Association Between Interleukin-4 Promoter and Receptor Polymorphisms and T-cell Count in Iranian HIV Positive Population

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Abstract

Background: Single Nucleotide Polymorphisms (SNPs) in the IL-4 promoter and receptor genes have been proposed to alter the individual's susceptibility to HIV infection and the rate of progression to AIDS. IL-4 -589 C/T and IL-4Rα I50V polymorphisms have been studied in different populations and were linked to differences in disease progression. No study has assessed the frequency of these polymorphisms in Iranian population.

Methods: In a cross-sectional study, after obtaining written informed consent, blood samples were collected from 120 HIV-seropositive individuals (91 males, 12 females) visiting a tertiary referral HIV center. All patients were primarily screened with the fourth-generation ELISA HIV test for the HIV-1 markers and subsequently with polymerase chain reaction (PCR). $CD4^+$ cell count of the patients was recorded by using fresh EDTA-whole blood samples. The genomic DNA was extracted from peripheral blood mononuclear cells (PBMC) using the standard extraction kit. Then, PCR-RFLP technique was used to analyze IL-4 -589 C/T, and IL-4Rα I50V SNPs.

Results: A frequency of 0.08 was found for IL-4 -589 T allele and 0.055 for IL-4Rα V allele. There was no significant difference between these polymorphisms regarding CD4 cell counts ($p = 0.44$ and $p = 0.08$ for IL-4 -589 and IL-4RαI50V, respectively).

Conclusion: This is the first study to assess the frequency of IL-4 promoter and receptor polymorphisms in the Iranian HIV-seropositive population. No significant association was found between IL-4 promoter and receptor polymorphisms and CD4 cell counts.

Keywords: CD4 lymphocyte count, HIV, Interleukin-4, Polymorphism, Promoter

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Introduction

Iran suffers from the largest HIV epidemic in the middle east (1). The official reports estimated total HIV-seropositive cases of 75,700 in 2014; however, the real number may be much higher (2). The country-specific cultural, political, and legal issues create barriers to research in the field (3). In spite of the efforts, the stigma of HIV infection still hinders access to proper treatment in the most vulnerable populations, including prisoners, sex workers, men who have sex with men, and injection drug users (2). Therefore, HIV has remained a public health issue, and research focusing on enhancing our understanding of the disease and development of new approaches to diagnosis and treatment is needed.

From a multitude of factors that determine the susceptibility of the individual to HIV infection, the rate of the progression, response to treatment, and host genetics have been studied the most (4). Polymorphisms of the genes coding for chemokine co-receptors, major histocompatibility complex (MHC), and cytokines have been found to regulate the transmission and pathogenesis of HIV infection (5). A 32 base-pair deletion in the CCR5 gene confers resistance to HIV infection in homozygous individuals and is the epitome of the impact of host genetics on HIV pathogenesis (6). Advances in our understanding of the intricate relationship between host genetics and HIV infection will undoubtedly result in the development of new therapeutics and preventive approaches.

Interleukin-4 (IL-4) is a multifunctional cytokine which regulates various aspects of the immune system. It modulates the immune response by inducing the production of immunoglobulin E (IgE) and IgG isotype switching in B lymphocytes (7). It also induces immature T-cells to assume a Th2 phenotype, and represses Th1-inducing signals (8). Various authors have studied the role of IL-4 gene polymorphisms in an array of immune-mediated diseases including atopy and asthma (9) and regulation of antimalarial responses (10). IL-4 also regulates the expression of chemokine receptors CCR5 and CXCR4, which, as noted above, are of utmost importance in the individual's susceptibility to HIV infection and its progression to AIDS (11).

In the IL-4 gene, a C- to T- Single Nucleotide Polymorphism (SNP) at position -589 upstream from the open reading frame has been associated with increased promoter activity and IL-4 transcription, and was first linked to elevated IgE levels in asthmatic families (12). Subsequently, Nakayama *et al* studied the link between the -589T/T genotype and HIV progression to AIDS (11). Although studies have reported divergent results regarding the effects of this SNP on HIV susceptibility and progression, the major role of IL-4 and its polymorphisms is not a matter of debate (13). A polymorphism located at +148 position of the interleukin-4 receptor (IL-4R) alpha subunit (transition A/G, Ile50 Val) also affects IL-4 induced IgE production and has been linked to many immune system disorders (14), and susceptibility to HIV infection (6).

There is a considerable variation in the allelic distribution and haplotypic relationships of SNP variants among different ethnic groups. Additionally, the effects of host genetic polymorphisms may differ in various geographical areas with discrete strains of HIV. Therefore, it is important to study the prevalence of genetic polymorphisms and their influence on HIV pathogenesis. The goal of this study was to estimate the prevalence of IL-4 589 C/T and IL4R-alpha I50V polymorphisms in Iranian HIV-seropositive patients and examine the association between these SNPs with CD4 cell counts.

