Interleukin-17A rs3819024 gene polymorphism in Iraqi Arab pulmonary tuberculosis patients

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Abstract
Pulmonary tuberculosis (PTB), a contagious, airborne infection occurs when M. tuberculosis primarily attacks the lungs. The aim of this study was to examine the association between IL-17A rs3819024genotypes and alleles with pulmonary tuberculosis susceptibility in Iraqi Arab population. From January to April 2017, 80 pulmonary tuberculosis (PTB) patients (MDR, RD and OC) were enrolled as patients group and 40 apparently healthy individuals as control group. Genotypes analysis of IL-17A rs3819024 was analyzed using quantitative real time-polymerase chain reaction (QRT-PCR).

The results revealed that the disease is more frequent in < 40 years (58%) than ≥ 40 years (42%) and in male (57%) than female (43%) with ratio of 1.3 : 1 in patient groups. Analysis of H-W equilibrium revealed that TB patient groups and control group were in a good agreement with the equilibrium and no significant variation between the observed and expected genotypes frequencies was observed. In RD-TBP patient group, AA genotype showed a significantly decreased frequency in patients (40% vs.75%, respectively; p=0.01) and the associated OR value of 0.22 with PF value of 0.58. Alleles A and G of IL-17Ars3819024 gene observed significantly differences in frequency between MDR and RD patients and control groups. At position rs3819024, IL-17 GG genotype showed a significant increased level of IL-17A (53.7 ± 0.98pg/ml) in OC- TBP patients group compared to AA genotype (44.87 ± 5.0pg/ml) and AG genotype (38.42 ± 7.1pg/ml) of the same group.

Keywords: Tuberculosis, IL-17Ars3819024 gene polymorphism, IL-17A serum levels, Odds ratio, QRT-PCR.

Introduction

Tuberculosis is an infectious disease in which the immune system plays an important role.[7] TB is the biggest killer infectious disease worldwide. Although the estimated incidence of TB has marginally declined over several years, it is out of control in some regions of world.[9] Even though DOTS strategy helps to reduce cost, accomplish powerful cure, and save the life of TB patients with observed patients as they take their medicine by healthcare workers, still patients need to spend for other investigations and other drugs.[19] Iraq has one of the highest rates of TB in the region with over 15,000 people affected in the country annually.[24]

The infection is caused by the bacterium *Mycobacterium tuberculosis*.[21,24] It is primarily a pulmonary infectious disease.[23] Cytokines represent an important part of the immune system that helps to differentiate tuberculosis infection from the non-infected state.[6] Cytokines play an essential role in the immune response to tuberculosis.[15] Interleukin 17 (IL-17) is an inflammatory cytokine[14] and plays a protective role against the infection.[8] It is induced during mycobacterial infection[13] which plays a crucial role in the causes of TB.[10] IL-17 has suggested a significant role in the first step of TB and granuloma formation.[22] Candidate gene approach and association studies have identified various host genetic factors that affect TB susceptibility, especially those genes that control immunological functions.[16]

Several studies have reported the relationship between the polymorphism of IL-17A and IL-17F genes with different inflammatory diseases in different population[3,11,21] and these polymorphisms play a crucial role in tuberculosis diseases.[17] A number of cytokine gene polymorphisms have shown associations with altered serum levels of cytokines, and various ethnic populations have shown different associations of various cytokine gene variants with susceptibility or resistance to TB.[1] The IL-17A gene polymorphism is associated with the susceptibility to various inflammatory diseases [11] and it is most sensitive to TB disease .[4] This study aimed to assess the link between IL-17A gene polymorphisms and TB development.

Material and Methods

Method: A total of 120 Iraqi Arabs individuals were enrolled in these studies; 80 patients with active tuberculosis (TB) and without HIV infection were diagnosed at National Reference Laboratory of Tuberculosis/ Baghdad for the period from January to April 2017 by specialized doctors. Patients were divided into three groups, MDR (multidrug resistant), RD (Recently diagnosed) and OC (Old recurrent cases). As well as,40 apparently healthy individuals were selected as a control group. All patients and control groups ages ranged between 16-68 years old. Five ml of venous blood samples were collected from both patients and control groups, and it was divided into two tubes; 2ml of whole blood sample was dispensed in the first tube containing ethylene diamine tetra acetic acid (EDTA), mixed gently and then stored by keeping in the freezer at -20^{o}C until further processing for DNA extraction. 3ml of whole blood

dispensed in the second tube (plane tube) were serum isolated by centrifuge at 3000 rpm for 10 min.

