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Research Article

Genotyping Study for the Promoter of IL-4 Gene in Iraqi Patients with Tuberculosis

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Abstract

Tuberculosis still represents a disaster that puts heavy shadow over all human societies. In spite of the availability of effective chemotherapy and vaccine, tuberculosis is a global health concern for both developing and developed countries. A defect in the genes of the immune response is the most acceptable explanation for susceptibility of some individuals and resistance of others to TB. Cytokines play critical role in interactions and integration between the cells of immune system, which leads to effective defense against TB, among cytokines IL-4, which have regulatory role in immune response in Iraqi patients with TB. Seventy-four blood specimens were collected from 74 patients in the Institute of the Tuberculosis and Chest Disease-Basra city, blood specimens also collected from 74 healthy individuals as control. Extracted DNA was amplified using two sets of specific primers for promoter regions of IL-4. Purified amplicons were sequenced and were analyzed using specific software. The genotyping analysis of promoter of IL-4 gene was indicated two alleles C and T, of them C allele is predominant (73%), while T allele is recessive (24%). Although those heterozygous genotypes (CT) were present in patient and healthy control (HC), but the frequency of CT in patients was significantly higher than those of controls. IL-4 promoters carry more than one type of mutations pushing toward increasing susceptibility of some individuals to TB. .

Keywords: Interleukin-4; Tuberculosis; Mycobacterium tuberculosis

Introduction

Tuberculosis (TB) is a real problem and a challenge for all health systems all over the world, *Mycobacterium tuberculosis* (*MTB*) is the major etiological agent of tuberculosis in human. Tuberculosis is a highly infectious disease, which is transmitted from person to person through the air droplet by cough, sneezing and song. A cough for three weeks or longer, weight loss, loss of appetite, high temperature or fever, drenching night sweats and extreme tiredness or lack of energy are the most common symptoms of TB [1]. TB has been ranked as the second cause of death after the human immune deficiency virus (HIV). The total numbers of new TB cases are rising worldwide [2]. Increasing in TB incidence in the developing countries and its re-emergence in the developed world, led the World Health Organization (WHO) to declare TB as a global emergency in 1993 [3]. Basra province is the second largest city in Iraq in terms of population. In Basra province also TB is serious problem, it threats all society sectors. Issa., *et al*. [2] studied the reality of TB in Basra from 2010 to 2014 and found that the total number of patient is 3218, including 1595 (49.6%) male and 1623 (50.4%) female and TB targeted all age groups even those who defiantly vaccinated. Mandatory vaccination and the use of early detection strategies and providing free treatment did not stand a barrier to

the spread of the disease in the province of Basra. Host genetic factors explain, at least in part why some people resistant to infection more successfully than others [4]. Rare gene disruptions cause fatal vulnerability to certain pathogens, but more clear differences are common and arise from minor variations in many genes to predict how much our genetic makeup determines the different ways in which individuals respond to some infectious agents is a difficult task [5]. Immune system of the person acts as wall off the disease; it leads to effective defense against TB. While cytokines play critical role in interactions and integration between the cells of immune system so any defect in the genes that coded for cytokines may lead to the inability of the immune system to rein in this pathogen [6,7]. Interleukin-4 (IL-4), a multifunctional pleiotropic cytokine mainly produced by activated T helper 2 (Th2) cells. IL-4 plays crucial roles as a mediator and as modulator of immune, also IL-4 has role in inflammatory responses. IL-4 involved in humeral and cell- mediated immunity and it necessary as regulator of immune response of B cells, T cells and macrophages to fight against infections and malignant cells [8,9]. IL-4 gene is located on chromosome 5 with plus strand orientation and includes 8,693 bases, any alterations occurs in the expression and function of IL-4 can lead to weakened immune responses and thus increase the susceptibility to infections and inflammation-related disease [10].

Aim of the Study

The main goal of this study is genotyping promoters region of IL-4 gene in Iraqi patients with TB and produce genetic explanation of innate receptivity to injury for some individuals without others.

Materials and Methods

The specimens were collected at the institute of the tuberculosis and chest disease-of Basra province from February to September 2015. Seventy-four patients (33) male and (41) female with pulmonary TB, with average age between (38.73 ± 23 years) were included in present study, while 74 volunteers, (36) male and (38) female, age mean between (35.19 ± 7.86 years) were accredited in the current study as healthy control (HC). Blood samples were collected from each patient and control by vein puncture using disposable syringes. For DNA extraction, 2 ml of collected blood was put in EDTA tubes, Reliaprep blood DNA Miniprep kit (Promega, USA) was used For DNA extraction, the extraction steps were done according to company instructions. The extraction process was verified by characterization of genomic DNA bands in agarose gel electrophoresis by loading of 6 µl DNA mixed with 3 µl of bromophenol blue in the wells of the 1% agarose gel. Set of primer for PCR amplification of IL-4 promoter was used as shown in table 1. For PCR reaction, the following reaction mixture was used: 1.5 µl of genomic DNA, 12.5 µl of Premix Taq v.2 plus dye (Takara Biomedical Technology, China), 0.5 µl $MgCl_2$, 0.5 µl of each primer (GeneScript Make Research Easy, China) and 9.5 µl of nuclease free water. PCR conditions for amplifying IL-4 were initial denaturation at 95°C for 3 minutes, followed by 30 cycles consisting of 30 seconds at 95°C, 30 sec. at 57°C and 30 seconds at 72°C with a final extension at 72°C for 1 minute. The amplified products were determined by electrophoresis on agarose gel containing 0.5 µg/ml Ethidium bromide. Before sequencing, PCR products were purified by Gel/ PCR Extraction Kit (BIOMIGA Ezgene, China) according to the manufacturer s recommendations. All samples were sent to GeneScript company (GeneScript Make Research Easy, China) for sequencing. Two types of file came back from the company ABI and text file, DNA Dynamo software was used to analyse the data results, multiple alignment for high-quality sequences were done for each other plus reference sequence at GenBank performed to find DNA polymorphism within sequences.

