

The role of Interferon- γ Receptor-1 Gene (-56 T < C) Polymorphism in development of susceptibility to pulmonary Tuberculosis in Central Sudan

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ABSTRACT

Background: It is estimated that every year 8 million new cases of tuberculosis occur globally and that 2 to 3 million people die annually from the disease. Human genetic variation is an important determinant of the outcome of infection with *Mycobacterium tuberculosis*. The aim of this study is to determine genetic susceptibility to tuberculosis among patients and their control by identifying the frequency of some IFN γ R1 polymorphisms in development of susceptibility to pulmonary tuberculosis in Gezira state of Central Sudan.

Methods: Polymorphism (-56 T < C) was analyzed in both 126 confirmed positive TB cases and 200 matched controls using PCR-RFLP method.

Results: The results showed that the distribution of IFN γ R1-56 T < C genotype differs significantly between tuberculosis patients and controls and those carrying the mutant C allele were associated with two times reduced risk for susceptibility to Tuberculosis (*P-value* = 0.017, odds ratio = 1.6, 95% CI = 1.025 – 1.441), that mean the mutation IFN γ R1 -56 T < C gives protection two times more than the wild type allele. However, more studies in IFN γ R1 gene, and polymorphisms in IFN γ gene, needs to be performed with larger sample size

Key words: IFN γ R1 gene, Tuberculosis, Sudan

INTRODUCTION

During the last few years it has become clear that; the genotype of the host plays an important role in the evolution and outcome of infectious diseases. Considering tuberculosis, there still no satisfactory explanation for the fact that small proportion of individuals infected with *Mycobacterium tuberculosis* develop a severe clinical disease while others developed immunity against mycobacterial infections.

Differences between *Mycobacterium* isolates from different patients with different clinical phenotypes of tuberculosis have not been fully demonstrated. Many host genes are involved in the control of the internal environment which the *Mycobacterium*

faces and should favor the survival internally within the host genes that confer some protection against the infections. Considerably, more evidences are available to indicate the importance of host factors in determining the clinical presentations of mycobacterial infection. The full knowledge of these factors is important not only to understand how the disease evolves within an individual, but also of great importance to understand, from an epidemiological point of view, how it behaves in a given population.

Many of the cytokine-binding receptors function in the immune and hematopoietic systems (Casanova *et al.*, 2001).

The level of secretion of both monokines (IFN γ IL-12) and

lymphokines (IFN γ) by peripheral blood mononuclear cells has been reported to be low in IFN γ R1-deficient children. Typically, these patients do not develop well differentiated and well circumscribed mycobacterial granulomas. This provides further evidence that impaired IFN γ mediated immunity affects both phagocytes and lymphocytes (Casanova *et al.*, 2001).

The polymorphisms in IFN γ R1 inhibit signaling that introduced by IFN γ to phagocytes that produced more activity against *M. tuberculosis* in many studies that confirm this fact. (Newport, 2004, Newport *et al.*, 1996).

Complete IFN- γ receptor ligand-binding chain (IFN γ R1) deficiency is a life threatening autosomal recessive immune disorder. Affected children invariably die of mycobacterial infection, unless bone marrow transplantation is undertaken. Pathogenic IFN γ R1 mutations identified to date include nonsense, splice mutations, frame shift deletions and insertions. All result in a premature stop codon upstream from the segment encoding the transmembrane domain, precluding cell surface expression of the receptors.

MATERIALS AND METHODS

The study was conducted in Wad Medani Chest Hospital during the period between June 2005 and Augustus 2006. Ethical approval was obtained from both the Federal Ministry of Health in Khartoum and the Research Committees of the University of Gezira. Consent was taken from the study subjects.

126 pulmonary tuberculosis patients who were diagnosed by ZN stain were selected for the study. Control group for candidate polymorphisms screening were selected from 200 healthy individuals or patients with diseases other than tuberculosis and they were matched in age, sex, socioeconomic status and never had tuberculosis before.

