The association between genetic polymorphism and susceptibility to pulmonary tuberculosis

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Abstract

Aim: the present study was started to find out the possible association of interferon gamma receptor 1 (IFNGR1) gene single nucleotide polymorphism(189T/G) and risk of pulmonary tuberculosis (PTB) and interferon gamma level in a sample of Iraqi population.

Subjects and Methods: Polymorphism of IFNGR1 +189 T/G were determined in 65 PTB patients and 65 healthy subjects by using PCR-RFLP and the concentration of IFNG were measured by using ELISA .

Results: our findings showed that IFNGR1 +189 TG genotype decreased the risk of PTB in comparison with TT genotype (OR =0.366, 95% CI =0.180-0.744, p =0.005 and preventive fraction 0.443) while the individuals with homozygous T alleles was significantly more common among the patients with PTB when compared with healthy control subjects and had a 3.143-folds increased risk of developing tuberculosis than the other genotype (TG), [p value 0.002, odds ratio, 3.143; 95% confidence interval(1.532-6.448), etiologic fraction 0.443]. and no significant association between IFNGR1 +189 T/G polymorphism and serum IFNG level.

Key words: pulmonary tuberculosis, single nucleotide polymorphism, IFNGR1 189 T/G.

{Citation: Sarhan Benyan AL-Saeedi, Ibtisam Habeeb Al-Azawi. The association between genetic polymorphism and susceptibility to pulmonary tuberculosis. American Journal of Research Communication, 2017, 5(11): 27-36} www.usa-journals.com, ISSN: 2325-4076.

1-Introduction

Tuberculosis (TB) caused by Mycobacterium tuberculosis, represent a major public health problem worldwide and result approximately 10.4 million new cases of TB and about 1.4 million HIV negative death in 2015 and more than two billion people (one third of world population) were estimated to be infected with Mycobacterium tuberculosis (WHO 2016). Mycobacterium tuberculosis is the major human pathogen that cause both pulmonary and extra pulmonary tuberculosis (Levinson 2010). The infection usually begins as an alveolar inflammatory reaction that progresses to atypical delayed type granulomatous reaction (Santucci *et al.*, 2011). The immune defense against M. tuberculosis is complex and involves interaction between TCD4+, TCD8+ lymphocytes, macrophages and monocytes with production of cytokines such as interferon gamma and tumor necrosis factor alpha (Philips and Ernst 2012). Interferon gamma signaling is mediated through the ligand binding (IFNGR1). Several studies demonstrated that susceptibility to TB might be determined by inherited host factors, such as polymorphisms in key genes that influence the outcome of the mycobacteria host interactions (Fol et al., 2015; Abel et al., 2014 Hanta et al., 2012 ;). Individuals defects in IFNGR1 have been reported as a cause of Mendelian susceptibility to mycobacterial disease, also known as familial disseminated atypical mycobacterial infection (Bustamante et al.,2014).

The defects in IFNGR1 is associated with impaired cellular responses to IFN-γ (Haverkamp et al., 2014).

2. Materials and Methods

2.1. clinical samples

A- patients

A total 65 patients were recruited in this case/control study during the period from October 2016 – august 2017. diagnosed clinically and radiologically for PTB and confirmed by conventional sputum smear (Ziehl -Neelsen method) and then confirmed by GeneXpert MTB/RIF. all patients were negative for HIV, hepatitis B, C, diabetes mellitus and not received prior immunosuppressive therapy or had serious medical illness were excluded from the study.

B- control subjects

The 65 control individuals was healthy blood donors free of history of TB or immune-related diseases and was recruited at blood bank center of AL- Nasiriyah and health care workers who work at the blood bank center they were nearly matched patients for age and gender.

C- Blood collection

five milliliters of the venous blood were collected from all study subjects at the beginning or shortly after treatment was started. then the blood samples were divided into ethylene diamine tetra acetic acid tubes (EDTA) as anticoagulant for the genomic DNA extraction and genetic studies and plain tube to separate the serum for measure IFN-ɣ concentration.

2.2. Genotyping

A- Genomic DNA extraction from fresh blood

The procedure was achieved according to the method recommended by the manufacturing company (ReliaPrep Blood gDNA Miniprep System) Promega- USA and the DNA was quantified by NanoDrop spectrophotometer and checked for purity , concentration and stored at ≤ -20 ˚C until use.

