

Analysis of *CTLA-4* (+49A/G) Gene Polymorphism and the Risk of Tuberculosis in Southeast of Iran

Edris Paad¹; Mohammad Kordi Tamendani^{1,*}; Mohammad Hosain Sangtarash¹; Mansoor Dehvari¹

¹Department of Biology, University of Sistan and Baluchestan, Zahedan, IR Iran

*Corresponding author: Mohammad Kordi Tamendani, Department of Biology, University of Sistan and Baluchestan, Zahedan, IR Iran. Tel: +98-5412452335, Fax: +98-5412446565, E-mail: dor_kordi@yahoo.com; dor_kordi@science.usb.ac.ir

Received: August 29, 2014; Revised: September 20, 2014; Accepted: October 9, 2014

Background: Tuberculosis (TB) is an acute or chronic bacterial infection caused by *Mycobacterium tuberculosis*. It primarily affects the lungs, but may spread to other organs (extra-pulmonary TB).

Objectives: Studies have shown that genetic predisposition can decrease the persistence and growth of *M. tuberculosis*. The current study highlights the effect of *CTLA-4* (+49A/G) gene polymorphism on the risk of TB.

Patients and Methods: This case-controlled study used tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) to analyze the *CTLA-4* (+49A/G) gene polymorphism in 100 patients with TB and 91 healthy individuals.

Results: Data analysis indicated that the frequencies of AA, AG and GG genotypes were 42%, 38%, and 20% in patients with TB and 43%, 47%, 1% in the control subjects, respectively. The GG genotype of *CTLA-4* (+49A/G) showed a significantly increased risk of disease (OR = 20.48; 2.63-159.60)

Conclusions: The results revealed a significant relationship between the GG genotype of *CTLA-4* and the increased risk of TB.

Keywords: Tuberculosis; *Mycobacterium*; *CTLA-4*

1. Background

Nearly one-third of the world's population (two billion people) is infected with *Mycobacterium tuberculosis* and is at risk of developing tuberculosis (TB). Every year, about nine million people get infected with active TB and approximately 1.5 million people die of this disease (1). More than 90% of the infections and deaths arising from TB occur in developing countries and 75% occur in the most economically-active age group (15-54 years old). In such regions, an adult infected with TB misses an average of 3-4 months of work each year, which accounts for a 20-30% loss of annual revenue (2). It is clear that TB, besides economic loss, has indirect negative effects on the quality of life of patients and their families. These include infected women being shunned by their families or expulsion from or dropping out of school of a patient's children. Concurrent infection by HIV and environmental factors can decrease the activity of immune T cells significantly and increase the risk of infection by TB. Countries with a high incidence of HIV, especially in southern Africa, have experienced a dramatic increase in the number of TB patients. The rates of reported patients with TB raised two to three folds over those recorded in the 1990's (2, 3). Genetic variation contributes to the risk of TB and polymorphism of different

genes can increase the risk of infection by active TB (4). Macrophages are the primary host cells for proliferation of intracellular mycobacteria. They are responsible for killing internalized bacilli by reacting with nitrogen and oxygen intermediates (ROI, RNI) and lysosomal destructive enzymes (5). The entry of *M. tuberculosis* into the body induces activation of cellular immune mechanisms which play important roles in the mechanism of T cells. TH1 cells induce macrophage activation and phagocytosis reactions. These cells mediate the acquired immune response against live phagocytic microbes in phagosomes of macrophages. They recognize microbial antigens and activate phagocytes to destroy the ingested microbes. Activated macrophages kill the microbes that exist inside the vesicle most effectively. Microbes that directly enter the cytoplasm (e.g. viruses) or are released from phagosomes into the cytoplasm, however, are relatively resistant. Eradication of these pathogens requires secondary administrative cellular immune mechanisms such as cytolytic T lymphocytes (CTL) (6). CTL-associated antigen 4 (*CTLA-4*) is a CD28 receptor that inhibits T cell proliferation through combination with B7 molecules (7). The human *CTLA-4* gene is located on chromosome 2q33 and the single-nucleotide polymor-