Genetic Analysis

2-3 ml of venous blood were collected from each study and control subject into 3.8% sodium citrate and DNA extraction was performed using salting out method (ref) and the quality of DNA was measured by UV spectrophotometer.

PCR reactions was performed in a total volume of 30 μ l containing 100 ng genomic DNA, 20 Pico moles of each primer, 200 μ M dNTP, 3 μ l from 10 x Taq Gold Buffer (100mM Tris HCl, pH 8.3, 500 mM KCl, 15 mM MgCl₂ and 0.01 % (w/v) gelatin (Perkin Elmer Cetus), 1.5 U AmpliTaq GoldTM polymerase (Perkin Elmer Cetus) and was completed to the final volume with deionized water.

Mutation Analysis

In this study, polymorphisms in Interferon gamma receptor 1 (IFN γ R1) IFN γ R1-56 in exon 1 were screened in cases and controls by PCR-RFLP.

Screening of IFN γ R1-56 T \rightarrow C polymorphisms by Restriction Fragment Length Polymorphisms (RFLP)

A DNA fragment 285 bp was PCR amplified using the primer pair:

IFN γ R-56 T \rightarrow C F: (5'-GGGCGTGGGCGGGGTCAA-3')

IFN γ R -56 T \rightarrow C R: (5'-CCTCCCTCCCTCTCGTCC-3')

PCR condition as initial temperature 95 C^o for 5 minute, followed by 35 cycles of 94 C^o as melting temperature for one minute, 66 C^o as annealing temperature for one minute and 72 C^o as extension temperature, then final prolongation step at 72 C^o for 5 minutes.

Digestion with Restriction Enzyme (Eco 47II)

In transition replacement of cyctine to thiamin in position -56 create a new restriction site for Eco 47II. In a total volume of 15 μ l, 5 μ l PCR products was digested overnight with 3 μ l Eco 47II enzyme and mixed with 1.5 μ l Buffer3

10x , 0.2 µl BSA 50µg and 7.8 µl deionized water.

The Digested DNA samples were mixed with 5 µl loading dye before being loaded on a 10 % Non-denaturing polyacrylamide gel and electrophoresed at 100 V for two hours. Then the gel was stained in 0.1 µg/ml ethidium bromide solution for 10-15 minutes and visualized under UV light in Gel Documentation System (GDS).

The genotype was assigned according to the length of obtained fragments. Complete cleavage of 285 bp into 193 and 92 bp fragments is characteristic profile of the homozygote mutant allele (CC), incomplete cleavage into 285 bp, 193 bp and 92 bp is of the heterozygote (TC). While absolute absence of digestion is characteristic of the homozygote wild (TT).

Statistical Analysis

Concordance of genotype frequencies with Hardy-Weinberg equilibrium was tested by a χ² goodness-of-fit test. The baseline value of these groups was

compared using the unpaired t test. The statistical tests were performed using statistical package of social sciences (SPSS)

RESULTS

Study Subjects

The mean age of the study subjects was (37.27 ± 16.1) years old; the minimum age was 10 years and the maximum was 95 years old. 92 (73%) were males and 34 (27%) were females

Genetic Analysis

IFNγR1-56 Genotypes in TB Patients and Controls

IFNγR1 -56 polymorphism were screened using PCR-RFLP method (Fig.1). Lane 10: M: 100-bp DNA marker. Lanes 2,3,4,5,6 and 9 were heterozygous (TC); Lanes 1 and 8 were homozygous (TT) for IFNγR1 -56 while Lane 7 is homozygous (CC) for IFNγR1-56.

The distribution of IFNγR1-56 T<C genotype show no significant difference between TB patients and controls (P = 0.071).

