28(17.5)		0(0.00)	
TLR 1- rs5743596				
CC	75(46.87)	<0.01	72(32.72)	<0.01
CT	22(13.75)		50(22.73)	
TT	63(39.38)		98(44.55)	
TLR 2- rs1816702				
CC	124(77.5)	<0.01	190(86.36)	<0.01
CT	24(15.0)		22(10.00)	
TT	12(7.5)		8(3.64)	
TLR 2- rs5743704				
CC	144(90.0)		212(96,36)	
CA	12(7.5)	<0.01	8(3.64)	<0.01
AA	4(2.5)		0(0.00)	
TLR 2- rs5743708				
GG	154(96.25)	<0.01	197(89.55)	<0.01
GA	4(2.5)		23(10.45)	
AA	2(1.25)		0(0.00)	
TLR 2- rs7656411				
TT	128(80.00)	<0.01	200(90.91)	<0.01
TC	16 (10.00)		20(9.09)	
CC	16(10.00)		0(0.00)	

(*Continued*)

Table 3 (Continued).

Genotyp	Tuberculosis Patients N=160(%)	HWE of Patient Population*	Control N=220(%)	HWE of Control Population*
TLR 2- rs11938228				
CC	56(35.00)	<0.01	75(34.09)	<0.01
CT	75(46.88)		78(35.45)	
TT	29(18.12)		67(30.45)	
TLR 2- rs893629				
GG	118(73.75)	<0.01	104(47.27)	<0.01
GA	26(16.25)		70(31.82)	
AA	16(10.00)		46(20.91)	
TLR 2- rs1898830				
AA	150(93.75)		216(98.18)	
AG	3(1.88)	<0.01	4(1.82)	<0.01
GG	7(4.37)		0(0.00)	
TLR 2- rs121917864				
CC	74(46.25)	<0.01	76(34.55)	<0.01
CT	46(28.75)		47(21.36)	
TT	40(25.0)		97(44.09)	
TLR 2- rs4696480				
TT	68(42.50)	<0.01	75(34.09)	<0.01
TA	26(16.25)		47(21.36)	
AA	66(41.25)		98(44.55)	
TLR 2- rs3804099				
TT	124(77.5)	<0.01	194(88.18)	<0.01
TC	24(15.0)		20(9.09)	
CC	12(7.5)		6(2.73)	
TLR 2- rs5743699				
TT	144(90.0)	<0.01	216(98.18)	<0.01
TC	10(6.25)		4(1.82)	
CC	6(3.75)		0(0.00)	
TLR 2- rs3804100				
TT	66(41.25)	<0.01	80(36.36)	<0.01
TC	26(16.25)		48(21.82)	
CC	68(42.5)		92(41.82)	
TLR 4 - rs7869402				
CC	128(80.00)	<0.01	190(86.36)	<0.01
CT	22(13.75)		22(10.00)	
TT	10(6.25)		8(3.64)	
TLR 4 - rs1927907				
GG	144(90.00)	<0.01	210(95,45)	<0.01
GA	12(7.5)		10(4.55)	
AA	4(2.5)		0(0.00)	
TLR 4 - rs1927911				
CC	70(43.75)		74(33.63)	
CT	22(13.75)	<0.01	48(21.82)	<0.01
TT	68(42.5)		98(44.55)	
TLR 4 - rs1927914				
TT	126(78.75)	<0.01	188(85.45)	<0.01
TC	22(13.75)		20(9.09)	
CC	12(7.5)		12(5.45)	

(*Continued*)

Table 3 (Continued).

Genotyp	Tuberculosis Patients N=160(%)	HWE of Patient Population*	Control N=220(%)	HWE of Control Population*
TLR 4 - rs6478317				
AA	140(87.5)	<0.01	182(82.73)	<0.01
AG	16(10.0)		38(17.27)	
GG	4(2.5)		0(0.00)	
TLR 4 - rs55912718				
AA	152(95.0)	<0.01	190(86.36)	<0.01
AG	4(2.5)		30(13.64)	
GG	4(2.5)		0(0.00)	
TLR 4 - rs5030719				
GG	124(77.5)	<0.01	202(91.82)	<0.01
GA	20 (12.5)		18(8.18)	
AA	16(10.0)		0(0.00)	
TLR 4 - rs10759931				
CC	60(37.5)	<0.01	70(31.82)	<0.01
CT	73(45.63)		78(35.45)	
TT	27(16.87)		72(32.73)	
TLR 4 - rs10759933				
AA	122(76.25)	<0.01	108(49.09)	<0.01
AC	24(15.00)		68(30.91)	
CC	14(8.75)		44(20.00)	
TLR 4 - rs2770150				
TT	150(93.75)	<0.01	216(98.18)	<0.01
TC	3(1.88)		4(1.82)	
CC	7(4.37)		0(0.00)	
TLR 4 – rs1554973				
CC	74(46.25)	<0.01	76(34.55)	<0.01
CT	46(28.75)		48(21.82)	
TT	40(25.00)		96(43.63)	
TLR 4 - rs11536878				
CC	72(43.13)	<0.01	74(33.63)	<0.01
CA	40(14.37)		48(21.82)	
AA	48(42.5)		98(44.55)	
TLR 4 - rs11536879				
AA	120(75.00)	<0.01	192(87.27)	<0.01
AC	28(17.5)		22(10.00)	
CC	12(7.5)		6(2.73)	
TLR 4 - rs7873784				
GG	144(90.00)	<0.01	212(96,36)	<0.01
GC	12(7.5)		8(3.64)	
CC	4(2.5)		0(0.00)	
TLR 4 - rs11536889				
GG	70(43.75)	<0.01	74(33.63)	<0.01
GC	22(13.75)		48(21.82)	
CC	68(42.5)		98(44.55)	
TLR 4 - rs4986790				
AA	128(80.00)	<0.01	194(88.18)	<0.01
AG	20(12.5)		18(8.18)	
GG	12(7.5)		8(3.64)	

