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Research Paper

Cytokine gene polymorphisms among North Indians: Implications for genetic predisposition?

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ABSTRACT

Variations in the production and activity of cytokines influence the susceptibility and/or resistance to various infectious agents, autoimmune diseases, as well as the post-transplant engraftment/ rejection. Differences in the production of cytokines between individuals have been correlated to single nucleotide polymorphisms (SNPs) in the promoter, coding or non-coding regions of cytokine genes. The present study aimed at understanding distribution of cytokine gene variants among HIV seropositive subjects including HIV + TB+ subjects of Indian origin. Our findings indicate significant association of pro-inflammatory (IL2, IFN-γ, TNF-α) and anti-inflammatory cytokine gene variants (IL4, IL10) with the risk to acquire the HIV infection and development of AIDS related illness in Indian population. Since distribution of genetic polymorphisms varies significantly across different populations, different genotypes might exhibit different disease-modifying effects. An understanding of the immunogenetic factors or AIDS restriction genes is important not only for elucidating the mechanisms of disease pathogenesis but also for vaccine design and its application.

1. Introduction

Since the beginning of HIV/AIDS epidemic, the Human Immunodeficiency Virus (HIV) has infected > 70 million individuals, of which approximately 35 million have died. As per the World Health Organization's (WHO) global data report, in 2017 about 36.9 million (31.1–43.9 million) people were living with HIV/AIDS and about 0.9 million died of HIV-related illnesses (WHO, 2017). In 2017, HIV prevalence in adults aged 15–49 years is estimated to be 0.8% (0.6–0.9%) worldwide, with India alone accounting for 0.22% (0.16–0.30%) [0.25% (0.18–0.34) among males and 0.19% (0.14–0.25) among females] (GOI, 2017). Adult HIV prevalence in India has gradually declined from its highest at 0.38% in 2001–03 to 0.22% in 2017 (GOI, 2017). However, given the huge population size in India (approx. 1.3 billion), even this seemingly small percentage accounts for 2.9 million individuals. Hence, HIV/AIDS remain a major public health issue with an urgent need of expansive investigations that may enhance our understanding to the disease and allow development of novel approaches to counter its global burden.

HIV-1 infection leads to reduction in the CD4+ T cells, dysfunctional thymic T lymphocytes and alters levels and functions of antigen presenting cells (APCs) (Kedzierska et al., 2000; Smith, 2006). However, a lot of inter-individual variation exists during the course of HIV infection. Not all the infected persons develop AIDS at the same pace. About 70–80% of infections remain clinically latent before progressing to full-blown AIDS years later (Pantaleo et al., 1993). Such unsteady pattern of disease progression and host susceptibility has been shown to be in conjunction to single nucleotide polymorphisms (SNPs) (Michael, 2002) in genes that encode molecules involved in HIV adsorption and penetration, regulation of anti-HIV immune responses and identification of viral epitopes by $CD4^+$ and $CD8^+$ T cells. Recent studies indicate the relevance of Interleukin signaling pathways during HIV infection and that the variations in interleukin genes might influence susceptibility/resistance to HIV acquisition (Tsiara et al., 2018). Among all of these, cytokine gene polymorphisms have been described as one of the most important factors that render individuals susceptible to HIV-1 infection and may also promote viral evolution.

In view of the above, the present study investigated 22 SNPs in the genes coding for 13 cytokines, viz., interleukin (IL)-1-α (T/C –889), IL-1 β (-511C/T, T/C + 3962), IL-1R (C/T pstI 1970), IL-1Rα (T/C mspaI

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1100), IL-4Rα (G/A + 1902), IL-12 (C/A –1188), IL-2 (T/G –330 G/T + 166), IL-4 (T/G –1098, T/C –590, T/C –33), IL-6 (G/C –174, G/A 560) and IL-10 (G/A –1082, C/T –819, C/A − 592), interferon (IFN)-γ (+874A/T), tumor growth factor (TGF)-β1 (C/T codon 10, G/C codon 25), tumor necrosis factor (TNF)-α (G/A –308, G/A − 238) in HIV infected individuals and healthy subjects.

Our findings indicated association between the cytokine SNPs with susceptibility/ resistance to HIV infection. Since the effect of gene variations on disease progression varies as per ethnicity of study population, the current study presents novel findings in reference to Indian population.