B - IFNGR1 genotyping

The genotyping of IFNGR1 polymorphism was done by PCR-RFLP. The primers sequences with respective annealing temperature and their amplicon size were shown in table 1.

Table 1. Primers sequences used for detection of INFGR1 gene polymorphism

Gene Polymorphism and location of SNP	Primer sequence (5′→3′)	Method	Product sizes of alleles (bp)		Annealing temperature
IFNGR1 exon (7) locus +189	(sense) 5'-gcc att tgg tgg tcc att ac-'3	PCR-RFLP	T	496	60 ˚C
	(antisense) 5'-tcc aga cag ctg gaa tca gt-'3		G	286 and 210	

In each 0.25 mL PCR reaction tube: Go Taq Green Master Mix 12.5µl (promiga,USA), Forward primer 1 µl, Revers primer 1 µl, Patient DNA template 3 µl and Nuclease-free water 7.5 µl for total volume 25 µl. The PCR cycling conditions for SNP were the initial denaturation at 95°C for 5 min followed by 30 cycles for 30 sec. at 95°C and annealing temperature 60˚C for 30 sec. and extension at 72°C for 30 sec, with a final extension of 72°C for 7 min.

The PCR products (15µl) and restriction enzyme TaqI(10 U/µl) 0.5 µl was mixed Then the mixture incubated at 37 ˚C for 3 hours and then at 65 for 10 minutes and hold at 4 ˚C for 10 min. then the digestion mixture was separated onto 1% agarose gels containing 0.5 µg/mL ethidium bromide and observed under UV light. The figure 1 show IFNGR1 genotypes.

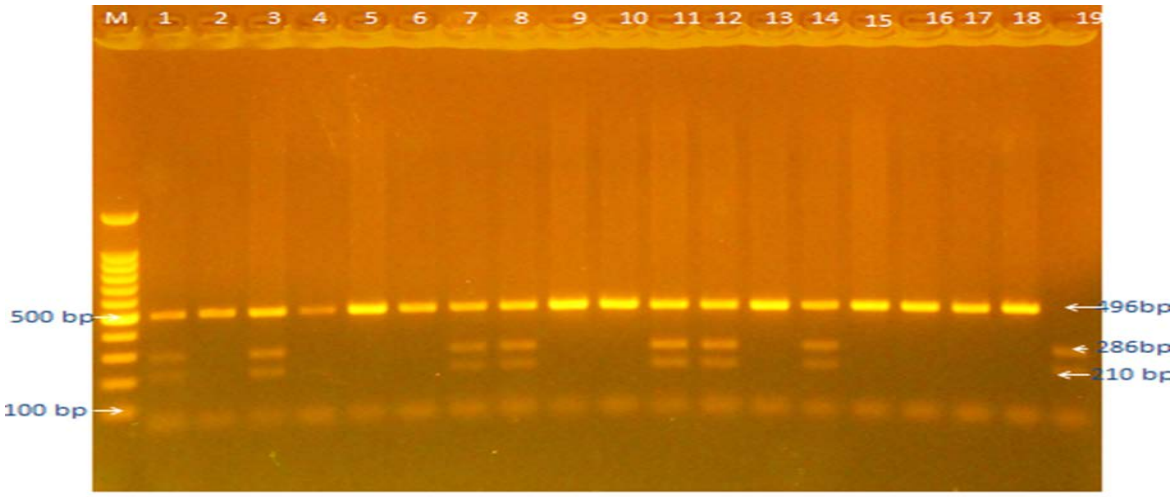

Figure 1 agarose gel electrophoresis (1%, 1X TBE) for 19 patients showing the RFLP fragments after digestion of PCR product by Taq1 for three genotypes. Lane 1 , 3, 7, 8, 11, 12 and 14 show TG genotype (496, 286 and 210 bp), lanes 2, 4, 5, 6, 9, 10, 13, 15, 16, 17 and 18 show TT genotype (496 bp) and lane 19 show GG genotype (286 and 210 bp).

2-3 ELISA for IFN-ɣ

ELISA assay was achieved according to the method described by the manufacturing company (Quantikine® ELISA) USA.

