Association between IL12A Gene of G/A genotype Polymorphism and Pulmonary Tuberculosis Risk in Baghdad Population

Anwar Abed Nasser Dhabaan[1], Mayes Abdul-Hadi Mahdi[2]

[1]Assit prof Dr., [1]Post graduate. AL-Iraqia University/ Collage of Education/department of biology, Baghdad, Iraq

Abstract

The study included eighty samples. This cases-controlled study was performed including fifty Pulmonary tuberculosis patients, their ages ranged from 12 to 77 year (27 female and 23 male) and thirty controls (healthy), their ages ranged from 19 to 58 year (15 female and 15 male). We confined the frequency of IL12A gene (G/A rs568408 genotype) polymorphism by Tetra-ARMS PCR (Tetra amplification refractory mutation system-polymerase chain reaction) technology. Also, we determined the association of IL12A Gene (G/A rs568408 genotype) polymorphism with Pulmonary tuberculosis patients in Baghdad. Statistical results showed significant difference in genotype frequency of IL12A Gene (G/A rs568408 genotype) polymorphism between Pulmonary tuberculosis patients and control (healthy). The G allele shows high frequency in Pulmonary tuberculosis patients comparison with control (healthy) and present related with etiological fraction risk of Pulmonary tuberculosis patients and its ratio 62% in patients and 51.67% in control (healthy), While A allele shows high frequency in control (healthy) comparison with Pulmonary tuberculosis patients and present related with protective fraction of Pulmonary tuberculosis patients and its ratio 48.33% in control and 38% in Pulmonary tuberculosis patients. The genotypes of GG and AA homozygotes shows high frequency in Pulmonary tuberculosis patients comparison with control (healthy), and its ratio 42% and 18% respectively in Pulmonary tuberculosis patients, while its ratio 3.33% and 0% respectively in control (healthy), also GG and AA genotypes appear related with etiological fraction risk of Pulmonary tuberculosis patients, while the GA heterozygote show high frequency in control (healthy) and its ratio 96%, GA genotypes related with preventive fraction of Pulmonary tuberculosis patients. Our findings demonstrate that the IL12A Gene (G/A rs568408 genotype) polymorphism may represent a significant risk factor for pulmonary tuberculosis patients in Baghdad population.

Keywords: IL12A Gene, Pulmonary Tuberculosis, Polymorphisms, Tetra-ARMS PCR

Introduction

The Pulmonary tuberculosis (TB) is a major cause of morbidity and mortality throughout the world, especially in Asia. The statistical data showed 9.6 million new cases and 1.5 million deaths, based on the WHO (World health organization) of 2015 year [(1)]. TB caused by Mycobacterium tuberculosis is an aerobic rode and intracellular pathogenic bacteria which have target the lungs and causative agent of tuberculosis [(2)]. Cytokines are pivotal in activation of the cell mediated immunity required for controlling of intracellular growth and eliminating of pathogens [(3)]. Interlukin-12 cytokine play an important role in immune response of Mycobacterium tuberculosis [(4)], and mainly produced by immune cells (macrophages and dendritic cells) [(5)]. IL-12 induces T lymphocyte cells and Natural Killer cells to produce pro-inflammatory cytokines such as TNF-α and IFN- γ in the immune response of pulmonary tuberculosis [(6)]. IL12A gene is located on chromosome 3 of short arm in region 12 (3p12) [(7)]. There is an association of IL-12A gene polymorphism with the risk of pulmonary tuberculosis [(8)]. The variability in the IL-12A gene circuit association studies probably confirm of the genetic heterogeneity underlying susceptibility to pulmonary tuberculosis [(9)]. The study present association between IL-12 gene and pulmonary tuberculosis risk, by using a panel of single

Corresponding author:
Anwar Abed Nasser Dhabaan
Email: anwer_naser@yahoo.com

nucleotide polymorphism providing comprehensive coverage of these genes [10]. IL12A gene is hypothesized to be involved in the progression and development of Pulmonary Tuberculosis. Genetic polymorphisms of *IL12A* gene, was found that genetic variants G/A rs568408 genotype associated with an increased risk of Pulmonary Tuberculosis. The results of this study demonstrate that genetic polymorphism of IL12 pathway may individually or jointly contribute to the sensibility to and prognosis of Pulmonary Tuberculosis TB [3]. The study aimed to the finding association between IL12A gene polymorphism in position G/A rs568408 genotype and risk of pulmonary tuberculosis development in Baghdad population.