phism (SNP) +49A/G (rs231775) is located in the first exon of *CTLA-4*. A +49A/G base substitution can cause a change from threonine to alanine amino acid in the coding region of *CTLA-4* (8). The *CTLA-4* +6230 (rs3087243) and *CTLA4* +49 SNPs have shown strong linkage disequilibrium, which have resulted in excellent combinations for haplotype analysis (9, 10). Previous studies have demonstrated that the +49 SNP of *CTLA-4* played a role in the development of Graves' disease (11), systemic lupus erythematosus (12) and other autoimmune diseases. *CTLA-4* +6230 SNP is associated with susceptibility to hepatitis B (13) and inflammatory bowel disease (14). Thye et al. (15) found that the *CTLA-4* +6230G allele contributed to the pathology of TB in the African population. Reif et al. found an association between genetic changes and extra-pulmonary TB (16).

2. Objectives

The present study investigated a possible correlation between *CTLA-4* gene polymorphism and the risk of TB in a sample of the Iranian population.

3. Patients and Methods

3.1. Sample Collection

Subjects diagnosed with TB and healthy controls were selected from Chabahar Health Center, Iran. After the subjects signed written informed consent forms, 5 mL of blood was taken from each individual in an EDTA K2 tube. The subjects with TB were selected from those who had had a confirmed diagnosis by a healthcare professional and who presented clinical symptoms, radiological evidence and positive sputum acid-fast bacillus (AFB) smears. The control subjects had no previous history of TB, autoimmune disease, diabetes, or any inflammatory or respiratory disorder. A total of 191 blood samples were collected (106 subjects with TB and 95 healthy controls). DNA extraction was accomplished using an IQ2000 kit according to the manufacturer's instructions. After estimation of the quality of DNA by spectrophotometer, the tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) method was used to analyze the genotyping of *CTLA4* (+49A/G) with the primers given in Table 1. The PCR conditions for the amplification of *CTLA-4* were as follows: initial phase comprised 10 minutes at 95°C; next 40 cycles (30 seconds at 94°C, 30 seconds at 62°C, and 30 seconds at 72°C); then, seven minutes at 72°C. The samples were stored at 4°C until electrophoresis.

3.2. Statistical Analysis

SPSS version 19.0 (SPSS, Chicago) was used for all the statistical analyses. These included categorical data (Pearson's χ^2), adjustment of P values with variables using the binary logistic regression test for estimation of

thee odds ratios (OR), 95% confidence intervals, and the relationship between *CTLA-4* gene polymorphism and the risk of TB. The significance level was set at $P \leq 0.05$ for all the tests.

4. Results

From a total of 100 patients with TB, 68 (35.6%) had positive smears and 15 (7.9%) had negative smears for pulmonary TB. An additional 9 (4.7%) had extra-pulmonary TB and 8 (4.2%) showed recurrent disease after the treatment. The AA genotype +49A/G was found in 42 (42%) subjects with TB as well as in 43 (47.3%) controls subjects. The AG genotype was observed in 38 (38%) subjects with TB and 47 (51.6%) control subjects. The GG genotype was observed in 20 (20%) patients with TB and 1 (1.1%) control subject.

The A allele frequency was 122 (61.4%) in subjects with TB and 133 (73.08%) in control subjects. Allele frequency for the G allele was 78 (38.6%) in subjects with TB and 49 (26.92%) in control subjects (Table 2).