2. Material and methods

2.1. Study population and sampling

2.1.1. Patients

A total of 240 HIV-1 infected individuals (with or without co-TB infection or other opportunistic infections) attending the Medical Out Patient Department (OPD) of All India Institute of Medical Sciences (AIIMS), New Delhi, India were included in the present study. Pregnant women, patients with any type of bleeding disorders, or a history of autoimmune disorder were excluded from the study. Majority of HIV infected individuals were recruited from Northern India (Delhi & the surrounding regions) and peripheral states, during the period January 2003 to December 2005. The age groups varied between 18 and 60 years with a median of 31 years. A large majority of the individuals (75%) in our cohort were believed to have been infected through heterosexual transmission of the virus.

As per the CDC criteria (CDC, 1993; Vajpayee et al., 2005), seropositive subjects were classified according to their $CD4^+$ T cell counts into different clinical categories, viz., A (n = 74), B (n = 60) and C (n = 106) as shown in the Table 1. $CD4^+$ T cell counts and $CD8^+$T cell counts were monitored as part of the standard clinical care program in the Department of Microbiology, AIIMS, New Delhi, India. The HIV-positive study subjects were also categorized as asymptomatic and symptomatic; or co-infected with tuberculosis (TB). The antiretroviral drug therapy status of the cohort was variable with some subjects receiving none while others on various combinations of 1–3 drugs (Nevirapine, Lamivir, Duovir, etc). Al1 relevant clinical laboratory information including plasma viral load (wherever available), $CD4^+$ and $CD8^+$ T cell counts, as well as each individual's symptoms and treatment histories were available from all seropositive participants. Informed consent for participation in the study was obtained from all the study subjects. The study was approved by the ethical committee of the institute.

2.1.2. HIV diagnosis

Confirmation of HIV infection was done by estimation of HIV specific antibodies by enzyme immunoassay (EIA) as per the guidelines of NACO (National AIDS Control Organization), India. ELISA was performed on plasma samples (ELISA, lab system OY, Finland) and the samples that were non-reactive in first test were considered HIV antibody- negative whereas those found to be reactive were re-tested in duplicate by the same test. All the diagnostic and confirmative tests were carried out in the Dept. of Microbiology, AIIMS.

2.1.3. Control subjects

A total of 130 age and sex matched, healthy HIV-seronegative volunteers, donors for stem cell transplant or renal transplant including their family members, students, unrelated healthy persons with the same ethnic background as that of the patients with HIV were randomly selected for inclusion as control subjects in the present study. None had a family history of TB or other related diseases.

All patients and controls subjects represented a fairly homogenous ethnic group of north Indian population.

2.1.4. Sample collection

Peripheral blood samples were collected by venipuncture into K3-EDTA vacutainer tubes (BD Biosciences) under complete aseptic conditions. All samples were collected and processed on the same day for DNA isolation and stored at 4 °C until further use.

2.2. DNA isolation, quantification and genotyping

DNA extraction and quantification was performed as described elsewhere (Singh et al., 2018). The isolated genomic DNA extracts were subjected to 0.8% agarose gel electrophoresis (Bio-Rad, Hercules, CA, USA) for evaluation of their integrity. The DNA bands were visualized by ethidium bromide staining.

2.3. Genotyping of cytokine gene polymorphism

Twenty two SNPs in 13 cytokine genes, viz., IL-1-α (T/C –889), IL-1β (-511C/T, T/C + 3962), IL-1R (C/T pstI 1970), IL-1Rα (T/C mspaI 1100), IL-4Rα (G/A + 1902), IL-12 (C/A –1188), IFN-γ (+874A/T), TGF-β1 (C/T codon 10, G/C codon 25), TNF-α (G/A –308, G/A –238), IL-2 (T/G –330 G/T + 166), IL-4 (T/G –1098, T/C –590, T/C –33), IL-6 (G/C –174, G/A nt560), IL-10 (G/A –1082, C/T –819, C/A − 592) were determined in HIV patients and healthy subjects. All genomic typing was done by PCR with sequence-specific assays using Heidelberg-cytokine genotyping kit (University of Heidelberg, Heidelberg, Germany) as per the manufacturer's instructions. PCR reactions were carried out at the following thermal conditions: 94 °C for 2 min, then 15 cycles of 94 °C for 10 s., 65 °C for 1 min., followed by 20 cycles of 94 °C for 10 s., 61 °C for 50 s., 72 °C for 30 s. A 10 µl aliquot of the amplified product from each well was resolved in a 2.0% agarose gel to identify the cytokine variants on the basis of their respective allele sizes. The results were interpreted as per the instructions provided with the kits.