(*Continued*)

Table 3 (Continued).

Genotyp	Tuberculosis Patients N=160(%)	HWE of Patient Population*	Control N=220(%)	HWE of Control Population*
TLR 4 - rs4986791				
CC	70(43.75)	<0.01	90(40.91)	<0.01
CT	22(13.75)		44(20.00)	
TT	68(42.5)		86(39.09)	
TLR 4 - rs11536897				
GG	124(77.5)	<0.01	192(87.27)	<0.01
GA	20(12.5)		24(10.91)	
AA	16(10.0)		4(1.82)	
TLR 4 - rs11536898				
CC	152(95.00)	<0.01	216(98,18)	<0.01
CA	6(3.75)		4(1.82)	
AA	2(1.25)		0(0.00)	
TLR 6- rs3796508				
TT	146(91.25)	<0.01	202(91.82)	<0.01
TC	10(6.25)		16(7.27)	
CC	4(2.5)		2(0.91)	
TLR 6- rs5743810				
CC	130(81.25)	<0.01	203(92.73)	<0.01
CT	15 (9.37)		15(7.27)	
TT	15(9.37)		2(0.91)	
TLR 6- rs5743831				
TT	60(37.5)	<0.01	70(31.82)	<0.01
TC	73(45.62)		78(35.45)	
CC	27(16.88)		72(32.73)	
TLR 6- rs1039559				
TT	70(43.75)	<0.01	100(45.45)	<0.01
TC	24(15.00)		48(21.82)	
CC	66(41.25)		72(32.73)	
TLR 6- rs6531670				
TT	74(46.25)	<0.01	102(46.36)	<0.01
TC	22(13.75)		40(18.18)	
CC	64(40.00)		78(35.45)	
TLR 6- rs5743788				
CC	128(80.00)	<0.01	186(84.55)	<0.01
CG	20(12.5)		30(13.63)	
GG	12(7.5)		4(1.82)	
TLR 6- rs5743794				
CC	122(76.25)	<0.01	216(98,18)	<0.01
CT	36(22.50)		4(1.82)	
TT	2(1.25)		0(0.00)	
TLR 8- rs4830805				
GG	75(46.88)	<0.01	88(40.00)	<0.01
GA	22(13.75)		42(19.09)	
AA	63(39.37)		90(40.91)	
TLR 8- rs4830808				
CC	128(80.00)	<0.01	194(88.18)	<0.01
CT	22(13.75)		22(10.00)	
TT	10(6.25)		4(1.82)	

(*Continued*)

Table 3 (Continued).

Genotyp	Tuberculosis Patients N=160(%)	HWE of Patient Population*	Control N=220(%)	HWE of Control Population*
TLR 8- rs3747414				
GG	150(93.75)	<0.01	216(98,18)	<0.01
GT	8(5.00)		4(1.82)	
TT	2(1.25)		0(0.00)	
TLR 8- rs3761624				
CC	146(91.25)	<0.01	200(90.91)	<0.01
CA	9(5.63)		20(9.09)	
AA	5(3.12)		0(0.00)	
TLR 8- rs1548731				
CC	131(81.88)	<0.01	204(92.73)	<0.01
CT	14 (8.75)		16(7.27)	
TT	15(9.37)		0(0.00)	
TLR 8- rs2109134				
AA	58(36.25)	<0.01	75(34.09)	<0.01
AT	73(45.63)		78(35.45)	
TT	29(18.12)		67(30.45)	
TLR 8- rs3788935				
AA	69(43.13)	<0.01	75(34.09)	<0.01
AG	23(14.37)		47(21.36)	
GG	68(42.5)		98(44.55)	
TLR 8- rs1013150				
GG	130(81.25)		204(92.73)	
AG	14 (8.75)	<0.01	16(7.27)	<0.01
AA	16(10.0)		0(0.00)	
TLR 8- rs5744068				
CC	52(32.5)	<0.01	80(36.36)	<0.01
CT	76(47.5)		73(33.18)	
TT	32(20.0)		67(30.45)	
TLR 8- rs3764879				
GG	80(50.0)	<0.01	70(31.82)	<0.01
GC	40(25.0)		60(27.27)	
CC	40(25.0)		90(40.91)	
TLR 8- rs5744080				
CC	70(43.75)	<0.01	83(37.73)	<0.01
CT	20(12.5)		47(21.36)	
TT	70(43.75)		90(40.91)	
TLR 8- rs3764880				
AA	155(96.87)	<0.01	218(99.09)	<0.01
AG	1(0.63)		2(0.91)	
GG	4(2.5)		0(0.00)	
TLR 8- rs17256081				
CC	86(53.75)	<0.01	210(95.45)	<0.01
CT	52(32.5)		10(4.55)	
TT	22(13.75)		0(0.00)	
TLR 8- rs5741883				
CC	75(46.88)	<0.01	216(98.2)	<0.01
CT	8(5.00)		4(1.8)	
TT	77(48.12)		0(0.0)	

(*Continued*)

Table 3 (Continued).