Table 1. Tetra-Primer Amplification Refractory Mutation System-Polymerase Chain Reaction Primer Sequences

Primers	Primer Sequence(5' → 3')	Length, bp
Outer primer		
Forward	GTGGGTTCAAACA-CATTTCAAAGCTTCAGG	229
Reverse	TCCATCTTCATGCTC-CAAAAGTCTCACTC	229
Inner primer		
Forward, A allele	ACAGGAGAGTGCAGGGCCAG-GTCCTAGT	162
Reverse, G allele	GCACAAGGCTCAGCT-GAACCTGGATG	120

Table 2. Genotypes and Alleles Frequency of *CTLA4* Gene +49A/G Polymorphism in Patients With TB and the Control Group ^{a, b}

Genotype	Cases	Controls	OR (95%CI)	P Value
AA	42 (42)	43 (47.3)	1.00	-
AG	38 (38)	47 (51.6)	0.83 (0.45-1.51)	0.645
GG	20 (20)	1 (1.1)	20.48 (2.63-159.60)	0.0001
AG + GG	58 (58)	48 (52.7)	1.24 (0.69-2.19)	0.471
Allele				
C	122 (61)	133 (73.1)	1.00	-
G	78 (39)	49 (26.9)	1.74 (1.13-2.68)	0.013

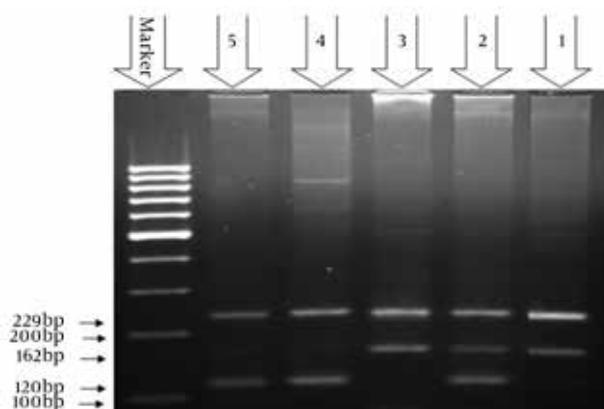
^a Abbreviation: CI, Confidence Interval; OR, Odd Ratio.

^b Data are presented No. (%).

5. Discussion

World Health Organization statistics has emphasized that in many parts of the world, females are more frequently diagnosed with TB than males and more frequently die from the disease. This disease is a major cause of death by causing infectious diseases in females and primarily affects females in their economically-active reproductive ages (17). The polymorphism in the promoter region of the *CTLA-4* gene may play a role in expression of the *CTLA-4* receptor in T cells. It can activate this receptor as a regulatory key to suppress the immune response induced, to inhibit transmission to T cells and interaction of these cells with antigen-acceptor cells. The result is the inability of the immune system to form an appropriate response to the pathogen to facilitate the disease progression. Zuh et al. demonstrated a significant association between the *CTLA-4* gene polymorphism and the risk of chronic bronchitis (18). Motsinger et al. found a significant correlation between the *CTLA-4* gene polymorphism and Graves' disease. Studies have reported a significant link between the *CTLA-4* gene polymorphism and inflammatory bowel disease, ulcerative colitis and Crohn's disease (16). The sparseness of data on the *CTLA-4* gene variation and TB allowed only limited comparison of the data with that from previous studies. The data showed a significant link between the GG genotype of *CTLA-4* and the increased risk of TB in this sample of the Iranian population.

Figure 1. Electrophoresis Pattern of Tetra-Primer Amplification Refractory Mutation System-Polymerase Chain Reaction for Detection of Polymorphisms



Sample 1, 229 bp; 2, 162 bp; 4, 120 bp.

Acknowledgements

The authors wish to thank the University of Sistan and Baluchestan, Chabahar Health Center and the individuals who participated in this study.

Authors' Contributions

Edris Paad performed data collection and laboratory

work. Mohammad Kordi Tamendani and Mohammad Hosain Sangtarash contributed to the study design, statistical analyses and interpretation of the results. Mansoor Dehvari drafted the manuscript. All the authors read, modified and approved the final version of the manuscript.

Funding/Support

This project was funded by the University of Sistan and Baluchestan.