Primers		Sequence	Product size	Reference
IL-4	Sense	5'- GTAAGGACCTTATG-GACCTGC -3'	209 bp	Vidyarani., *et al*. [8]
IL-4	Antisense	5'- CATCTTGGAAACT-GTCCTGTC -3'		

Table 1: Primers using in PCR amplification of TLR-2.

Results

Promoters of IL-4 Gene were successfully amplified. The amplicons were visualized by agarose gel electrophoresis, whilst PCR product sizes were determined by comparison with marker as in figure 1.

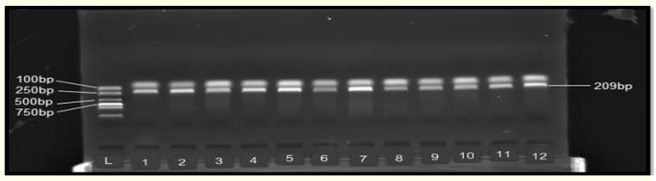

Figure 1 : PCR products of the DNA amplicons of IL-4 visualized by 1% agarose gel electrophoresis, for 1 hour in (50V), product sizes were determined by comparison with 2000 bp marker. Lane L: 2000 bp DNA marker, lanes 1-12: IL-4 bands.

In the population of patients under the focus of current study, the genotyping analysis divided into two alleles C and T, of them C allele is predominant (75%), while T allele is recessive (20.2%). Although those heterozygous genotypes (CT) were present in patient and healthy control (HC), but the frequency of CT in patients was significantly higher than those of controls (Table 2). Figure 2 was show the homozygous and heterozygous genotypes sequencing results.

Locus IL-4	Genotype	Patients (%) (n = 74)	Healthy control (n = 74)	Allele frequency	Allele frequency	p-value
				P.	HC	P.
-590	CC TT CT	56 (75%) 3 (4.8%) 15 (20.2%)	71 0 3	0.75 0.048 0.202	0.96 0 0.04	0.0008

Table 2: The genotype and allele frequencies of the identified IL-4 SNPs in the completely studied population (148 samples).

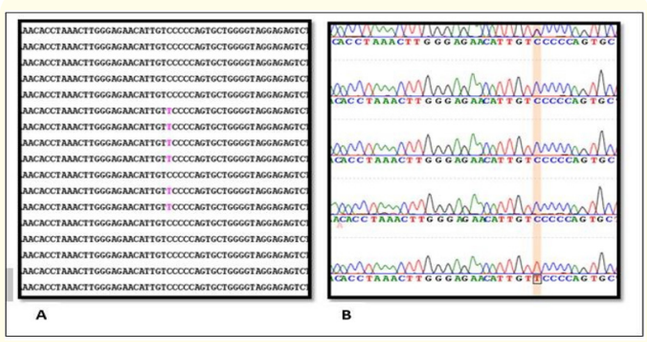

Figure 2: (A) Alignment of sequences different allele appears with red letter. (B) Alignment of chromatograms appears its peaks quality.

Discussion

Iraq, like other countries Tuberculosis represents a real problem should be studied all its dimensions. Basra province is the second largest city in Iraq in terms of population, with a population of about (2,774,600). While the Basra province area is 19,070 sq. km (4.4% of Iraq). From the administrative point of view, the province of Basra subdivided into seven districts. Abu Al-Khaseeb, Al-Midaina, Al-Qurna, Al-Zubair, Fao, Shatt Al-Arab and center of Basra district, the last one is included first center and second center [2]. In Basra province applied since early all the programs for controlling of the TB, early detection and compulsory treatment and compulsory vaccination, but statistics remained indicate an increasing number of cases of injuries, especially among individuals who vaccinated against this disease. A defect in the genes of the immune response is the most plausible explanation for susceptibility of some individuals and resistance of others to TB [11]. Human Interleukin 4 (IL-4) located in the chromosome 5 (5q31-33), is an anti-inflammatory cytokine produced by CD4+ Th2 cells, basophils and mast cells. IL-4 regulates variety of cell types IL-4 response in tuberculosis downregulates protective Th1 responses [12]. IL-4 plays an essential role in differentiation of Th2 effector cells, suppression of Th1 signaling, promoting humoral immunity and Ig class switching and a dominant role in immunopathology. Human IL-4 gene promoter contains six conserved binding sites of NFAT (nuclear factor of activated T-cells), a transcription factor; along with activator protein 1 (AP1) regulates IL-4 transcription. Vidyarani., *et al.* [8] mentioned that A single nucleotide polymorphism at position -590 in the IL- 4 promoter region has been shown to be associated with enhanced IL-4 promoter strength and altered IL-4 activity and production. Our results agree with Vidyarani., *et al.* [8] and Butov., *et al.* [13] they also found that 'T' allele may be associated with susceptibility and the 'C' allele with resistance to tuberculosis, Gharagozlou., *et al.* (2015) also found that T/C polymorphism in (-590) association with atopic dermatitis which is inflammatory skin disease.

Conclusion

There are one type of SNP pushing toward increasing susceptibility of some individuals to TB. Mutation in promoter region of cytokines coded genes such as IL-4 plays critical role in altering immune response.

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