Table 1
Classification of HIV positive study subjects.

Characteristics	Median (min. to max.)			
	Total HIV +	CDC-A	CDC-B	CDC-C
	(N = 240)	(N = 74)	(N = 60)	(N = 106)
Age (in years)	31 (18–60)	31 (19–60)	32 (19–56)	31 (18–45)
Absolute CD4 + Tcells/mm3	346 (21–2180)	460 (40–2180)	280 (21–1188)	145 (40–410)
Absolute CD8 + Tcells/mm3	1060 (230–4330)	1035 (450–4330)	1064 (230–4087)	1180 (321–2854)

Clinical symptoms in Category A: Asymptomatic HIV infection, persistent generalized lymphadenopathy; Category B: Oropharyngeal and vulvovaginal candidiasis, constitutional symptoms such as fever (38.5 °C) or diarrhea lasting > 1 month, herpes zoster; Category C: *Mycobacterium tuberculosis* (pulmonary and disseminated), *Pneumocystis carinii* pneumonia, candidiasis of bronchi; trachea or lungs, extra pulmonary cryptococcosis, CMV, HIV-related encephalopathy, Kaposi's sarcoma, wasting syndrome due to HIV (Vajpayee et al., 2005).

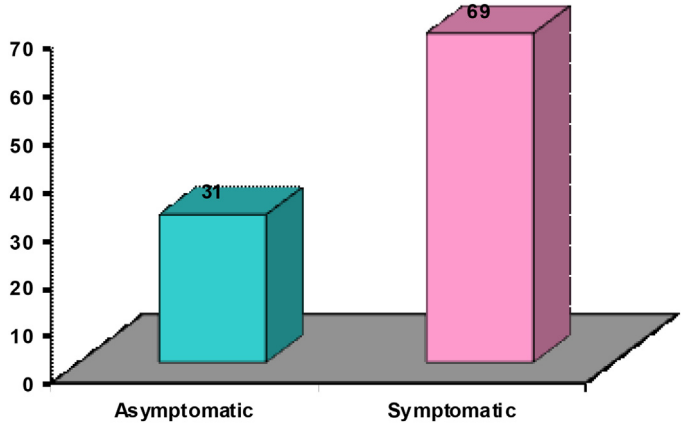

Fig. 1. Clinical status of HIV subjects. A, B, C are the CDC- defined categories of HIV infected subjects.

2.4. Statistical analysis

Genotypic and allelic frequencies were calculated by direct counting method. The significance of the distribution of alleles between HIV infected subjects and healthy controls were tested by the Chi-square test with Yates correction or Fischer's exact test wherever applicable. The p values $\leq .05$ were considered as statistically significant. The risk contributed by genetic variant allele was assessed by calculating odd ratio (OR) or relative risk (RR) with 95% confidence interval. The statistical calculations were performed using MSTAT software.

3. Results

3.1. Study group

The study cohort consisted of 240 HIV seropositive individuals (31% CDC-A and 69% CDC- B & C categories) (Fig. 1) with median age of 31 years (range 18–60 yrs). This is in conformity with the UNAIDS and NACO estimates which report that almost 90% of the reported cases in India fall within the most economically productive age group. The male to female ratio of 5:8 of the study cohort revealed that the number of young infected females in the age group 15–24 yrs. were nearly > 1.5 times higher than the young infected males. The heterosexual contact was the most predominant mode of HIV transmission (75%) followed by blood transfusion and blood product infusion (8%), intravenous drug abuse (2%), mother to child transmission (3%) and others (12%). Of the opportunistic infections, tuberculosis was found to be the commonest in > 40% of the cohort studied.