IL-17A Serum levels: Serum levels of IL-17A from TB patients and control groups were measured by using a commercial ELISA kit (Human IL-17A, Biosource, USA).

IL-17A rs3819024 Genotyping: DNA samples were prepared from whole blood by using Genomic DNA Extraction Kit (Promega, USA) following the manufacturer's instructions, and checking the concentrations and purity by using Nanodrop Software (Bioneer /korea) at 260 /280 nm.

The IL-17Ars3819024 A>G SNP was genotyped by using QRT-PCR with the following primers: forward; 5′-CCGGAATTGTCTCCACAACAC-3`; reverse 5′-GTACCTTGATTTTCCATTTGATCTT-3` and probes sequence: forward, G:FAM- AATCTGTGAGGGAAAG-MGB, reverse; A: HEX- AGGAATCTGTGAGGAAA –MGB. The primers and probes used in this study were obtained from Macrogen, Korea. Amplification was performed under the following conditions: an initial denaturation step at 95 º C for 4 min followed by 45 cycles at 95 º C for 15s, 60 º C for 60 s, 60 º C for 60 s,60 º C for 60s and a final extension at 72 º C for 7 min.

Statistical analysis: All statistical analysis was performed using Statistical Package for Social Science (SPSS) program version 17 for windows (SPSS INC., Chicago IL, USA). Results were expressed as mean ± SD. Comparisons between two groups were performed using T test for categorical data. P values of <0.05 were considered to indicate statistical significance. Genotypes of IL-17A rs3819024 was presented as percentage frequencies and significant differences between their distributions in pulmonary tuberculosis patients and controls were assessed by two-tailed Fisher's exact probability (P).

In addition, odds ratio (OR), etiological fraction (EF) and preventive fraction (PF) were also estimated to define the association between a genotype with the disease. These estimations were calculated by using the latest version of the WINPEPI package (including the programs and their manuals) available free online at http://www.brixtonhealth.com. Allele frequencies of genes were calculated by direct gene counting methods, while a significant departure from Hardy-Weinberg (H-W) equilibrium was estimated using H-W calculator for two alleles which is available free online at http://www.had2know.com/academics/hardy-weinbergequilibrium-calculator-3-ale;es.html.

Results and Discussion

Demographic characteristics of the study groups: Patients' age was distributed into two age groups (< 40 years and ≥ 40 years). The results revealed that the disease is more frequent in < 40 years (58%) than ≥ 40 years (42%). In addition, tuberculosis disease is more frequent in male (57%) than female (43%) with ratio of 1.3 : 1 in patient groups (table 1).

Genotypic and allelic frequencies of the IL-17A rs3819024 SNP in TB and control populations: Analysis of H-W equilibrium revealed that TB patient groups and control group were in good agreement with the equilibrium, and no significant variation between the observed and expected genotypes frequencies was observed as shown in table 2.

Comparing TB patient groups to control group revealed that in MDR group, none of genotypes showed significant differences between patients and control, while alleles *A* and *G* observed to have significant frequency differences between them; *A* alleles have significantly decreased frequency (70% *vs.* 85%, respectively; p= 0.036), with OR value of 0.41 and PF value 0.5 and *G* have significantly increased frequency (30% *vs.* 15%, respectively; p= 0.036), with OR value of 2.43 and EF value 0.17.

In RD patient group, AG and GG genotypes showed insignificant difference between patients and control, while AA genotype showed a significantly decreased frequency in patients (40% *vs.*75%, respectively; p=0.01), and the associated OR value of 0.22 with PF value of 0.58.

Alleles frequency showed significant decrease for *A* allele in patients (63% *vs.* 85%; p= 0.01) with OR value of 0.29 and PF value of 0.60. *G* allele showed significant increase in patient than control (38% *vs.* 15%; p= 0.01) with OR value of 3.4 and EF value of 0.26. The OC patient group showed none of the genotypes or alleles have a significant difference between patients and controls (table 3). The results are indicated that genotypes or alleles of *IL-17A* rs3819024 gene were either associated with an increased risk to promote TB infection in Iraqi Arab population or protection against them, and the recorded OR, EF and PF values are in favor of such conclusion. In Chinese population, IL-17A rs3819024 alleles and genotypes showed no association with the risk of TB development[23] which does not agree with the results of present study.