Materials and Method

Population samples Study

The population samples Study consisted of 50 patients with pulmonary tuberculosis (27 female and 23 male), there ages range from 12 to 77 years, and 30 control healthy individuals, (15 female and 15 male), and there ages range from 19 to 58 years. All the samples of pulmonary tuberculosis patients were collected from The National Center for Chest and Respiratory Diseases/ Medical City in Baghdad. They had an established diagnosis of pulmonary tuberculosis by the clinical examination and laboratory test.

Genotyping of IL12A gene (G/A rs568408 genotype)

Genomic DNA was extracted by take five ml of blood from each patient and healthy control by venipuncture, later, 2.5 ml was added in to EDTA tubes then DNA was extracted by DNA isolation kit (Promega, USA) and according to manufacture instructions manual. DNA purity was qualified by Nano drop and it was about 1.6 ±1.8. All samples were kept at 20 º for further study. Polymorphism of IL12A gene (G/A rs568408 genotype) was examined by using Tetra-ARMS-PCR technology. The PCR reaction was carried out on a DNA template with a pair of specific primers (Alpha DNA, Canada) that designed according to [11]. Table (1), 20 µl was the total volume of reaction mix (PioNeer, Korea), and the molecular marker size (Promega, USA) 100-2000 base pair. Tetra-ARMS-PCR programs were summarized in table (2). The genotypes were established by analyzing electrophoresed 2.5% agarose gel stained with diamond dye (Promega).

Table (1): primer sequences of IL12A gene (G/A rs568408 genotype) by Tetra-ARMS PCR technology

Target Gene	primer	Primer sequences (5`→ 3`)	Size (bp)
IL12A gene (G/A rs568408)	Forward outer	5′-AATTTTGGAATACCATGTAAGTCATGCT-3′	556 bp
	Reverse outer	5′-AGTTAGCTCAGATGCTTTCATGATTACC-3′	
IL12A gene (G and A allele)	Forward inner (A allele)	5′-GAAGGATGGGACTATTACATCCACCTA-3′	271 bp
	Reverse inner (G allele)	5′-AAATGTCAAAAATACTTGATCAGAGGTCTC-3′	352 bp

Table (2): The cycling condition for Tetra-ARMS PCR program for detection of IL12A gene (G/A rs568408) by outer primer in pulmonary tuberculosis patient and control groups (healthy) samples.

Target gene	steps	Temperature (co)	Number of cycles	Time (seconds)
IL12A gene (G/A rs568408)	Pre-denaturation	94		300
	Initial denaturation	94	35	30
	Annealing	65		30
	Extension	72		30
	Final Extension	72		5

Statistics

Differences in the frequencies of of IL12A gene (G/A rs568408 genotype) for pulmonary tuberculosis patient in this study with control groups were analyzed with a value $P<0.05$ by Fisher's exact test. Odds ratios (OR) and confidence intervals (CI) were calculated using Compare 2 Ver.3.04 software J. H. Abramson (2003-2013). Preventive Fraction (PF) and Etiologic Fraction (EF) results were compared with Hardy-Weinberg equilibrium and according to the software within the following website www. had2know.com.

Results

The genetic polymorphisms of IL12A gene (G/A rs568408) in fifty pulmonary tuberculosis patients with mean age 34.95±1.4 year, and thirty of healthy individuals as a control samples with mean age 26.7 ±1.9 year. Notably, the two alleles G/A are more present for IL12A gene (G/A rs568408) with GG, GA and AA genotypes in pulmonary tuberculosis group and control (figure 1), use of tetra-ARMS PCR technology in study. The allelic frequency and genotypes distribution for each tested polymorphisms for healthy control and pulmonary tuberculosis patient are presented in table (3). With respect to the IL12A gene (G/A rs568408) polymorphisms, there was a significance in pulmonary tuberculosis patient in compare with control group ($P>0.05$), and the G and A alleles were different in frequency, so allele G frequency was 62% for pulmonary tuberculosis patient while allele A frequency was 38%, as compared with G and A alleles in control group that it's frequency was 51.7% and 48.3% (figure 2). The odds ratio (OR) for G allele was 1.53 with confidence intervals (CI) 0.80 to 2.90 at 95 % (Table 3), and it was 0.21 as an etiological fraction (EF), while for allele A there is no significance in pulmonary tuberculosis patient comparison with control and OR was 0.6 with CI 0.34 to 1.25 at 95%, and the value of allele A as preventive fraction (PF) was 0.17 (Table 3). The previous report on polymorphisms of IL12A gene (G/A rs568408) show that may G allele be an etiological fraction and also, it's describe that the A allele may be a preventive fraction that correlated with the risk of pulmonary tuberculosis patients. The genotyping polymorphisms for IL12A gene (G/A rs568408) by tetra-ARMS PCR technology, there are a genotypes frequency significance in pulmonary tuberculosis patients, so GG and AA genotypes showed the high frequency in pulmonary tuberculosis patients as compared with control (health) group, and it was 42 % and 18 % respectively (Figure 3), also the OR for GG and AA genotypes was 21 and 14 respectively, with CI 2.74 to 161.1 and 0.82 to 238.1 respectively. The GG and AA genotypes presented of association with etiological fraction for risk pulmonary tuberculosis, while for GA genotype the frequency was 40% and 96% for pulmonary tuberculosis patients and control (health) group respectively (Figure3), also the OR was 0.02 and CI was 0.00 to 0.18 and the value for GA genotype as protective fraction was 0.94. Briefly, the result showed that GG and AA genotypes were correlated with the risk of pulmonary tuberculosis, while GA genotype was correlated with the protective fraction of pulmonary tuberculosis in Baghdad Population. The results are