Materiala and Methods

Study population

A prospective study at the HIV clinic was conducted in a tertiary referral center. All adult patients with confirmed HIV-1 seropositivity who attended the clinic from March 2019 to August 2019 were invited to participate in this study. All participants were interviewed by one of the main investigators and clinically examined by two trained general physicians.

HIV status and CD4 count

After obtaining EDTA-treated blood from each individual, all plasma samples were primarily screened with ELISA HIV test (4thgeneration, BIO-RAD, USA) for the HIV-1 markers and subsequently with polymerase chain reaction (PCR) . The CD4^{+} cell count was measured with flow cytometry (Partec CyFlow SL) using the fresh EDTA-whole blood samples.

Genomic analysis

Peripheral Blood Mononuclear Cells (PBMCs) were obtained using the Ficoll-Hypaque method and the genomic DNA was extracted using the highly pure PCR template preparation kit (Roche LifeScience, Germany). Samples were stored at -80°C until use.

A single base pair (bp) polymorphism at the -589 position in the promoter of IL-4 gene was analyzed by the PCR-restriction fragment length polymorphism (PCR-RFLP) method. Forward primer 5′-TAAACTTGGGAGAACATGGT-3′ and reverse primer 5′-TGGGGAAAGATAGAGTAATA-3′ were used to amplify a 195-bp fragment spanning position -589. PCR amplification cycling conditions included an initial denaturation at 93°C for 5 min, followed by 40 cycles of 94°C for 30 s, 48°C for 30 s, and 72°C for 30 s. The PCR product was digested with AVAII (MBI Fermentas, Vilnius, Lithuania) restriction enzyme which yields a 177-bp and 18-bp fragment only when a C is present.

For the IL-4Rα I50V, forward primers 5′-GGCAGGTGTGAGGAGCATCC-3′ and reverse primer 5′-GCCTCCGTTGTTCTCAGGGA-3′ were used. Cycling conditions included an initial denaturation at 93°C for 5 min, followed by 40 cycles of 93°C for 60 s, 60°C for 60 s, and 72°C for 60 s. The 273-bp PCR-amplified product was digested with RsaI (MBI Fermentas, Vilnius, Lithuania) restriction enzyme, which yields a 273-bp fragment for I allele and a 254-bp fragment when V allele is present.

Ethical consideration

The protocol of the present study was reviewed and approved by the Institutional Review Board (IRB) of Tehran Univerity of Medical Sciences (Ethic code: IR.TUMS.VCR.REC.1396.2856). All the patients were informed regarding the purpose of the study. The written consent was obtained from all participants.

Statistical analysis

Statistical analysis was done by SPSS software version 22.0 (SPSS, Chicago, IL). The direct gene counting method was used to determine the frequency of genotypes and alleles. The Chi-square test was used to determine differences in allele/genotype frequencies. A p-value <0.05 was considered statistically significant.

Results

A total of 120 HIV-seropositive patients (91 male, 12 female) enrolled in this study. Only 99 patients (mean age of 36±1.2 years) had acceptable bands in the post-PCR electrophoresis. Table 1 summarizes the distribution of IL-4 promoter and receptor polymorphisms among the patients.

Table 1. Distribution of IL-4 promoter and receptor genotypes among HIV-positive patients

Genotype	No. of patients	% of total	Mean CD4 cell counts/*µl*
IL-4 589			
CC	81	74.3	468
CT	11	10.1	412.6
TT	3	2.8	286
IL-4 receptor			
II	93	85.3	441.4
IV	1	0.9	87
VV	5	4.6	421.8
CC: homozygous wild; TT: homozygous mutant; CT: heterozygous; I: isoleucine; V: valine			

Table 2. Distribution of CD4 cell count among HIV-positive patients by groups

Group	No.	% of total
Group 1 (<200/*µl*)	24	24.7
Group 2 (200-350/*µl*)	16	16.5
Group 3 (351-500/*µl*)	18	18.5
Group 4 (>500/*µl*)	39	40.2

There was no significant difference between these polymorphisms regarding CD4 cell counts (p=0.44 and p=0.08 for IL-4 -589 and IL-4RαI50V, respectively). Table 2 summarizes the CD4 cell counts of the patients in this study.

Discussion

To our best of knowledge, this is the first study reporting the prevalence of IL-4 -589 and IL-4RαI50V polymorphisms in Iranian HIV-seropositive patients. In the present study, an attempt was made to determine the frequency of IL-4 promoter and receptor polymorphisms and whether CD4 cell count has a relationship with any of these genetic polymorphisms in the Iranian population.