On the other hand, the association of this SNP (IL-17 A rs3819024) was studied with other diseases;[25] it was observed that the rs3819024-GG polymorphisms of *IL-17A* rs3819024 gene was more likely to have a decreased risk of gastric cancer in Chinese population and[18] genotyped IL-17A rs3819024 and found that the AG genotype was associated with an increased risk of gastric cardiac adenocarcinoma in Chinese population. These results and irrespective of their different observed associations, may highlight the role of IL-17A gene polymorphism in conferring susceptibility or resistant against the progression of TB infection and other investigated diseases.

The impact of IL-17A rs3819024 on the IL-17A serum level in PTB patients and control groups: In OC patients group, *IL-17* rs3819024GG genotype showed a significant increased level of IL-17A (53.7±0.98pg/ml) compared to AA genotype (44.87±5.0pg/ml) and AG genotype (38.42± 7.1pg/ml) of the same group and its percentage of the total sum of IL-17A was 10% while no such difference was observed MDR, RD patients and control groups (table 4 and figure 1).

The result show increased level of IL-17A serum level in GG genotype, that is it may be due to its exerted effect in the production of this interleukin in patient but not in control. The positive was associated allele G in progress of PTB infection in Iraqi Arabs population although of low frequency compared with A alleles in patients and control groups of this study suggested that IL-17 rs3819024 GG genotypes are risk factors of PTB, probably through up regulation the protein expression of IL-17A gene in patients but no controls groups. Furthermore, the present results are in line with other reports showing correlation of IL-17A production in TB with disease severity[12] and with an elevated bacterial burden.[2] Butov et al[5] proved the association between polymorphisms and circulating cytokine levels in patients with pulmonary TB.

Conclusion

The present study findings revealed a significant association between IL-17A rs3819024 AA genotype, *A* or *G* alleles with a risk of TB patients through its role in the susceptibility to tuberculosis infection or its impact in the production of IL-17A in the Iraqi Arabs population.

Table 1
Demographic characteristics of the study groups.

Percentage	Patients groups						Control group	
	MDR		RD		OC			
Age	< 40 years	≥ 40 years	< 40 years	≥ 40 years	< 40 years	≥ 40 years	< 40 years	≥ 40 years
	50%	50%	65%	35%	60%	40%	70%	30%
Sex	Male	Female	Male	Female	Male	Female	Male	Female
	57.5%	42.5%	60%	40%	55%	45%	50%	50%

Table 2
Observed and expected number with the percentage frequencies and Hardy-Weinberg (H-W) equilibrium of IL-17A rs3819024 genotypes and alleles in TB patients and control groups.

Studied groups				IL-17A rs3819024 genotypes or allele					H-W P ≤
				AA	AG	GG	*A*	*G*	
TB Patient Groups (N:80)	MDR (N:40)	Observed	No.	22	12	6	56	24	Not significant
			%	55	30	15	70	30	
		Expected	No.	19.6	16.8	3.6	Not estimated		
			%	49	42	9			
	RD (N:20)	Observed	No.	8	9	3	25	15	Not significant
			%	40	45	15	62.5	37.5	
		Expected	No.	7.81	9.38	2.81	Not estimated		
			%	39.05	46.9	19.05			
	OC (N:20)	Observed	No.	12	6	2	30	10	Not significant
			%	60	30	10	75	25	
		Expected	No.	11.25	7.5	1.25	Not Estimated		
			%	56.25	37.5	6.25			
Control Group (N:40)		Observed	No.	30	8	2	68	12	Not significant
			%	75	20	5	85	15	
		Expected	No.	28.9	10.2	0.9	Not estimated		
			%	72.25	25.5	2.25			

MDR=Multidrug resistant, RD= Recently diagnosed, OC= Old cases.

Table 3
Statistical evaluations of association between IL-17A rs3819024 genotypes or allele in TB patients and control groups.