consistent with (8, 10 and 12).

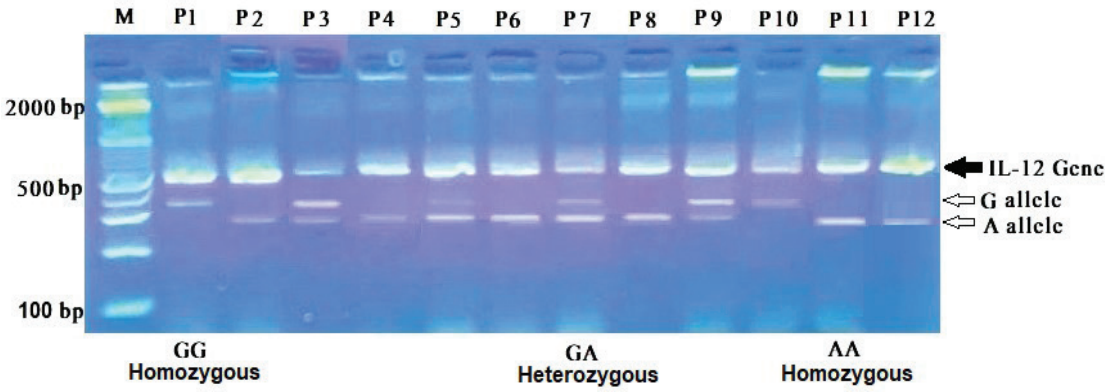

Figure (1): The genotypes of IL12A gene (G/A rs568408) polymorphisms for Pulmonary tuberculosis patient samples. Electrophoretic scheme (M is DNA marker, IL-12 gene 565bp, samples of 1 and 10 GG homozygous (352bp), 2, 4, 6, 8,11 and 12 AA homozygous(271bp) and 3,5,7 and 9 heterozygous).

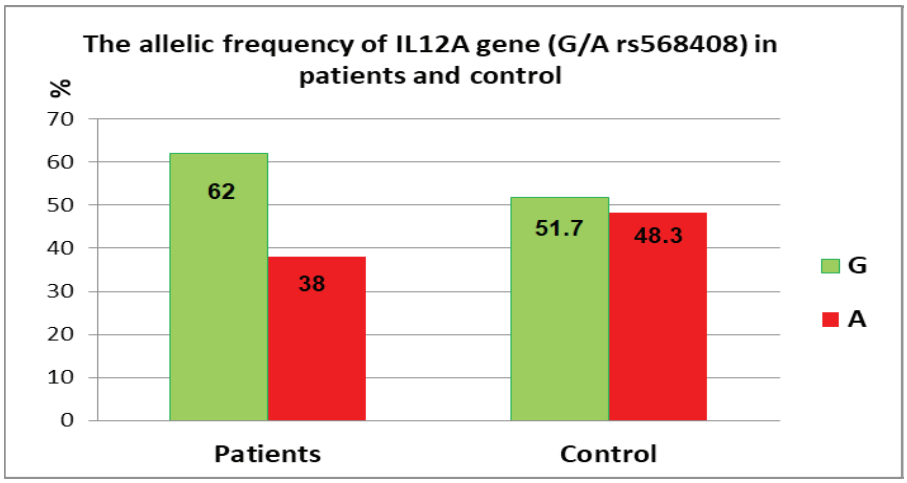

Figure (2): allelic frequencies of IL12A gene (G/A rs568408) polymorphisms for Pulmonary tuberculosis patient and healthy samples