CD4 cell count has been an important biomarker of disease progression in HIV infection. In our study,

the median CD4 cell count was 438 cells/µl (Range; 6-1127 *cells/µl*) which is higher than previous reports and may explain some differences in findings (13).

Gene polymorphism has been shown to change the levels or activity of specific proteins resulting in altered function (14). Therefore, studying the frequency of polymorphisms in different populations which affects disease severity and prognosis is critical to improve our understanding of HIV.

The frequency of IL-4 -589 T allele was 0.08 in our study of Middle Eastern patients, which is significantly lower than what has been reported in Thai (0.78), Japanese (0.69), and Chinese (0.77) population. However, it is more in line with the reported frequency (0.15) in other Caucasian populations (7,15-17). This may explain the difference in the CD4 cell counts between the previous and the present study.

The presence of IL-4 -589 T allele has been previously linked to an increase in promoter activity. Nakayama *et al* reported an increase in the IL-4 and IgE levels in association with this SNP. As IL-4 down-regulates CCR5, which is an HIV coreceptor, they proposed that the polymorphism will decrease the rate of HIV virus acquisition. On the contrary, because of the cellular immunity suppressing effects of IL-4, they speculated that IL-4 -589 T allele would accelerate disease progression (11). Subsequently, in their second study, they reported on 427 HIV-seropositive subjects with known seroconversion dates and confirmed that the presence of IL-4 -589 C/T polymorphism makes delay in progression to AIDS (7).

There are other studies with conflicting results. Chatterjee *et al* found no association between the IL-4 -589 T allele SNP and HIV acquisition or progression (14). Furthermore, Kwa *et al* observed a delayed HIV acquisition in homosexual men with the IL-4 -589 C/T polymorphism but did not observe any effects on disease progression (15). Singh *et al*, in an study on HIV-infected children from the United States, found no association between the IL-4 -589 polymorphism and disease progression and suggested that this polymorphism is unlikely to play an important role in the rate of progression to AIDS (18). In this study, no significant relationship between the IL-4 -589 C/T polymorphism and CD4 cell count could be found. One could argue that viral factors such as subtype, phenotype, and viral load could explain the conflicting results. Also, the differences in populations and racial groups which is common in polymorphic systems could explain the diverging results.

Several studies have found that IL-4RαI50V polymorphism alters the IL-4 activity and IgE levels. Soriano *et al* found that the V homozygosity results in slower disease progression in IV drug users (19). Some evidence suggest that I50 and V50 homozygosity involves hyper- and hypo-responsiveness, respectively of IL-4R to IL4 concerning IgE production. Chatterjee *et al* found an association between IL-4RαI50 allele and susceptibility to HIV infection among North Indians and argued that the hyperresponsiveness of IL-4R to IL4 favors a Th2 response, which results in a rapid progression to AIDS (14). A lower CD4 cell count in the variants of IL-4Rα was not observed so the fact that our patients were not followed over the time might limit the generalizability of our results. Also, a frequency of 0.055 for the V allele was found. Chatterjee *et al* found a frequency of 0.23 for the V allele in HIV-seropositive patients, and 0.35 in seronegative patients (14). Soriano *et al* found a frequency of 0.36-0.6 for the V allele, which is also higher than our findings (19). Both of these studies have found associations between disease progression and IL-4R polymorphism. The low frequency of the V allele in our study, together with the small number of patients, might partly explain why no association could be found.

Limitations

This study has some limitations. First, this study is not a prospective cohort of seroconverters with a defined start date of infection; therefore, the effect of SNPs on HIV progression cannot be documented. The second limitation is the fact that most of our patients were undergoing antiretroviral treatment (ART), and this poses some heterogeneity in CD4 cell counts. Third limitation is that there was no control group and thus differences in the host polymorphism between the HIV-seropositive and seronegative populations could not be determined.

Conclusion

This is the first study that determined the frequency of IL-4 -589 C/T and IL-4RαI50V polymorphisms in Iranian HIV-seropositive patients. The frequency of IL-4 -589 T allele was more in line with previous reports on the patients. No link between IL-4 and

IL-4R polymorphisms and CD4 cell count could be detected in this study. The difference in frequency of alleles or the discrepancy in study design may explain some disparate findings. Population studies are important to demonstrate the disease pathogenesis and develop screening tests for identifying people at risk and measuring their response to different medicines. Therefore, more population-based studies are needed to elucidate the exact effect of different polymorphisms in the pathogenesis of HIV infection.

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Conflict of Interests

The authors declare no conflict of interests.

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