Genotyp	Tuberculosis Patients N=160(%)	HWE of Patient Population*	Control N=220(%)	HWE of Control Population*
TLR 8- rs2407992				
GG	136(85.00)	<0.01	170(77.27)	<0.01
GC	10(6.25)		40(18.18)	
CC	14(8.75)		10(4.55)	
TLR 8- rs178998				
GG	98(61.25)		198(90.00)	
GA	22(13.75)	<0.01	20(9.09)	<0.01
AA	40(25.00)		2(0.91)	
TLR 9- rs5743836				
AA	69(43.13)	<0.01	75(34.09)	<0.01
AG	23(14.37)		47(21.36)	
GG	68(42.5)		98(44.55)	
TLR 9- rs164637				
GG	126(78.75)	<0.01	192(87.27)	<0.01
GA	22(13.75)		20(9.09)	
AA	12(7.5)		8(3.64)	
TLR 9- rs352139				
TT	146(91.25)	<0.01	213(96,82)	<0.01
TC	10(6.25)		7(3.18)	
CC	4(2.5)		0(0.00)	
TLR 9- rs352140				
CC	155(96.88)	<0.01	197(89.55)	<0.01
CT	4(2.5)		23(10.45)	
TT	1(0.62)		0(0.00)	
TLR 9- rs352143				
TT	129(80.63)	<0.01	202(91.82)	<0.01
TC	15 (9.37)		18(8.18)	
CC	16(10.0)		0(0.00)	
TLR 9- rs352162				
TT	58(36.25)	<0.01	75(34.09)	<0.01
TC	73(45.63)		78(35.45)	
CC	29(18.12)		67(30.45)	
TLR 9- rs352165				
AA	120(75.00)		106(48.18)	
AG	26(16.25)	<0.01	70(31.82)	<0.01
GG	14(8.75)		44(20.00)	
TLR 9- rs352167				
TT	152(95.00)	<0.01	216(98.18)	<0.01
TC	1(0.63)		4(1.82)	
CC	7(4.37)		0(0.00)	
TLR 9- rs187084				
AA	76(47.5)	<0.01	74(33.64)	<0.01
AG	44(27.5)		49(22.27)	
GG	40(25.0)		97(44.09)	
TLR 10- rs4129009				
AA	75(46.88)	<0.01	88(40.00)	<0.01
AG	22(13.75)		42(19.09)	
GG	63(39.37)		90(40.91)	

(*Continued*)

Table 3 (Continued).

Genotyp	Tuberculosis Patients N=160(%)	HWE of Patient Population*	Control N=220(%)	HWE of Control Population*
TLR 10- rs7694115				
AA	128(80.00)	<0.01	194(88.18)	<0.01
AG	22(13.75)		22(10.00)	
GG	10(6.25)		4(1.82)	
TLR 10- rs10856839				
AA	150(93.75)	<0.01	216(98,18)	<0.01
AC	8(5.00)		4(1.82)	
CC	2(1.25)		0(0.00)	
TLR 10- rs11466645				
TT	146(91.25)	<0.01	200(90.91)	<0.01
TA	9(5.63)		20(9.09)	
AA	5(3.12)		0(0.00)	
TLR 10- rs4274855				
GG	131(81.88)	<0.01	204(92.73)	<0.01
GA	14 (8.75)		16(7.27)	
AA	15(9.37)		0(0.00)	
TLR 10- rs11096955				
AA	58(36.25)	<0.01	75(34.09)	<0.01
AC	73(45.63)		78(35.45)	
CC	29(18.12)		67(30.45)	
TLR 10- rs11096956				
GG	69(43.13)	<0.01	75(34.09)	<0.01
GT	23(14.37)		47(21.36)	
TT	68(42.5)		98(44.55)	
TLR 10- rs11096957				
AA	130(81.25)	<0.01	204(92.73)	<0.01
AC	14 (8.75)		16(7.27)	
CC	16(10.0)		0(0.00)	
TLR 10- rs7698870				
GG	52(32.5)	<0.01	80(36.36)	<0.01
GA	76(47.5)		73(33.18)	
AA	32(20.0)		67(30.45)	
TLR 10- rs10776483				
TT	80(50.0)	<0.01	70(31.82)	<0.01
TC	40(25.0)		60(27.27)	
CC	40(25.0)		90(40.91)	

Note: *HWE was performed with Pearson's χ^2 test.

TLR6 were determined. To determine if the SNPs reached Hardy-Weinberg equilibrium (HWE), the Pearson's χ^2 test was performed. It appeared that in the patients and control population, all genotype distributions were in Hardy-Weinberg disequilibrium.