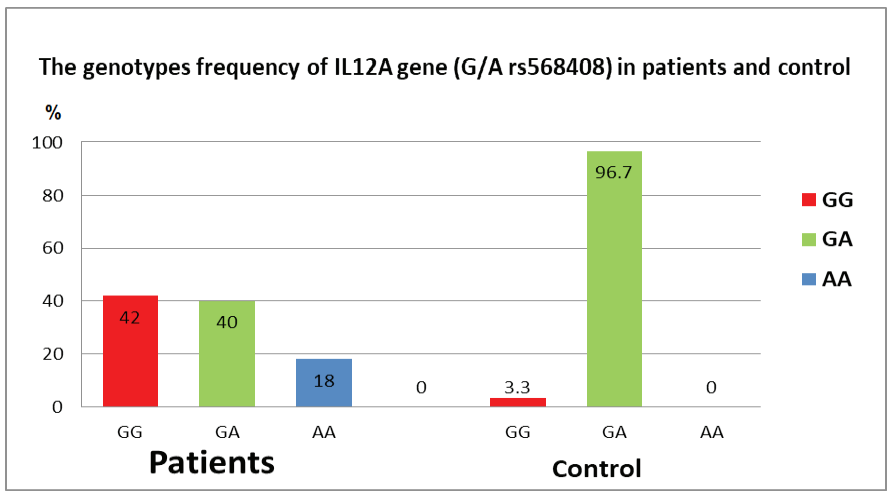

Figure (3): The genotypes frequencies of IL12A gene (G/A rs568408) polymorphisms for Pulmonary tuberculosis patient and healthy samples

Table (3): The allelic frequency of IL12A gene (G/A rs568408) for Pulmonary tuberculosis patient and healthy samples

Target Gene	Allele	pulmonary tuberculosis Patients (%)Number	Control (%)Number	OR (95%CI)	P-Value
IL12A gene (G/A rs568408)	G	62 (62%)	31 (51.7%)	1.53(0.80 to 2.90)	0.247
	E.F				
	A	38 (38%)	29(48.3%)	0.6(90.34 to 1.25)	
	P.F				

Notes: OR= Odds ratio, CI= Confidence Interval, P.F= Preventive fraction E.F= Etiological fraction , P<0.05 by Fisher's test.

Discussion

Interleukine-12 is an immuno-regulatory cytokine, which linked innate and acquired immune responses to mycobacterium through induction of IFN-γ production [13]. A series of recent reports in the cytokine pathway suggest that more subtle variants of relevant genes may contribute to susceptibility to tuberculosis at the general population level. To investigate whether polymorphism in the interleukin-12 (IL-12) gene predispose individuals to tuberculosis, we studied these genes by single-strand conformational polymorphism analysis and direct sequencing [6]. The most potent enhancer of reactivated T-cells and interferon production which is necessary for killing intracellular bacteria like mycobacteria is interleukin-12 (IL-12), and confirms to be an effective and successful adjuvant to a standard anti-tuberculotic medication in patients suffering from progressive pulmonary tuberculosis (TB) [14]. IL-12, produced mainly by macrophages and dendritic cells, has a important role in the immune response to Mycobacterium tuberculosis, also, IL-12 induces T cells and NK cells to produce pro-inflammatory cytokines such as Interferon-γ and Tumor necrosis factor-α while also regulate the production of IL-17 in immunity response for Mycobacterium tuberculosis [6]. The genetic polymorphisms of the IL-12 pathway may individually contribute to the susceptibility to and prognosis of pulmonary tuberculosis TB [12]. We investigated the impact of IL12A rs568408 gene polymorphisms on risk pulmonary tuberculosis in a sample was living in southeast [13]. The IL12A rs568408 variant was not a risk factor for susceptibility to pulmonary tuberculosis in codominant, dominant and recessive tested inheritance models [11], but another study showed association between IL12A rs568408 polymorphisms and risk of pulmonary tuberculosis in Chinese population and It was found that genetic variants AG/GG of rs2243115 (IL12A) were associated with a decreased risk of pulmonary tuberculosis [12].

Conclusion

The statistical data of current study proved the association between of IL12A gene (G/A rs568408) polymorphism and Pulmonary tuberculosis risk in Baghdad Population.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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