

Original Article

Lack of Association between Interleukin-10 Gene Promoter Polymorphisms with HIV Susceptibility and Progression to AIDS

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ABSTRACT

Background & Objective: Interleukin (IL)-10 is an important anti-inflammatory and immunomodulatory cytokine. Some authors believe that single nucleotide polymorphisms (SNP) in the promoter region of the IL-10 gene have been associated with susceptibility to HIV infection and progression to AIDS, but its role is not clearly defined yet. The present study was undertaken to evaluate the association between HIV infection susceptibility and progression with SNP in the promoter region of the IL-10 gene.

Methods: This study was carried out on 70 HIV infected patients (39 treatment naïve and 31 under treatment) and 31 matched healthy controls. The biallelic polymorphisms in the IL-10 gene promoter (-592, -1082) were analyzed by polymerase chain reaction and direct sequencing.

Results: At position -1082, G/A was the most common genotype and A was the most prevalent allele and at position -592, A/C was the most prevalent genotype and -592 C was the most common allele in HIV positive patients; although there was not any significant difference between cases and controls regarding genotypes and alleles of these regions.

Conclusion: Genetic polymorphisms of IL-10 promoter region may not associate with HIV infection outcome and the lack of this association suggests that other genes may influence on HIV infection course.

Keywords: Human Immunodeficiency Virus (HIV); Interleukin (IL)-10 Gene; Genotype

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Introduction

Human immunodeficiency virus (HIV) infection represents a major global health problem and is the fourth leading cause of death worldwide (1). Clinical course of HIV infection and progression to AIDS, can vary considerably in population and is associated significantly with host genetic factors such as cytokines encoded by Interleukin (IL)-10 (2).

IL-10 is an immunoregulatory cytokine mainly produced by monocytes, macrophages, T helper 2 cells and B lymphocytes and can inhibit secretion of interferon γ (IFN- γ) and IL-2 from T cells and IL-1, IL-6, IL-8 and tumor necrosis factor α (TNF- α), from monocytes and macrophages. Because of this inhibitory effect on cytokine production, it is generally defined as anti-inflammatory cytokine (3, 4). IL-10 also inhibits different immunologic reactions, like antigen presentation, macrophage activation, antigen specific T cell proliferation and cell mediated immunity (5, 6).

IL-10 has been associated with pathogen persistence and poor clinical outcome of several infectious diseases including HIV (7). Animal models suggest that generally IL-10 has specific influence on HIV infection through T cell activity (8, 9). IL-10-positive CD8⁺ T cells have a regulatory role in the immune response against HIV infection (10). IL-10 levels are higher in HIV infected patients with lower CD4⁺ cell counts and higher HIV viral loads than cases newly infected with HIV (11). While, in long term non-progressors HIV infected patients, IL-10 level is comparable to individuals without HIV (12).

In contrast some authors reported that polymorphisms associated with decreasing of IL-10 production causes increase the risk of acquiring HIV infection and accelerates the rate of reducing CD4⁺ cell counts particularly in advance stage of disease, and they suggested that high IL-10 production may decrease the risk of HIV acquisition and protect against developing AIDS (13-15). Therefore, it seems that IL-10 provides a biologi-

cal balance in the immune system by suppressing or reinforcing immunological reactions and can act helpfully in certain cases and harmfully in the others (16).

Several polymorphic sites within the IL-10 gene promoter region have been described, including three biallelic polymorphisms at positions -1082, -819, and -592 from the transcription start site (17). The association of IL-10 promoter polymorphisms with HIV progression demonstrated in some population, like European and African American, Indian and Thais (3,4,15,16,18); but this association is not universal in different investigated population.

Due to contradictory reports about the impact of IL-10 promoter polymorphisms and the susceptibility to HIV infection and disease progression, the present study was undertaken to evaluate the role of single nucleotide polymorphisms (SNP) in the promoter regions of the IL-10 gene on HIV infection susceptibility and progression.