To determine if there was an association between any of the studied SNPs and tuberculosis, the allele frequencies between the control population and the patient population were compared with the Fisher Exact test. It appeared that in the tuberculosis population the TLR1 (rs5743557, rs4833095, rs5743596), TLR2 (rs5743704, rs5743708, rs3804099), TLR4 (rs4986790, rs4986791), TLR6 (rs5743810), TLR8 (rs3764879, rs3764880), TLR9 (rs352165, rs352167, rs187084) and TLR10 (rs4129009) were significantly more often encountered ($p<0.0001$) (Table 4) than in the control population and were associated with tuberculosis in the

Table 4 Allele Frequencies of Tuberculosis Patients in Comparison to a Matching Healthy Control Population

Genotype	Tuberculosis Patients N=160(%)	Control N=220(%)	P-value for Association*	OR (95% CI)
TLR 1- rs5743604 T/C				
T-allele	152	192	0.352	1.03 (0.79–1.63)
C-allele	168	239		
TLR 1- rs5743611C/G				
C-allele	270	405	0.674	1.09 (0.75–1.45)
G-allele	50	35		
TLR 1- rs5743618G/T				
G-allele	304	419	0.456	1.18 (0.75–1.74)
T-allele	24	21		
TLR 1- rs76600635A/G				
A-allele	311	410	0.182	1.20 (0.80–1.26)
G-allele	9	30		
TLR 1- rs5743551A/G				
A-allele	275	412	0.472	1.26 (0.66–2.24)
G-allele	45	28		
TLR 1- rs5743557C/T				
C-allele	191	238	**<0.0001**	1.39 (0.77–2.40)
T-allele	129	202		
TLR 1- rs5743565A/G				
A-allele	266	297	0.672	1.33 (0.67–2.17)
G-allele	54	143		
TLR 1- rs5743566C/G				
C-allele	278	438	0.344	1.22 (0.85–1.45)
G-allele	42	2		
TLR 1- rs5743580A/G				
A-allele	197	206	0.455	1.15 (0.76–1.76)
G-allele	123	234		
TLR 1- rs5743594C/T				
C-allele	162	199	0.621	1.23 (0.78–1.65)
T-allele	158	241		
TLR 1- rs4833095 G/A				
G-allele	278	414	**<0.0001**	1.17 (0.77–1.89)
A-allele	42	26		
TLR 1- rs5743595 T/C				
T-allele	254	432	0.662	1.05 (0.64–1.78)
C-allele	66	8		
TLR 1- rs5743596 C/T				
C-allele	172	194	**<0.0001**	1.00 (0.54–1.45)
T-allele	148	246		
TLR 2- rs1816702C/T				
C-allele	272	402	0.705	1.57 (1.40–2.25)
T-allele	48	38		

(*Continued*)

Table 4 (Continued).

Genotype	Tuberculosis Patients N=160(%)	Control N=220(%)	P-value for Association*	OR (95% CI)
TLR 2- rs5743704C/A				
C-allele	300	432	**<0.0001**	1.15 (0.75–1.45)
A-allele	20	8		
TLR 2- rs5743708 G/A				
G-allele	312	417	**<0.0001**	1.23 (0.95–1.45)
A-allele	8	23		
TLR 2- rs7656411 T/C				
T-allele	272	420	0.234	1.97 (0.75–2.12)
C-allele	48	20		
TLR 2- rs11938228 C/T				
C-allele	187	228	0.648	1.93 (0.54–2.15)
T-allele	133	212		
TLR 2- rs893629 G/A				
G-allele	262	278	0.870	1.95 (0.65–2.19)
A-allele	58	162		
TLR 2- rs1898830 A/G				
A-allele	303	436	0.239	1.93 (0.58–2.98)
G-allele	17	4		
TLR 2- rs121917864 C/T				
C-allele	194	199	0.544	1.99 (0.68–2.45)
T-allele	126	241		
TLR 2- rs4696480 T/A				
T-allele	162	197	0.635	1.12 (0.76–1.45)
A-allele	158	243		
TLR 2- rs3804099 T/C				
T-allele	272	408	**<0.0001**	1.19 (0.87–1.57)
C-allele	48	32		
TLR 2- rs5743699 T/C				
T-allele	298	436	0.681	1.01 (0.90–1.35)
C-allele	22	4		
TLR 2- rs3804100 T/C				
T-allele	158	208	0.569	1.08 (0.65–1.19)
C-allele	162	232		
TLR 4 - rs7869402 C/T				
C-allele	278	402	0.528	1.90 (0.60–2.35)
T-allele	42	38		
TLR 4 - rs1927907 G/A				
G-allele	300	430	0.536	1.84 (0.63–2.12)
A-allele	20	10		
TLR 4 - rs1927911 C/T				
C-allele	162	196	0.255	1.02 (0.65–1.61)
T-allele	158	244		

(*Continued*)

Table 4 (Continued).

