Interleukin 17A and F Single Nucleotide Polymorphisms with Tuberculosis in Sudanese Population

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Abbreviations used in this paper: IL-17, Interleukin-17;PCR-RFLP, polymerase chain reaction and restriction fragment length polymorphism; SNPs, single nucleotide polymorphism; HWE, Hardy-Weinberg equilibrium.

Abstract - Background: Several lines of evidence had suggested the importance of host genetic factors in TB susceptibility. Polymorphisms in genes encoding for cytokines usually influence the efficiency of the immune response to infection and are associated with disease susceptibility and progression. Therefore ,we aims to describe the first association between the IL-17A G-197A (rs227593) and IL-17F A7488G (His161Arg, rs763780) gene SNPs and susceptibility to tuberculosis 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 the IL-17A G-197A (rs227593) and IL-17F A7488G (His161Arg, rs763780) gene SNPs and susceptibility to tuberculosis disease in Sudanese population using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). Result: From our results it appeared that significant differences in allele distribution was demonstrated for IL-17A G-197A (rs227593)in tuberculosis patients(p<0.0001) compared to healthy controls but no significant IL-17F A7488G (His161Arg, rs763780) gene SNPs in tuberculosis patients (p=0.3). Conclusion: This indicates that the genotypes obtained for IL-17A G-197A have a significant role in the genetic susceptibility to development tuberculosis in Sudanese population .

Keywords: Tuberculosis, cytokine, IL-17A, IL-17F, and PCR-RFLP.

I. INTRODUCTION

Tuberculosis(TB), caused by *Mycobacterium tuberculosis*(*M. tuberculosis*),is an infectious disease with a high morbidity and mortality in humans [1] .It kills approximately 2 million people and 5.7 million of new TB cases are reported worldwide annually[2].

Genetic factors are important contributors to the development of a wide range of complex disease. 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 [3].

Several lines of evidence had suggested the importance of host genetic factors in TB susceptibility including the most striking example of apopulation acquiring resistance to TB occurred in the population of Qu' Appelle Indians[4] , twin studies [5], genome-wide linkage studies [6-10], and recently published genome-wide association studies (GWAS) [11,12].

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.

Polymorphisms in several cytokine genes have been shown to be involved in the susceptibility, the severity and clinical outcomes of TB in humans [13, 14].

The interleukin (IL)-17 cytokine family includes several cytokines among which IL-17A and IL-17F are pro-inflammatory cytokine being important to the innate and adaptive immune responses [15-18]. Several studies were found increased expression of IL-17A in different tumor tissues, including prostate cancer, colorectal cancer, breast cancer and gastric cancer[26-29]. Recent studies of IL-17A cytokine has revealed its important role in protective mechanisms against infectious diseases [15, 16, 19] and in studies of BCG-induced immunity [20,21],has also been play critical roles with TNF in the first steps of TB and granuloma formation [15-17].

In the other hand, IL-17F was discovered to share strongest similarity to IL-17A. IL-17F was reported to induce the expression of various cytokines, chemokines and adhesion molecules by human airway epithelial cells, vein endothelial cells and fibroblasts [30-31].

Few studies have investigated the importance ofgenetic factors such as single nucleotide polymorphisms (SNPs) in IL17Aand IL-17F genesand TB outcome [22-24]. The most commonly SNPs described for IL17A was -197 G /A (rs2275913) and for IL-17F was +7488 A /G(His161Arg,rs763780) alleles were be associated with high IL-17 production [22-25].

Du *et al.* observed that the rs763780-CC polymorphisms of the IL-17F gene were more likely to have an increased risk [22] .Ocejo-Vinyals*et al.* investigated the IL-17A rs2275913 polymorphisms and suggested that the GG genotype was related to an increased risk of tuberculosis [24]. Shi *et al.* genotyped rs2275913 and rs3748067 in IL-17A and rs763780 in IL-17F and found that the CC genotype of rs763780 was associated with an increased risk of tuberculosis [23]. Peng*et al.* conducted a study in a Chinese population and found that those carrying the CT/TT genotype of rs763780 were more susceptible to tuberculosis, but no significant association was found for rs2275913[25] .Therefore in this study we aims to describe the first association of these expression SNPs in a sub-Saharan Sudanese tuberculosis patients .

II. MATERIALS AND METHODS

Study population

A prospective, cross sectional, case–control study was carried out during the period between 2015 and 2016 atAbu-AngahHospital , 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). The present study was approved by the Ethics Committee of University of Khartoum, Khartoum, Sudan. Written informed consents were obtained from all participants in the study. 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 Cassete test , Clinotech Diagnostics & Pharmaceuticals, Canada). Blood samples were stored at -20˚C until use.

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 IL17-A and IL-17 F genes were detected by PCR followed by restriction enzyme fragment analysis (PCR-RFLP). All PCR primers and restriction enzymes 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 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).

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 in the control population with Pearson's $\chi 2$ test. The effect of the IL17-A and IL-17 Fpolymorphisms 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.