Patients and Methods

Study population:

Overall, 70 HIV positive patients referred to Iranian Research Center for HIV/AIDS in Tehran, Iran and 31 matched (age and sex) HIV-seronegative healthy subjects were enrolled. Thirty-nine HIV infected patients were treated naive and 31 cases were under antiretroviral treatment.

Informed consent was obtained from all patients. A questionnaire that gathered epidemiological and clinical data was completed by clinicians. The project was approved by Iranian Research Center for HIV/AIDS Ethics Committee.

HIV-antibody was determined by ELISA (MP Biomedicals, Illkirch, France); with positive tests confirmed by Western blot assay (Diaplus, San Francisco, USA). All assay protocols, cut-offs, and result interpretations were carried out according to the manufacturers' instructions. CD4⁺ cell counts was determined by flowcytometry and defined as cells/mm³.

IL-10 Genotyping

Genomic DNA was extracted from 300 µl whole blood using a DNA Purification Kit QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The 2 biallelic IL-10 promoter polymorphisms were detected by PCR using primers that amplified a short fragment of DNA containing the polymorphisms sites in combination with direct sequencing. The parameters for thermocycling were as follows: denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec; annealing at 65 °C for 30 sec; and extension at 72°C for 30 sec. This was followed by final extension at 72°C for 10 min. The primers used in the PCR were as follows:

IL-10-1082

F: 5'-CTCGCTGCAACCCAACTGC-3'

R: 5'-TCTTACCTATCCCTACTACTTCC-3'

IL-10-592

F: 5'-GTGAGCACTACCTGACTAAGC-3'

R: 5'-CCTAGGTCACAGTGACGTGG-3'

PCR products were electrophoresed on a 1.5% agarose gel, containing ethidium bromide and visualized on a gel documentation system.

PCR products were purified using the High Pure PCR product purification kit (Roche Diagnostics GmbH, Mannheim, Germany) and sequenced directly by the ABI sequencer.

Statistical Analysis

Data were analyzed by the Chi-square and Fisher's exact test using the SPSS 16.0 data analysis software package; *P* values <0.05 were considered statistically significant. The odds ratio (OR), which reflects the likelihood of a subject carrying a specific genotype and the 95% confidence interval (95 % CI) were calculated. Logistic regression analysis was also used to

determine the association of a specific genotype with HIV infection progression.

Results

A total of 70 HIV positive patients (39 treatment naïve and 31 under treatment) with mean age 36 ±9.1 years and 31 matched healthy controls were enrolled in the study. 58.2% of patients were male and 41.8% were female. The mean CD4⁺ cell counts of patients were 383.8±229.9 (10-1031) cells/mm³. The possible routes of HIV transmission were intravenous drug use (42.9%), infected husband (21.4%), heterosexual contact (14.3%), infected blood and blood products transfusion (4.3%), tattooing and heterosexual contact (1.4%), tattooing and intravenous drug use (1.4%), heterosexual contact and intravenous drug use (4.3%), and in 10%, the route of HIV acquisition was not identified.

Polymorphisms of the IL-10 -592 in HIV infected patients and healthy controls

The prevalence of the C/C genotype was 31 (44.3%) in patients and 16 (51.6%) in controls, the frequency of the A/C genotype was 35 (50%) and 11 (35.5%) in patients and controls, respectively, and A/A genotype in the patient group was 4 (5.7%) and in controls was 4 (12.9%). The frequency of the C allele was 97 (69.3%) and 43 (69.3%) in patients and controls, respectively. A allele was seen in 43 (30.7%) and 19 (30.6%) of cases and controls respectively. Statistical analysis showed no significant difference between cases and controls regarding these genotypes and alleles (Table 1).

The -592 A/C was the most prevalent genotype (50%) and the -592 C was the most common allele (69.3%) in HIV positive patients.