Genotype	Tuberculosis Patients N=160(%)	Control N=220(%)	P-value for Association*	OR (95% CI)
TLR 4 - rs1927914 T/C				
T-allele	274	396	0.225	1.98 (0.77–2.25)
C-allele	46	44		
TLR 4 - rs6478317 A/G				
A-allele	296	402	0.564	1.18 (1.03–1.33)
G-allele	24	38		
TLR 4 - rs55912718 A/G				
A-allele	308	410	0.587	1.06 (0.76–1.26)
G-allele	12	30		
TLR 4 - rs5030719 G/A				
G-allele	268	422	0.434	1.36 (0.72–2.55)
A-allele	52	18		
TLR 4 - rs10759931 C/T				
C-allele	193	218	0.737	1.96 (0.76–2.23)
T-allele	127	222		
TLR 4 - rs10759933 A/C				
A-allele	268	284	0.767	1.90 (0.77–2.19)
C-allele	52	156		
TLR 4 - rs2770150 T/C				
T-allele	303	436	0.307	1.12 (0.94–1.28)
C-allele	17	4		
TLR 4 – rs1554973 C/T				
C-allele	194	200	0.482	1.20 (0.99–2.23)
T-allele	126	240		
TLR 4 - rs11536878 C/A				
C-allele	184	196	0.881	1.58 (0.35–2.04)
A-allele	136	244		
TLR 4 - rs11536879 A/C				
A-allele	268	402	0.633	1.11 (0.95–2.25)
C-allele	52	34		
TLR 4 - rs7873784 G/C				
G-allele	300	432	0.566	1.14 (0.93–1.32)
C-allele	20	8		
TLR 4 - rs11536889 G/C				
G-allele	162	196	0.235	1.12 (0.60–2.07)
C-allele	158	244		
TLR 4 - rs4986790 A/G				
A-allele	276	406	**<0.0001**	1.31 (0.65–2.66)
G-allele	44	34		
TLR 4 - rs4986791 C/T				
C-allele	162	224	**<0.0001**	1.32 (0.88–1.187)
T-allele	158	216		

(*Continued*)

Table 4 (Continued).

Genotype	Tuberculosis Patients N=160(%)	Control N=220(%)	P-value for Association*	OR (95% CI)
TLR 4 - rs11536897 G/A				
G-allele	268	408	0.663	1.40 (0.86–2.23)
A-allele	52	32		
TLR 4 - rs11536898 C/A				
C-allele	310	436	0.773	1.37 (0.83–2.26)
A-allele	10	4		
TLR 6- rs3796508 T/C				
T-allele	302	420	0.235	1.54 (1.05–2.16)
C-allele	18	20		
TLR 6- rs5743810 C/T				
C-allele	275	421	**<0.0001**	1.19 (0.85–1.76)
T-allele	45	19		
TLR 6- rs5743831 T/C				
T-allele	193	218	0.698	1.22 (0.73–2.35)
C-allele	127	222		
TLR 6- rs1039559 T/C				
T-allele	164	248	0.623	1.11 (0.96–1.50)
C-allele	156	192		
TLR 6- rs6531670 T/C				
T-allele	170	244	0.690	1.09 (0.96–1.45)
C-allele	150	196		
TLR 6- rs5743788 C/G				
C-allele	276	402	0.543	1.23 (0.53–243)
G-allele	44	38		
TLR 6- rs5743794 C/T				
C-allele	280	436	0.598	1.18 (0.95–2.45)
T-allele	40	4		
TLR 8- rs4830805 G/A				
G-allele	172	218	0.530	1.92 (0.85–4.32)
A-allele	148	222		
TLR 8- rs4830808 C/T				
C-allele	278	410	0.827	1.08 (0.56–2.09)
T-allele	42	30		
TLR 8- rs3747414 G/T				
G-allele	308	436	0.517	1.56 (0.93–2.62)
T-allele	12	4		
TLR 8- rs3761624 C/A				
C-allele	301	420	1.000	1.90 (1.40–2.56)
A-allele	19	20		
TLR 8- rs1548731 C/T				
C-allele	276	424	0.629	1.07 (0.74–1.55)
T-allele	44	16		

(*Continued*)

Table 4 (Continued).

Genotype	Tuberculosis Patients N=160(%)	Control N=220(%)	P-value for Association*	OR (95% CI)
TLR 8- rs2109134 A/T				
A-allele	189	228	0.299	1.68 (1.07–2.65)
T-allele	131	212		
TLR 8- rs3788935 A/G				
A-allele	161	197	0.652	1.28 (0.74, 2.22)
G-allele	159	243		
TLR 8- rs1013150 G/A				
G-allele	274	424	0.851	1.32 (0.74, 2.38)
A-allele	46	16		
TLR 8- rs5744068 C/T				
C-allele	180	233	0.245	1.98 (1.45–2.60)
T-allele	140	207		
TLR 8- rs3764879 G/C				
G-allele	200	200	**<0.0001**	1.81 (1.31–2.44)
C-allele	120	240		
TLR 8- rs5744080 C/T				
C-allele	160	213	0.540	1.23 (0.67, 2.27)
T-allele	160	227		
TLR 8- rs3764880 A/G				
A-allele	311	438	**<0.0001**	1.43 (0.79–2.760)
G-allele	9	2		
TLR 8- rs17256081 C/T				
C-allele	224	430	1.000	1.10 (0.76, 1.60)
T-allele	96	10		
TLR 8- rs5741883 C/T				
C-allele	158	436	0.618	1.20 (0.92–1.57)
T-allele	162	4		
TLR 8- rs2407992 G/C				
G-allele	282	380	0.543	1.26 (0.97–1.65)
C-allele	38	60		
TLR 8- rs178998 G/A				
G-allele	218	416	0.833	1.15 (0.77, 1.70)
A-allele	102	24		
TLR 9- rs5743836 A/G				
A-allele	161	197	0.09	1.25 (0.96–1.66)
G-allele	159	243		
TLR 9- rs164637 G/A				
G-allele	274	404	0.06	(0.90–0.10)0.28
A-allele	46	36		
TLR 9- rs352139 T/C				
T-allele	302	433	0.26	1.46 (0.79–2.75)
C-allele	18	7		

(*Continued*)

Table 4 (Continued).