3.2. Assessment of allelic and genotypic frequencies of cytokine gene polymorphisms

Distribution of the allele and genotype frequencies corresponding to various cytokine genes among total HIV+ subjects, different HIV+ sub-groups, with or without TB and healthy controls are represented in the Table 2 and Table 3 respectively. The allele C of -889C/T polymorphism of IL-1α gene exhibited a frequency of about 75% in healthy subjects, while the allele T showed higher frequency in asymptomatic (32.1%) patients as compared to the symptomatic subjects (17.5%, $\chi^2 = 9.2$; $p = .002$). The genotype CC was found to be the predominant one (> 55%) followed by CT (> 20%) and TT (> 10%) in all subject categories. An over-representation of TT genotype was observed in CDC-A asymptomatic category (19.6%) when compared to symptomatic (CDC-B + C) group of patients (6.2%, $\chi^2 = 6.0$; $p = .01$) indicating its negative correlation with AIDS related symptoms. The alleles A and T at position +874 of IFN-γ gene occurred at comparable frequencies, 57.0% and 43.0%, respectively in healthy controls. However, the frequency of allele T (high producer) was lower in total HIV+ subjects (31.5%) as compared to healthy controls (43%; $\chi^2 = 7.75$; $p = .005$). Two SNPs at *codon* 10C/T and *codon* 25C/G were investigated for TGF-β1 gene. The frequency of G allele at codon 25 was comparatively higher in symptomatic (93.8%) subjects as compared to the asymptomatic patients (86.7%, $\chi^2 = 3.9$, $p = .04$). Also, the frequency of the CT genotype at codon 10 was observed to be significantly higher in total HIV subjects (61.6%) in comparison to healthy controls (48.4%, $\chi^2 = 4.66$, $p = .031$). While, the predominant genotype was GG (> 75%) in all the study groups, an increased trend of genotype CT at codon 10 was observed in HIV+ patients with TB (73.3%) as compared to those without TB (55.6%, $\chi^2 = 4.5$; p = .03) (Table 3).

The G allele of −330G/T polymorphism of IL-2 gene was observed at marginally increased frequency in asymptomatic group of patients (51.1%) as compared to symptomatic CDC-C group (37.5%, $\chi^2 = 3.9$; $p = .046$) (Table 2). A low occurrence of -330 GT genotype in CDC-B + C groups (36.7%) of patients as compared to CDC-A (55.6%, $\chi^2 = 6.0$; $p = .01$) was recorded. Frequency of +160GT genotype was also found to be decreased in symptomatic group (B + C) (23.7%) as compared to asymptomatic subjects (40%, $\chi^2 = 5.6$; $p = .02$). A significant increase of T allele (high producer) of −590 polymorphism of IL-4 gene was observed in total HIV infected participants (27.6%) as compared to healthy controls (14.2%, $\chi^2 = 13.7$; $p = .0001$). Similarly, the other allele -33 T allele also occurred with a higher frequency in seropositive subjects (27.3%) as compared to seronegative individuals (14.2%, $\chi^2 = 13.1$; $p = .0004$). A significantly increased frequency of IL-4 -590 TT genotype was also observed in HIV infected subjects (12.4%) in contrast to healthy controls (0.9%, $\chi^2 = 11$; $p = .001$). A significant increase in frequency of G allele of IL-6 gene at position −174 was observed in total HIV positive subjects (91.3%) as compared to healthy controls (81.9%, $\chi^2 = 10.0$; $p = .002$). A significant increase in the frequency of GG genotype at position −174 was found in total HIV positive subjects (83.2%) compared to healthy controls (68.9%, $\chi^2 = 7.169$; $p = .007$). The high producer 174 GG genotype showed significantly higher frequency in CDC-C symptomatic group of patients (90.6%) also in comparison to CDC-A asymptomatic group of subjects (78.7%, $\chi^2 = 5.0$, $p = .04$). An increased occurrence of IL-6565 GG genotype was also recorded in the CDC-C symptomatic group of patients (91.8%) as compared to CDC-A asymptomatic subjects (74.5%, $\chi^2 = 5.9$, $p = .014$). A significant increase of −1082 AG genotype of IL-10 in symptomatic (B + C) cohort (49.1%) compared to asymptomatic cohort (31.3%, $\chi^2 = 4.2$; p = .04). Interestingly, the HIV infected individuals with and without tuberculosis infection showed decreased frequency of −1082 AG genotype in dually infected subjects (33.3%) with respect to HIV+ patients (50%, $\chi^2 = 3.9$; $p = .05$).

3.3. Analysis of haplotype frequencies

For several cytokine genes with multiple SNPs (TGF β1, TNF α, IL-2,

Table 2
Cytokine allele frequencies among healthy and HIV + subjects.