WT hetro hetro hetro hetro hetro WT mutant hetro
1 2 3 4 5 6 7 8 9 10

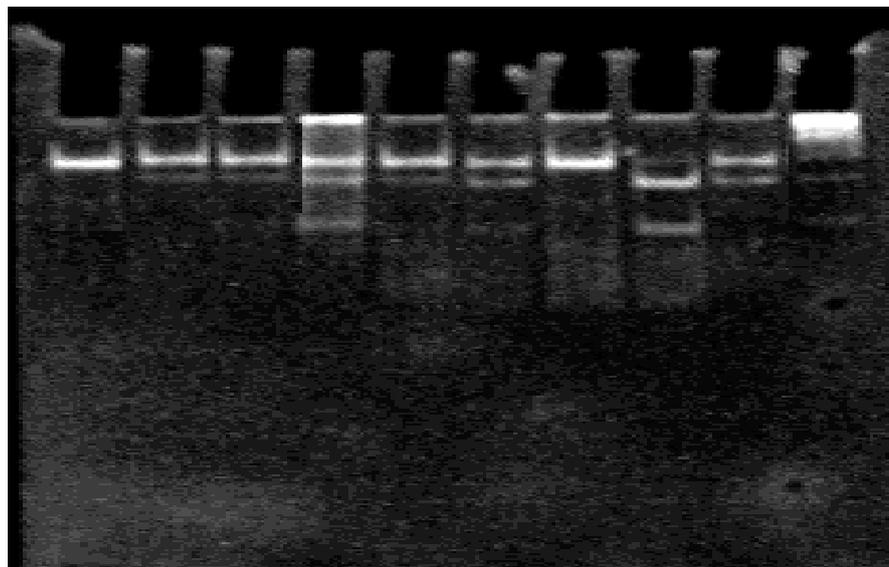


Fig 1: Detection of IFNγR1 -56 polymorphism using PCR-RFLP method.

The distribution of IFNγR1 -56 C >T genotype differ significantly between TB patients and controls (P = 0.017) (Fig.2).

That indicate the T allele is associate with two times increased risk to pulmonary tuberculosis than C allele (P -value = 0.017, odds ratio = 1.6, 95% CI = 1.025 – 1.441).

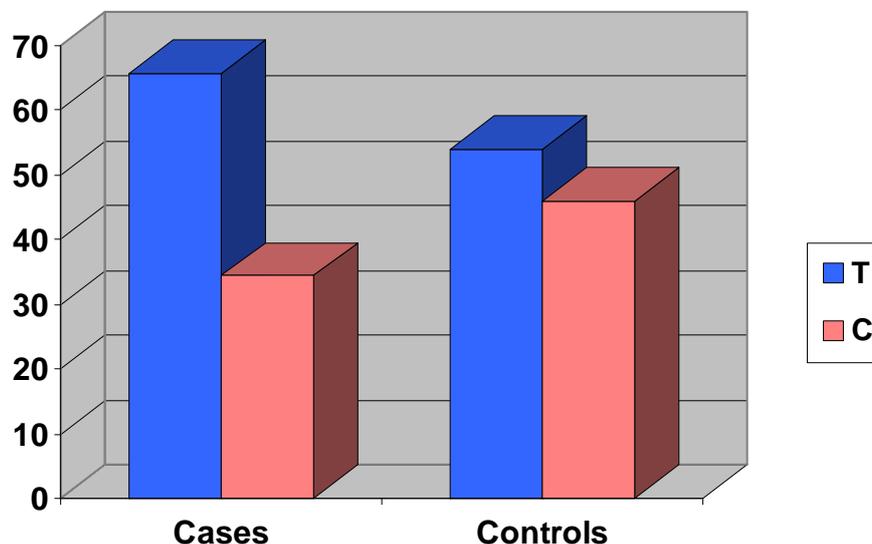


Fig. 2: The percentages of Allele frequency of IFN γ R1 -56 in study subjects and controls

DISCUSSION

Tuberculosis is a social disease that is extremely sensitive to changes in the standard of living. The disease is an infection that is primarily spread and transmitted directly from human being to human being. The degree of crowding and the congested houses are therefore important factors in the spreading of the disease (Puranen, 2003) that is the same factors that is found in rural community beside the direct contact between the populations. In this study the distribution of study subject according to locality showed that 89 (71%) were from rural area and 37 (29%) were from the urban areas.