2-4 Statistical analysis

Data were collected, summarized, analyzed and presented using three statistical software programs: the statistical package for social science (SPSS version 22), Microsoft office excel 2013 and medCalc 2014.

Categorical variables were presented as number and percentage whereas numeric variables were presented either as mean and standard deviation (SD) or median and interquartile range (IQR) according to the results of kolmogrov Smirnov test of normality distribution for numeric variables . Odds ratio was used to estimate the risk and etiologic and preventive fractions were assessed accordingly. P-value was considered significant when it was equal to or less than 0.05.

3. Results and discussion

3-1 Demographic characteristic of the study and control groups

The study consisted of 65 PTB (37 male and 28 female), mean age 38.86 ±17.44 years and 65 healthy donors (44 male and 21 female), mean age 35.75±13.61 years. There was no significant difference between the groups concerning sex (P=0.205)and age (P=0.259).

The figure(2) show the patients were evenly distributed along the age intervals scale which reflects all age groups were susceptible for TB infection. Also showed most incidence occurs with ages less than 50 year.

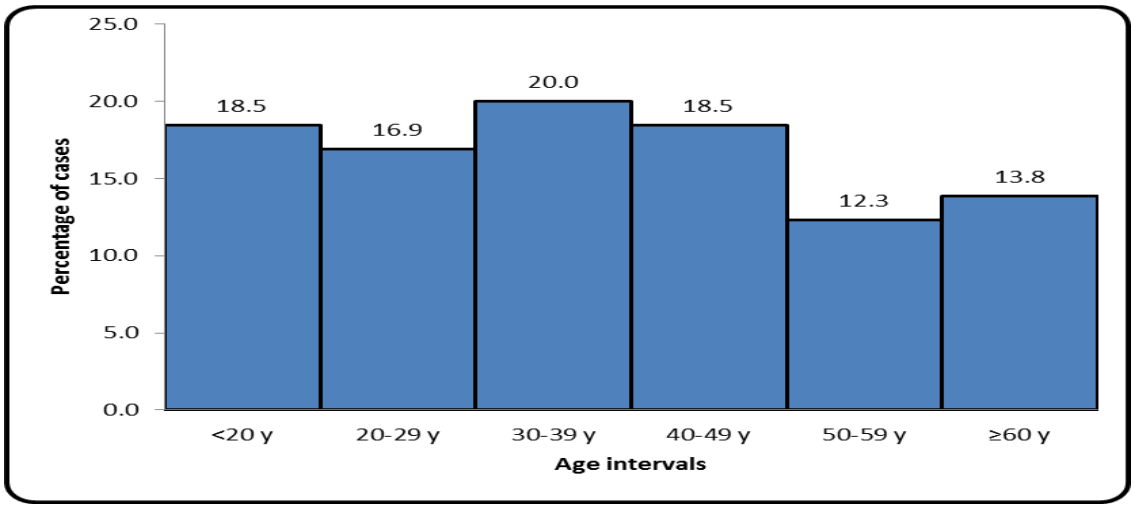

Figure 2: Histogram showing the distribution of patients according to 10 years age interval

3-2 IFN-γR1 (+189 T/G) SNP and susceptibility to pulmonary TB

In the present study we examined the possible of association between IFNGR1 (+189 T/G) gene polymorphism in the exonic region seven (effect of synonymous codon change) and the risk of PTB.

The table (2) show the genotypes and alleles frequencies of IFNGR1

IFNGR1 SNP 189 T/G		Control (n = 65)		Study group (n = 65)		P-value	OR	95 % CI		EF	PF
		n	%	n	%			Lower	Upper		
Genotype	TT	21	32.3	39	60.0	0.002	3.143	1.532	6.448	0.443	---
	TG	40	61.5	24	36.9	0.005	0.366	0.180	0.744	---	0.394
	GG	4	6.2	2	3.1	0.403	0.484	0.086	2.740	---	0.262
	Total	65	100.0	65	100.0						
Allele	T	82	63.1	102	78.5	0.006	2.132	1.231	3.693	0.294	---
	G	48	36.9	28	21.5	0.006	0.469	0.271	0.812	---	0.294
	Total	130	100.0	130	100.0						

n: Number; OR: Odds ratio; CI: Confidence interval; EF: etiologic fraction; PF: preventive fraction.