IL-17A rs3819024 genotypes or allele	Patients (N:80)		Controls (N:40)		OR	Etiological or Preventive Fraction	Fishers Exact Probability	95%CI
	No.	%	No.	%				
Genotypes	**MDR(N:40)**							
AA	22	55	30	75	0.41	0.44	0.1	0.16 to 1.04
AG	12	30	8	20	1.71	0.12	0.439	0.62 to 4.73
GG	6	15	2	5	3.35	0.10	0.263	0.65 to 17.37
Alleles								
A	56	70	68	85	0.41	0.5	0.036	0.19 to 0.89
G	24	30	12	15	2.43	0.17	0.036	1.12 to 5.26
Genotypes	**RD (N:20)**							
AA	8	40	30	75	0.22	0.58	0.011	0.07 to 0.68
AG	9	45	8	20	3.27	0.31	0.067	1.04 to 10.34
GG	3	15	2	5	3.35	0.10	0.322	0.53 to 21.21
Alleles								
A	25	63	68	85	0.29	0.60	0.01	0.12 to 0.71
G	15	38	12	15	3.4	0.26	0.01	1.41 to 8.18
Genotypes	**OC (N:20)**							
AA	12	60	30	75	0.5	0.37	0.249	0.16 to 1.54
AG	6	30	8	20	1.71	0.12	0.519	0.51 to 5.73
GG	2	10	2	5	2.11	0.05	0.595	0.29 to 15.59
Alleles								
A	30	75	68	85	0.53	0.4	0.214	0.21 to 1.35
G	10	25	12	15	1.89	0.11	0.214	0.74 to 4.80

MDR=Multidrug resistant, RD= Recently diagnosed, OC= Old recurrent cases, OR=Odds ratio, CI=Confidence Intervals.

Table 4
Serum level of IL-17A in PTB patients and control groups distributed by *IL-17A* rs3819024 genotypes.

Groups		IL-17A serum level Mean ± SD pg/ml		
		AA	**AG**	**GG**
Patients (N:80)	MDR (N:40)	27.96 ± 14.0^{A}	30.58 ± 14.4^{A}	33.24 ± 10.36^{A}
	RD (N:20)	37.26 ± 8.3^{A}	39.12 ± 5.34^{A}	40.3 ± 4.5^{A}
	OC (N:20)	44.87 ± 5.0^{A}	38.42 ± 7.1^{AB}	53.7 ± 0.98^{C}
	Total	36.11 ± 12.5^{A}	35.77 ± 10.49^{A}	39.46 ± 10.9^{A}
Control (N:40)		42.14 ± 6.2^{A}	41.06 ± 6.5^{A}	44.75 ± 7.8^{A}

MDR=Multidrug resistant, RD= Recently diagnosed, OC= Old cases, Similar letters: No significant difference ($P > 0.05$) between means of rows.

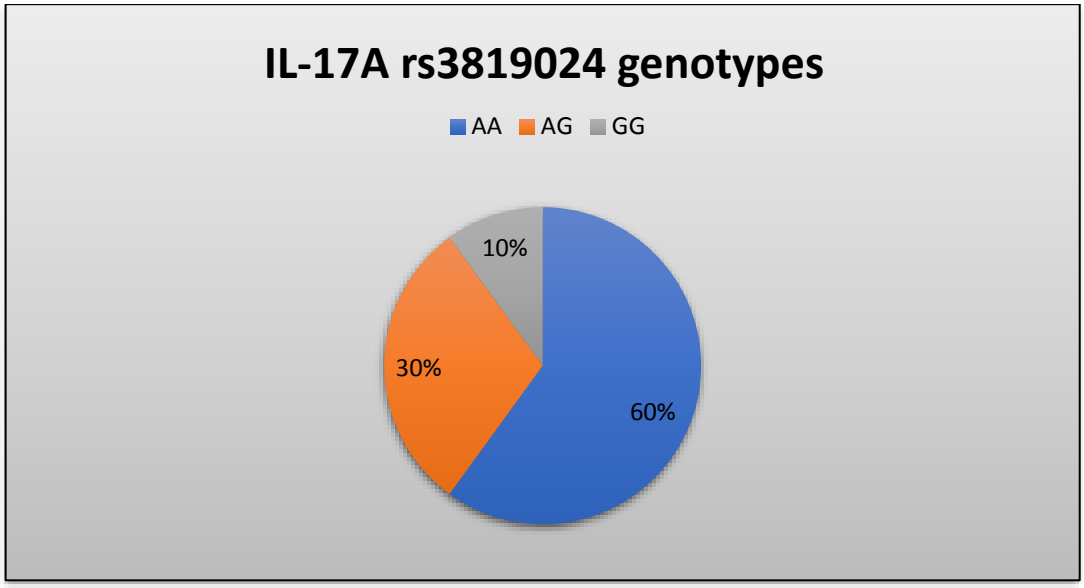

Figure 1: The percentage of IL-17A rs3819024genotypes.

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