Genotype	Tuberculosis Patients N=160(%)	Control N=220(%)	P-value for Association*	OR (95% CI)
TLR 9- rs352140 C/T				
C-allele	314	417	0.34	1.90 (1.40–2.56)
T-allele	6	23		
TLR 9- rs352143 T/C				
T-allele	273	422	0.20	1.20 (0.92–1.57)
C-allele	47	18		
TLR 9- rs352162 T/C				
T-allele	189	228	0.25	1.26 (0.97–1.65)
C-allele	131	212		
TLR 9- rs352165 A/G				
A-allele	266	282	**<0.0001**	1.91 (1.41–2.57)
G -allele	54	158		
TLR 9- rs352167 T/C				
T-allele	305	436	**<0.0001**	1.47 (0.78–2.76)
C-allele	15	4		
TLR 9- rs187084 A/G				
A-allele	196	197	**<0.0001**	1.98 (1.45–2.60)
G-allele	124	243		
TLR 10- rs4129009 A/G				
A-allele	172	218	**<0.0001**	1.36 (1.01–1.81)
G-allele	148	222		
TLR 10- rs7694115 A/G				
A-allele	278	410	0.468	1.92 (1.19–3.12)
G-allele	42	30		
TLR 10- rs10856839 A/C				
A-allele	308	436	0.729	1.94(1.14–3.12)
C-allele	12	4		
TLR 10- rs11466645 T/A				
T-allele	301	420	0.154	1.13 (0.84–1.45)
A-allele	19	20		
TLR 10- rs4274855 G/A				
G-allele	276	424	0.370	1.52 (0.86–2.58)
A-allele	44	16		
TLR 10- rs11096955 A/C				
A-allele	189	228	0.084	1.36 (0.89–2.06)
C-allele	131	212		
TLR10- rs11096956 G/T				
G-allele	161	197	0.446	1.34 (0.79–2.28)
T-allele	159	243		
TLR10- rs11096957 A/C				
A-allele	274	424	0.275	1.58 (1.01–2.47)
C-allele	46	16		

(*Continued*)

Table 4 (Continued).

Genotype	Tuberculosis Patients N=160(%)	Control N=220(%)	P-value for Association*	OR (95% CI)
TLR 10- rs7698870 G/A				
G-allele	180	233	0.296	1.35 (0.77–2.38)
A-allele	140	207		
TLR10- rs10776483 T/C				
T-allele	200	200	0.543	1.47 (0.62–3.56)
C-allele	120	240		

Notes: *P-values are calculated with the Fisher exact test. Significant p-value (<0.05) are shown in bold.

Sudanese population (Table 4). For the other polymorphisms tested, no association with tuberculosis was found in the population tested (Table 4).

Discussion

Association Between TLR1, TLR2, TLR4, TLR6, TLR8, TLR9 and TLR10 Genes Polymorphisms and Tuberculosis

Tuberculosis is a complex disease in which environmental, immunological and genetic factors are contributed. It has been estimated that 10% of the infected population with *M. tuberculosis* may develop TB disease sometime in their life, suggesting that the majority of those infected are endowed with a protective immune response. Previous association studies have indicated a potential involvement of genetic variation within innate immune response genes as risk factors for TB. In particular, TLR1,[39] TLR2,[40–45] TLR4,[46] with TLR8[47] and TLR9[30] genetic variants have been associated with TB, suggesting that TLR-mediated responses may be important for protection to *M. tuberculosis* infection in humans.

TLRs are a family of PRRs consisting of 12 members in human and other mammals. TLRs play a crucial role in the recognition of *M. tuberculosis*, this immune activation occurs only in the presence of functional TLRs. Variants of TLRs may influence their expression, function and alters the recognition or signaling mechanism, which leads to the disease susceptibility.[48,49] The polymorphisms of TLRs have been hypothesized to affect the tuberculosis susceptibility. However, the direct evidence remains controversial.

Therefore, in the present study, we genotyped 86 SNPs including TLR1, TLR2, TLR4, TLR6, TLR8, TLR9 and TLR10 in the Sudanese tuberculosis patients to determine whether they are associated with susceptibility to TB in Sudanese tuberculosis patients.

Our results describes novel mutations in the TLR1, TLR2, TLR4, TLR6, TLR8, TLR9 and TLR10 genes and describes their association with pulmonary tuberculosis infection. Studies of the genetic factors involved in complex diseases have not yet provided clear explanations for the onset of such diseases, though they may help to identify the risk factor to get the infection.