Polymorphisms of the IL-10 -1082 in HIV infected patients and healthy controls

Evaluation of polymorphisms within the -1082

region of the IL-10 gene showed that homozygous A/A genotype was detected in 28 (40%) of patients and 13 (41.9%) of controls, G/A genotype was seen in 32 (45.7%) and 15 (48.4%) of cases and controls, respectively, and G/G genotype was reported in 10 (14.3%) and 3 (9.7%) of patients and healthy controls respectively. Statistical analysis showed no significant difference between groups regarding the genotypes of this locus. A allele was seen in 88 (57.1%) and 41 (66.1%) of patients and controls, respectively

and 52 (37.1%) of the patients and 21 (33.8%) of controls had G allele respectively. Statistical analysis showed that the alleles frequency were also not significantly different between groups. The OR and 95% CI of the genotypes of IL-10 are shown in Table 1.

At position IL-10 -1082, G/A was the most common genotype (45.7%) and A was the most prevalent allele (57.1%) in HIV infected subjects.

Table 1- Genotype distribution of IL-10 in HIV positive and negative subjects

Locus	Genotypes	Cases n (%)	Controls n (%)	OR*	95% CI**	P value
-592	C/C	31(44.3)	16(51.6)	0.75	0.3-1.7	NS***
	C/A	35 (50)	11(35.5)	1.8	0.8-4.5	NS
	A/A	4(5.7)	4(12.9)	0.3	0.06-1.4	NS
-1082	G/G	10(14.3)	3(9.7)	1.4	0.3-5.5	NS
	G/A	32(45.7)	15(48.4)	0.9	0.4-2.1	NS
	A/A	28(40)	13 (41.9)	0.9	0.4-2.2	NS

*: Odds ratio; **: Confidence interval; ***: Not significant

Polymorphisms of the IL-10 -592 in treated and untreated HIV infected patients

The homozygous variant C/C genotype was reported in 11 (35.5%) of treated HIV infected patients and 20 (51.3%) of untreated HIV infected cases, the frequency of the heterozygous A/C genotype was 19 (61.3%) and 16 (41%)

in treated and untreated HIV infected patients, respectively, and the A/A genotype was detected in 1 (3.2%) and 3 (7.7%) of treated and untreated patients respectively. No significant difference was seen between these groups regarding the genotypes of -592 region (Table 2).

Table 2- Genotype distribution of IL-10 promoter region among HIV treated and untreated groups

Locus	Genotypes	HIV treated group n* (%)	HIV untreated group n (%)	P value
-592	C/C	11 (35.5)	20(51.3)	NS**
	C/A	19 (61.3)	16(41)	NS
	A/A	1(3.2)	3(7.7)	NS
	G/G	2(6.4)	8(20.5)	NS
-1082	G/A	15(48.4)	17(43.6)	NS
	A/A	14(45.2)	14 (35.9)	NS

*: Number **: Not significant

Polymorphisms of the IL-10 -1082 in treated and untreated HIV infected patients

A/A genotype was revealed in 14 (45.2%) of treated HIV infected patients and 14 (35.9%) of untreated cases, the frequency of the G/A genotype was 15 (48.4%) and 17 (43.6%) in treated and untreated HIV infected patients, respectively, and the values for the G/G genotype in the treated patient group was 2 (6.4%) in comparison to untreated cases which was 8 (20.5%). Statistical analysis showed no significant difference between groups regarding these genotypes (Table 2).

Associations of IL-10 promoter SNPs with CD4⁺ cell counts

To examine the relationship between IL-10 promoter SNPs and CD4⁺ cell counts in HIV infected cases, the patients were classified into three groups on the basis of their CD4⁺ cell counts [low (<200); moderate (200–350) and high CD4⁺ cell counts (>350)]. No significant differences in -592 and -1082 genotypes and CD4⁺ cell counts were seen.

Discussion

In this study, we evaluated the association of IL-10 promoter gene polymorphisms with HIV acquisition and disease progression. At position -1082, the G/A was the most common genotype and A was the most prevalent allele and at position -592, the A/C was the most prevalent genotype and the -592 C was the most common allele in HIV positive patients; although there was not any significant difference between cases and controls regarding genotypes and alleles of these regions.