References

- Sandhu GK. Tuberculosis: current situation, challenges and overview of its control programs in India. *J Glob Infect Dis.* 2011;**3**(2):143-50.
- Joshi JM. Tuberculosis chemotherapy in the 21 century: Back to the basics. *Lung India.* 2011;**28**(3):193-200.
- Mihret A. The role of dendritic cells in Mycobacterium tuberculosis infection. *Virulence.* 2012;**3**(7):654-9.
- Bellamy R. Susceptibility to mycobacterial infections: the importance of host genetics. *Genes Immun.* 2003;**4**(1):4-11.
- Schaible UE, Collins HL, Kaufmann SH. Confrontation between intracellular bacteria and the immune system. *Adv Immunol.* 1999;**71**:267-377.
- Abbas AK, Lichtman AH. *Basic Immunology: Functions and Disorders of the Immune System.*: Saunders; 2004.
- Anderson DE, Sharpe AH, Hafler DA. The B7-CD28/CTLA-4 costimulatory pathways in autoimmune disease of the central nervous system. *Curr Opin Immunol.* 1999;**11**(6):677-83.
- Harper K, Balzano C, Rouvier E, Mattei MG, Luciani MF, Golstein P. CTLA-4 and CD28 activated lymphocyte molecules are closely related in both mouse and human as to sequence, message expression, gene structure, and chromosomal location. *J Immunol.* 1991;**147**(3):1037-44.
- Munthe-Kaas MC, Carlsen KH, Helms PJ, Gerritsen J, Whyte M, Feijen M, et al. CTLA-4 polymorphisms in allergy and asthma and the TH1/ TH2 paradigm. *J Allergy Clin Immunol.* 2004;**114**(2):280-7.
- Amundsen SS, Naluai AT, Ascher H, Ek J, Gudjonsdottir AH, Wahlstrom J, et al. Genetic analysis of the CD28/CTLA4/ICOS (CELIAC3) region in coeliac disease. *Tissue Antigens.* 2004;**64**(5):593-9.
- Kouki T, Sawai Y, Gardine CA, Fisfalen ME, Alegre ML, DeGroot LJ. CTLA-4 gene polymorphism at position 49 in exon 1 reduces the inhibitory function of CTLA-4 and contributes to the pathogenesis of Graves' disease. *J Immunol.* 2000;**165**(11):6606-11.
- Ulker M, Yazisiz V, Sallakci N, Avci AB, Sanlioglu S, Yegin O, et al. CTLA-4 gene polymorphism of exon 1(+49 A/G) in Turkish systemic lupus erythematosus patients. *Int J Immunogenet.* 2009;**36**(4):245-50.
- Thio CL, Mosbrugger TL, Kaslow RA, Karp CL, Strathdee SA, Vlahov D, et al. Cytotoxic T-lymphocyte antigen 4 gene and recovery from hepatitis B virus infection. *J Virol.* 2004;**78**(20):11258-62.
- Repnik K, Potocnik U. CTLA4 CT60 single-nucleotide polymorphism is associated with Slovenian inflammatory bowel disease patients and regulates expression of CTLA4 isoforms. *DNA Cell Biol.* 2010;**29**(10):603-10.
- Thye T, Scarisbrick G, Browne EN, Chinbuah MA, Gyaopong J, Osei I, et al. CTLA4 autoimmunity-associated genotype contributes to severe pulmonary tuberculosis in an African population. *PLoS One.* 2009;**4**(7).
- Motsinger-Reif AA, Antas PR, Oki NO, Levy S, Holland SM, Sterling TR. Polymorphisms in IL-1beta, vitamin D receptor FokI, and Toll-like receptor 2 are associated with extrapulmonary tuberculosis. *BMC Med Genet.* 2010;**11**:37.
- World Health Organization. *Global Tuberculosis Control: Surveillance, Planning, Financing WHO Report 2008.*; 2008.
- Zhu G, Agusti A, Gulsvik A, Bakke P, Coxson H, Lomas DA, et al. CTLA4 gene polymorphisms are associated with chronic bronchitis. *Eur Respir J.* 2009;**34**(3):598-604.