Our results revealed that TLR1 rs5743557, TLR1 rs4833095, and TLR1 rs5743596 allele were more frequently found in the patients population compared to the healthy controls population. The association of the TLR1 rs5743557, TLR1 rs4833095 and TLR1 rs5743596 allele with tuberculosis were also found in other populations originating from Caucasian, Indian population and South Asian for TLR1 rs4833095[32,50] and, East Asian Population for TLR1 rs5743557,[32] but not in populations originating from in North China[32] and African American subpopulation.[39] Further, in vitro experiments showed that the TLR1 rs4833095 is in strong linkage disequilibrium (LD) with TLR1 rs5743618 and determining how these two SNPs contribute to TB susceptibility has proven difficult. TLR1 rs5743618 -GT genotype was related to reduction in surface expression of TLR1 in monocytes and granulocytes. In addition, after stimulated by inactivated H37Rv, samples from children with the rs5743618-GT genotype showed a decreased production of TNF-α and CXCL10, invariable production of IL-6 and IL-8 and increased production of IL-10.[51] In Spanish population, rs5743618 G allele and GG genotype influenced the susceptibility to PTB;[33] in Han Chinese population, Ma et al

reported that rs5743618 was not associated with adult TB.[39] Zhang et al[32] conducted a meta-analysis on TLR1 rs5743618 and also found no association with TB susceptibility, which corresponds with our results.[32] Salie et al performed an association study of 23 polymorphisms in five TLR genes (TLR1, TLR2, TLR4, TLR8, and TLR9) in TB cases and healthy controls in a South African population. The study found that TLR1 rs5743618, TLR8 rs3764879, and TLR8 rs3764880 polymorphisms were associated with TB susceptibility in both sexes.[52]

Our results also revealed that TLR2 rs5743708, TLR2 rs3804099 were more frequently found in the patient population compared to the healthy control population. The association of the TLR2 rs5743708, with tuberculosis were also found in other populations originating from Asian and Hispanic populations[40,53,54] but not European subgroup.[52] Zhang et al[32] showed a significant association with TB susceptibility for the rs5743708 A allele and AA genotype across different ethnic groups in Asians and Europeans, but decreased risk in the Hispanic population.[32]

In contrast, the TLR2 rs5743708 and TLR2 rs5743704 were not associated with TB meningitis in an Indian population.[36,55] TLR2 rs3804099 was associated with susceptibility to TB meningitis rather than with susceptibility to pulmonary TB in a case-control study of a Chinese cohort.[56] Another study investigated possible associations of 16 polymorphisms of six TLR genes and TIRAP with TB susceptibility in a Chinese population. It found that TLR2 rs5743708, polymorphism was associated with pulmonary TB.[53]

Several studies have demonstrated critical role of TLR4 in *M. tuberculosis* recognition and verified necessity of these TLR for development of a protective response against *M. tuberculosis* infection.[41] Variants in TLR4, rs4986790 and rs4986791, were investigated for their association with susceptibility or resistance to pulmonary tuberculosis.[27,28,30,34,46]

The results of our study are consistent with the reports of Najmi et al in India[28] Ferwerda et al in Tanzania[46] and Pulido et al in Spain[57] but not similar to that reported previously in other Sudanese[31] and South India[58] Asian populations[34] European Caucasians,[34] North and South American[34,39], African populations[34,39], Gambian TB population[59], a south-eastern Chinese population,[60] Mexico,[35] and USA.[30]

It is known that for TLR6 rs5743810 SNP have a protective effect against TB development. The T allele was found by Shey et al[61] to reduce NF-kB signalling which led to an altered level of IL–6 production, while Randhawa et al[62] showed that it leads to increased IFN-γ production and thus protection against *M. tuberculosis*. These functional studies correlate with the results found in this meta-analysis as well as that of Zhang et al[32] where the T allele and TT genotype was also associated with resistance to TB disease.

The TLR8 polymorphism plays a significant part in the immune response in regulating the induction of interferon (IFN) and inflammatory cytokines.[47,52] Our results revealed that TLR8 rs3764879 and rs3764880 allele were more frequently found in the patients population compared to the healthy controls population. The association of the TLR8 rs3764879 and rs3764880 alleles with tuberculosis were also found in other populations originating from Russian and Indonesian populations.[47] Furthermore, associations have also been found in Turkish male children,[63] Pakistan population,[64] and South African population.[52] However, neither Kobayashi et al[27] nor Chimusa et al[65] showed any association between rs3764880 and TB susceptibility.