Different genotypes seem to respond differently to environmental risk factors. Host genetic factors have an important role in the development of clinical disease following infection with tuberculosis, but inheritance of TB susceptibility in the general population is non-Mendelian. The correlation between the molecular pathology, mycobacterial virulence, and clinical phenotype in

inherited IFN γ R1 deficiency suggests that more subtle variation in IFN γ R1 could contribute to *M. tuberculosis* disease susceptibility in an outbred population (Awomoyi *et al.* 2004).

The distribution of IFN γ R1 -56 C >T genotype differs significantly between TB patients and controls and those carrying the mutant C allele were associated with two times reduced risk for susceptibility to TB (P -value = 0.017, odds ratio = 1.6, 95% CI = 1.025 – 1.441).

That means the mutation IFN γ R1-56 CC give protection two times according to this result, that consistent with result of the study uses population collections from the Gambia, Guinea Bissau, and the Republic of Conakry. All cases were confirmed by either two consecutive smear-positive samples or a positive *Mycobacterium tuberculosis* (MTB) culture, and that found there is evidence from an *in vitro* model of cell expression that constructs bearing the IFN γ R1-56 C allele produce less transcriptional activity in a standard

assay system. If these findings can be translated into the clinical setting, might expect individuals with the IFN γ R1-56 CC genotype to express less IFN γ R1 receptor on the cell surface. It is perhaps surprising that this genotype is associated with protection from pulmonary tuberculosis in West African populations. A reduced immune response mediated by IFN γ R1 could protect against pulmonary immunopathology, but given everything else that is known about IFN γ R1, this explanation seems unlikely. More plausibly is that either another variant exists in linkage disequilibrium that might explain the disease association or that the functional role of this polymorphism has not yet been fully characterized (Graham *et al.* 2006) In conclusion, the mutant C allele in the IFN γ R1-56 is associated with two times increased protection to tuberculosis infection. However, more genetic studies are needed, to identify genes associated with susceptibility to tuberculosis.

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ARABIC SUMMARY

دور تعدد الأشكال في مستقبلات الإنترفيرون - 1g جين (-56 T > C) في تطوير القابلية للإصابة بمرض السل الرئوي في وسط السودان

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خلفية: تقدر معدلات الإصابة بمرض السل ب ٨ ملايين حالة جديدة على الصعيد العالمي بالإضافة إلى ٢ إلى ٣ ملايين شخص يموتون سنويا من جراء هذا المرض. اختلافات الجينات البشرية هو أحد المحددات الهامة لنتائج الإصابة بالمتطفرة السلية. الهدف من هذه الدراسة هو تحديد علاقة الإصابة بمرض السل مع تواتر بعض من الأشكال المتعددة لمستقبلات الجاما انترفيرون لمرضى و أصحاب بولاية الجزيرة في وسط السودان.

الأسلوب: تم تحليل تعدد الأشكال للموقع (-56 T > C) في كل الحالات المؤكدة الإيجابية السل (١٢٦) و عينات الضبط الملائمة (٢٠٠) باستخدام PCR - RFLP.

النتائج: أظهرت النتائج أن توزيع IFN γ R1 في النمط الجيني - 56 C>T يختلف بشكل كبير بين مرضى السل والضوابط وارتبطت تلك التي تحمل متحولة الأليل C مع خفض مخاطر التعرض لمرض السل بمعدل مرتين (قيمة P = ٠.٠١٧، نسبة الأرجحية = ١.٦ او بمدى ثقة = ٩٥ (1)، 441,025-1، يعني ذلك أن الطفرة IFN γ R1 -56 C> يعطي حماية مرتين أكثر من أليل نوع البرية. ومع ذلك، يتعين القيام بالمزيد من الدراسات في جينات IFN γ ، وتعدد الأشكال في الجين IFN γ R1 على عدد أكبر من العينات.