IL-10 is an important anti-inflammatory, immunosuppressive and immunomodulatory cytokine, which is associated with many diseases (19) and is involved in the regulation of

inflammatory response, autoimmunity, infection progression, tumorigenesis and transplantation tolerance (20-23). IL-10 can affect several aspects of immune reaction and suppresses HIV replication in vivo (4). Polymorphisms in the IL-10 promoter region and its influence on HIV infection acquisition and progression have been implicated in different populations throughout the world (3,4,15-18).

Naicker *et al.* (13) reported that cases with -592 A/A genotype were at more risk of HIV acquisition in South Africa. They reported that there was no significant difference in the distribution of IL-10 genotypes at the position -1082 between the HIV negative and HIV positive groups. These results are reinforced by the observation that lower IL-10 producer haplotypes were more likely to become HIV infected. Carriers of -592 C/C and A/C genotypes showed lower viral loads during the early chronic phase of infection.

Shin *et al.* (15) also demonstrated that patients with -592 A allele were more likely to become HIV infected in comparison to subjects with wild type allele in North America. They found that the carriers of G allele at position -1082 had a better prognosis and lower progression to AIDS.

Another study by Chatterjee *et al.* (4) also revealed that the frequency of IL-10 -592 A allele was significantly higher in patients with HIV in comparison to HIV negative subjects. They also reported that HIV infected patients with IL-10 -592 A allele have greater risk of developing AIDS. This study reported that individuals carrying -592 A/C might be at more risk for HIV transmission and progression to AIDS in North India.

Oleksyk *et al.* (16) found no differences in IL-10 allelic or haplotype frequencies between HIV negative and positive cases in European Americans, and they demonstrated that carriers of low producer haplotype (ATA) progress to AIDS faster.

Another study in Denmark demonstrated that carriers of G at position -1082 and C at position -592 showed a better survival and lower progression to AIDS, especially in the late stages of infection (14).

Variants of IL-10 and related genes may influence various immunological and virological outcomes of HIV infection and immunological response to antiretroviral treatment in African Americans (6). In a study on Northern Thais, IL-10 -1082 A/A genotype and A allele and -592 A/C genotype and A allele were the most prevalent genotypes and alleles in HIV infected patients. No associations of any of the IL-10 SNPs with CD4⁺ cell counts or with viral load were found in this study (3).

At position IL-10- 592, the A/A and C/C genotypes were a little more frequent in cases than in controls; meanwhile A/C genotype was more detected in controls than patients (18). The authors reported that there was no statistically significant association for risk of HIV susceptibility and developing to AIDS with IL-10- 592 variation in North of India.

Our results showed no statistically significant difference between genotypes of IL-10 and risk of HIV susceptibility and disease progression at position -1082 and -592 among cases and controls, which are in agreement with Kingkeow *et al.* (3) and Sobti *et al.* (18) results.

The discrepancy between the studies may be due to geographical and epidemiological variations and study conditions, the presence of different HIV genotypes and composition and number of the study cohort.

Previously we demonstrated that IL-10 levels increased with HIV disease progression and remained high in under treatment patients. In treatment naïve patients, IL-10 was relatively lower than under treatment patients. However, we did not find any significant difference between HIV positive and negative cases regarding IL-10 levels (24). Therefore, it seems that in Iranian

HIV infected patients, IL-10 levels has not significant role in HIV disease course.

Conclusion

Our study showed that genetic polymorphisms of IL-10 promoter region may not associate with HIV infection outcome and the lack of this association suggests that other genes may influence on HIV infection course. However, due to limited number of patients in the current study, a conclusion cannot be reached regarding the association of IL-10 gene promoter polymorphisms and HIV infection acquisition and progression, so further studies are warranted with large cohort groups and long time follow up in order to more clarify this association.

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