III. 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.

Distribution of IL17-A and IL-17 F gene polymorphisms:

To detect the possible deficiencies in IL-17A and IL-17F production among tuberculosis patients, genotype (table 3) and allele frequencies (table 4) in the promoters of the genes encoding for IL-17A andIL-17F were determined. To determine if the SNPs reached Hardy-Weinberg equilibrium (HWE), the Pearson's $\chi 2$ test was performed. It appeared that in the control population, the genotype distributions for the IL-17A -197G/AandIL-17 F+7488A/GSNPs reached Hardy-Weinberg equilibrium (HWE) (table 3). In the patient 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 IL-17A -197G/A was significantly more often encountered ($p<0.0001$) (Table 4) than in the control population .Furthermore, no association with tuberculosis was found in the population tested for IL-17 F+7488A/G ($p=0.3$) (Table 4).

IV. DISCUSSION

Association between IL17-A and IL-17 F gene polymorphisms and tuberculosis

IL-17 is a pro-inflammatory cytokine family containing six isoforms (IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F) [33]. Produced by the T helper 17 (Th17) subsets of CD4+ T cells, IL-17 plays an important role in the development and progression of inflammatory and autoimmune diseases [26-29].Recent studies of IL-17A and IL-17 F cytokines has revealed its important role in protective mechanisms against infectious diseases [15, 16, 19]

In this study we analyzed two single-nucleotide polymorphisms (SNPs) within the IL-17A and IL-17F genes including the G-197A (rs227593) and A7488G (His161Arg, rs763780), respectively, and TB susceptibility in Sudanese population. Our results revealed that -197A allele was more frequently found in the patient population compared to the healthy control population. The association of the -197A allele with tuberculosis was also found in other populations originating from Southern Brazil [41] and Northern Spain [24], but not in populations originating from Croatian [34] and China [25]. No association for the IL-17 F+7488A/G polymorphisms was found in our Sudanese population.This SNP was associated with TB in a Chinese Han population who had the genotype AG/AA of IL-17F A7488G (His161Arg, rs763780) gene SNP were more susceptible to acquiring tuberculosis, compared to the GG genotype [25] .The IL-17A -197G/A variant was previously reported to elevate promoter activity and increases IL-17A protein expression [35-38]. Therefore, the overproduction of IL-17Acould be leading to pathological conditions. The ability of IL-17A to induce neutrophils migration , activation of bronchial fibroblasts, epithelial cells, and smooth muscle cells and in inducing the secretion of cytokines and chemokines, which in concert leads to the accumulation of neutrophils with proteolytic enzymes that may burden the airway.[39, 40] and contributing in formation of mature granuloma and blocking disease progression [39, 40].In conclusion our result indicates that the genotypes obtained for IL-17A G-197A have a significant role in the genetic susceptibility to development tuberculosis in Sudanese population .

Conflict of interest

The authors declare no conflict of interest.

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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)**	
Gender (male/female)		**111/49**	**65/155**	**0.0041**
Occupation	**Governmental employee**	**22 (13.8)**	**21 (9.55%)**	
	Workers	**63 (39.4%)**	**44 (20%)**	
	Other job	**40 (25%)**	**40 (18.3%)**	
	Jobless	**3 (1.8%)**	**13 (5.9%)**	
	Housewife	**13 (8.1%)**	**17 (7.7%)**	
	Student	**19 (11.9%)**	**85 (38.6%)**	
BCG vaccination		**112 (70.6%)**	**212 (96.8%)**	**0.923**
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**	

Table 2: PCR primers and restriction enzymes for genotyping the different Single Nucleotide Polymorphisms

Gene(s)	Rs number	Primer sequence(s) (5'→-3')	Restriction endonuclease	Allele	Length (bp[1])	Reference
IL-17A						
-197G/A	rs2275913	FAACAAGTAAGAATGAAAAGAGGACATGGT	XagI	G	68 + 34	[26]
		R CCCCCAATGAGGTCATAGAAGAATC		A	102	
IL-17F						
+7488A/G	rs 763780	F ACCAAGGCTGCTCTGTTTCT	NlaIII	A	63 + 80	[26]
		R GGTAAGGAGTGGCATTTCTA		G	143	

[1] bp: number of base pairs

Table 3: Genotype distributions and Hardy Weinberg Equilibrium in Sudanese tuberculosis patients and healthy controls

Genotype	Tuberculosis patients N=160(%)	HWE of patient population*	Control N=220(%)	HWE of control population*
-197G/A GG AG AA	105(65.6) 3(1.8) 52(32.5)	<0.01	115 (52.3) 70(31.8) 35(15.9)	<0.01
+7488A/G AA AG GG	150(93.8) 10(6.2) 0(0.0)	<0.01	216(98.2) 4(1.8) 0(0.0)	<0.01

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)
-197G/A G-allele A-allele	213(50.2) 107(48.8)	300(44.4) 140(55.6)	**<0.0001**	1.91 (1.39-2.55)
+7488A/G A-allele G-allele	310(96.8) 10(3.2)	436 (99.0) 4 (1.0)	0.3	1.49 (0.76-2.74)

P-values are calculated with the Fisher exact test.