The individuals with homozygous T alleles was significantly more common among the patients with PTB and had a 3.143-folds increased risk of developing tuberculosis than the other genotype (TG), [p value 0.002, odds ratio 3.143, 95% confidence interval(1.532-6.448), etiologic fraction 0.443]. While heterozygous(TG) was relatively more frequent in control individuals and had a [p value 0.005, OR 0.366, 95% CI (0.180-0.744), preventive fraction 0.394] and homozygous (GG) rate was not significantly different between study group and control group(p=value 0.403). because its rare in frequencies in control and study groups so it cannot be regarded as a protective or as a preventive factor.

In this study suggest the effect of the allele +189 T (recessive allele) which seems to be a predisposing factor to *M. tuberculosis* infection in opposition to a protective role for the +189 G allele (dominant allele) so the individuals with homozygous TT was contributed to

increased risk of developing tuberculosis also the results appear to support the association of the +189 TT genotype with the susceptibility to the *M. tuberculosis*, whereas the heterozygous TG may be related to protection against *M. tuberculosis* infection. also the results showed no significant association between +189 T/G SNP and serum IFNG level.

The association between IFNGR1 +189 T/G polymorphism and risk of PTB showed by (Mohammed Naderi *et al.,* 2015) which demonstrated that TG genotype decreased the risk of PTB 36% when compared with TT genotype, also showed the G allele decreased the risk 41% when compared with T allele. Also the association between IFNGR1 and susceptibility to pulmonary TB was investigated in a Gambian adult population. the promoter and coding regions of IFNGR1 including exon 7 at position +189T/G SNP there was no association between the IFNGR1 variants studied and PTB in this Gambian population sample (Awomoyi *et al.*, 2004). one reason for the differing results is various genotype and allele incidences between different ethnic populations that confirm heterogeneity of populations hence a genetic risk factor in one population may not change disease susceptibility in another population (Laine *et al.,*2012). at present there is little data to determine whether this +189 T/G locus variants are functional in determining IFNGR1.

Single nucleotide polymorphisms (SNPs) within a coding sequence do not essentially change the amino acids sequence of the protein that is produced (synonymous polymorphism), due to degeneracy of the genetic code that code same amino acid, or result in the insertion of an alternative amino acid with similar properties to that of the original amino acid in either case there is no significant change in phenotype. however, synonymous mutations only occur within exons, and are not always silent mutations (Zhou et al., 2012). use of synonymous codons in the coding regions of gene are not random and codon usage bias is an essential feature of most genomes (Plotkin and Kudla 2011).

Some researches suggests that such changes to the triplet code do effect protein translation efficiency and protein folding and function (Czech et al .,2010 ; Komar 2007) also Goymer and Patrick (2007) demonstrated the synonymous mutations can affect translation, and alter phenotype of gene and convert the synonymous mutation to non-silent. Zhang *et al.*, (2012) demonstrated the silent mutations can affect the timing and rate of protein folding, which can lead to impairment of functions. Also recent experimental studies demonstrated that codon usage regulates translation, elongation speed and co-translational protein folding (Yu 2015; Zhou 2013; Spencer et al.,2012).

The position +189T/G in exon 7 of IFNGR1 constitute codon TCT or TCG which coded the serine there is a specific tRNA molecule for the mRNA codon UCU and another specific for the mRNA codon UCG, both of which codes for the amino acid serine if there was a thousand times less UCU tRNA than UCG tRNA, then the incorporation of serine into a polypeptide chain would occur a thousand times more slowly after a mutation causes the codon to change from UCG to UCU. If transportation of amino acid to the ribosome is delayed the translation will be carried out at a much slower rate this can result in lower expression of a particular gene containing that silent mutation. Also slow translation rates temporally separate the synthesis of defined protein portions and can coordinate the synthesis with the concurrently folding process of the proteins domains (Buhr *et al.*2016).

Conclusion

From these results the IFNGR1 +189 TT genotype were found to be associated with increased susceptibility to PTB while the TG genotype were associated with protection against PTB in Iraq population.

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