Ethnic differences in TLR polymorphisms may in part reflect the ethnic diversity of host TB susceptibility. Davila et al found that around 30% of the Indonesian male subjects carried the A allele (rs3764880) associated with risk for TB, whereas this same allele was present in 78% of Russian patients.[47] 34.3% of the Turkish male children with pulmonary TB had the A allele associated with risk for TB, which is similar to Indonesian population.[63] The rs3764880 polymorphism of TLR8 was observed more than 16% among the healthy Chinese adult population by Cheng et al.[66]

The gene of TLR9 is located on chromosome 3p21.3. The total length of TLR9 gene is approximate 5 kb. Its coding gene has two exons, and the major coding region is in the second exon.[53,67] TLR9 is an intracellular pathogen recognition receptor (PRR) that recognizes non-methylated cytosine-phosphate-guanine (CpG) motifs in bacterial DNA.[53,67] Based on NCBI SNP database, twelve SNPs have been identified for TLR9 gene. Studies have indicated certain race population with special genotype of TLR9 polymorphism might have higher risk for TB. Our results revealed that TLR9 rs352165, rs352167 and rs187084 allele were more frequently found in the patients population compared to the healthy controls population. Sanchez et al found that TLR9 rs352165, rs352167 were not associated with TB risk in a Colombian population.[37]

In previous study, a meta-analysis was performed to assess the association between seven extensively studied TLR9 polymorphisms (rs187084, rs352165, rs5743836, rs5743842, rs352139, rs352140 and rs352167) and TB risk. The analysis revealed an association between certain TLR9 polymorphism and TB risk. The studies included Indians, Iranian and West African, Indonesians, Vietnamese, Chinese and Mexicans. The results showed that rs187084 and rs5743836 polymorphisms were not associated with TB risk, while the association between rs352139 polymorphism and TB risk may vary by race.[68,69]

Bharti et al found that rs187084 locus may be associated with susceptibility to TB in Indian population.[69] The SNP rs187084 and rs5743836 SNPs located in the promoter are the most important and have been associated with various inflammatory diseases located in the promoter of TLR9 gene.[70–72] Previous functional analyses have shown that both rs187084 and rs5743836 SNPs influence the transcription of TLR9 by regulation of promoter activity.[70,72,73] Some studies found that the rs187084 in TLR9 showed no association with TB in Vietnam and Iran.[74,75]

Our result revealed that rs5743836, rs164637, rs352139, rs352140, rs352143, and rs352162 allele may not be risk factors for susceptibility to pulmonary tuberculosis in Sudanese populations. On the other hands rs352139 has been strongly associated with susceptibility to TB in Indonesian[72] and Vietnamese populations,[27,73] African-Americans and in Mexican Amerindians but not with Caucasians and African patients from Guinea-Bissau.[30] Sanders et al found that TLR9 rs5743836 and TLR9 rs352140 alleles have protective effect against meningococcal meningitis in Dutch children.[76]

The rs5743836 in TLR9 showed a strong association with tuberculosis in African-Americans and Caucasians,[31] while the association was not found in Vietnam[75] or Mexico population[74] and Chinese population.[53] Different variants of TLR9 rs352140 polymorphisms are described in the genome database[38] and in in the Asian population, in Japan.[77]

TLR9 rs352142 polymorphism was positively associated with meningeal TB, while variant *TWF2* rs352143 was associated with pulmonary TB in a Vietnamese cohort.[75]

Recently, a number of studies indicated that TLR10 serves as a modulatory pattern-recognition receptor with mainly inhibitory properties on TLR2-derived immune responses, which are involved in the progression of TB.[39,40] Our result revealed that TLR10 rs4129009 that was associated with TB susceptibility in Sudanese tuberculosis patients. Various studies found that single-nucleotide polymorphisms in TLR10 were associated with susceptibility to tuberculosis in different ethnic groups. Ma et al demonstrated that polymorphisms of TLR10 were significantly associated with TB in African and European Americans.[39]

Bulat-Kardum et al found that the rs11096957 AA genotype was associated with a predisposition to TB in the Caucasian population.[78] However, in the present study, we did not observe association between rs11096957 and risk of TB, no association was also found in Han Chinese population.[79] This inconsistent result is likely due to the ethnic difference.

There are some limitations in our study which need to be mentioned. The sample size was relatively limited and the methods were limited to PCR-RFLP. Moreover, some genotypes and alleles of SNPs were found to have low frequency in the studied population which may limit the statistical power. Moreover, we did not correlate the TLR SNPs with demographic characteristics or with others clinical parameters. Moreover, functional exploration inferring these candidate SNPs should be conducted further to confirm our findings. With these limitations, the study provides evidence for the association of SNPs present in TLR1, TLR2, TLR4, TLR6, TLR8, TLR9 and TLR10 genes with pulmonary tuberculosis. The presence of associated SNPs with pulmonary tuberculosis may give us the clue that it may provide susceptibility to pulmonary tuberculosis.

In conclusion, since TLRs are the key modulator of inflammatory processes, the associated specific TLRs with PTB may be a potential target for attenuation of specific TLR pathways to reduce the severity of the disease and testing for TLR SNPs may be helpful for early prediction of the course of the disease and early identification of patients who at risk. This the first published results that studies 86 SNPs and their association to pulmonary tuberculosis. Future studies are warranted in a larger sample to replicate our findings and understand the mechanism of association of TLR polymorphism in PTB.

Abbreviations

TLRs, Toll-like Receptors; PCR-RFLP, polymerase chain reaction and restriction fragment length polymorphism; SNPs, single nucleotide polymorphism; HWE, Hardy-Weinberg equilibrium.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

The present study was approved by the Ethics Committee of University of Khartoum, Khartoum, Sudan (5/2018).This study adheres to the Declaration of Helsinki (1964). Written informed consents were obtained from all participants in the study or legally responsible guardians for participants less than 18 years old.

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Disclosure

The authors declare that they have no competing interests in this work.

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