LOCUS	Healthy		Total HIV		Asymptomatic (CDC-A)		Symptomatic CDC-B		Symptomatic CDC-C		HIV + TB-		HIV + TB +	
	n +	F (%)	n +	F (%)	n +	F(%)	n +	F(%)	n +	F	n +	F(%)	n + (60)	F(%)
IL1α − 889	*N* = 130		*N* = 196		*N* = 56		*N* = 46		*N* = 94		*N* = 136		N = 60	
C	196	75.4	307	78.3	76	67.9	83	90.2	148	78.7	215	79	92	76.7
T	64	24.6	85	21.7	36	32.1[f]	9	9.3[f]	40	21.3[f]	57	21	28	23.3
IL1β-511	N = 130		*N* = 197		*N* = 55		*N* = 47		*N* = 95		*N* = 137		N = 60	
C	97	37.3	151	38.3	39	35.5	35	37.2	77	40.5	102	37.2	49	40.8
T	163	62.7	243	61.7	71	64.5	59	62.8	113	59.5	172	62.8	71	59.2
IL1β + 3962	N = 130		*N* = 194		N = 55		*N* = 45		N = 94		*N* = 134		N = 60	
C	221	85	325	83.8	89	80.9	76	84.4	160	85.2	219	81.7	106	88.3
T	39	15	63	16.2	21	19.1	14	15.6	28	29.8	49	18.3	14	11.7
IL1Rpstl1970	N = 130		*N* = 195		N = 55		N = 46		N = 94		*N* = 135		N = 60	
C	151	58.1	221	56.7	65	59.1	50	54.3	106	56.4	152	56.3	69	57.5
T	109	41.9	169	43.3	45	40.9	42	45.7	82	43.6	118	43.7	51	42.5
IL1RAmspa111100	N = 130		*N* = 175		*N* = 51		*N* = 38		*N* = 86		*N* = 115		N = 60	
C	34	13.1	51	14.6	17	16.7	7	9.2	27	15.7	33	14.3	18	15
T	226	86.9	299	85.4	85	83.3	69	90.8	145	84.3	197	85.7	102	85
IL-4Rα-1902	N = 130		*N* = 188		N = 51		*N* = 42		N = 95		*N* = 128		N = 60	
A	186	71.5	292	77.7	81	79.4	66	78.6	145	76.3	201	78.5	91	75.8
G	74	28.5	84	22.3	21	20.6	18	21.4	45	23.7	55	21.5	29	24.2
IL-12B-1188	*N* = 123		*N* = 174		*N* = 48		*N* = 36		*N* = 90		*N* = 114		N = 60	
A	169	68.6	250	71.8	71	74	55	76.4	124	68.9	166	72.8	84	70
C	77	31.3	98	28.2	25	26	17	23.6	56	31.1	62	27.2	36	30
γ-IFN + 874	*N* = 127		*N* = 173		*N* = 39		N = 36		*N* = 91		*N* = 113		N = 60	
A	145	57	237	68.5	49	62.8	50	69.4	118	64.8	152	67.3	85	70.8
T	109	43.0[a]	109	31.5[a]	29	37.2	22	30.6	64	35.2	74	32.7	35	29.2
TGFβ1Cdn10	*N* = 126		*N* = 177		*N* = 49		*N* = 37		N = 91		*N* = 117		N = 60	
C	125	49.6	175	49.4	50	51	37	50	88	48.4	113	48.3	62	51.7
T	127	50.4	179	50.6	48	49	37	50	94	51.6	121	51.7	58	48.3
TGFβ1Cdn 25	N = 126		N = 177		N = 49		N = 37		N = 91		N = 117		N = 60	
C	20	7.9	29	8.2	13	13.3	6	8.1	10	5.5	24	10.3	5	4.2
G	232	92.1	325	91.8	85	86.7[g]	68	91.9[g]	172	94.5[g]	210	89.7	115	95.8
TNFα-308	N = 127		N = 175		N = 48		N = 37		N = 90		N = 115		N = 60	
A	22	8.5	29	8.3	11	11.5	4	5.4	14	7.8	19	8.3	10	8.3
G	234	91.5	321	91.7	85	88.5	70	94.6	166	92.2	211	91.7	110	91.7
TNFα-238	N = 127		*N* = 172		*N* = 48		*N* = 34		N = 90		N = 115		N = 60	
A	18	7.1	22	6.3	3	3.1	4	5.4	15	8.3	15	6.5	7	5.8
G	236	92.9	332	93.7	93	96.9	70	94.6	165	91.7	215	93.5	113	94.2
IL-2-330	*N* = 121		N = 172		N = 45		N = 34		*N* = 88		*N* = 112		N = 60	
G	131	54.2	204	59.3	46	51.1[e]	47	69.1	66	37.5[e]	143	63.8	74	61.7
T	111	45.8	140	40.7	44	48.9	21	30.9	110	62.5	81	36.2	46	38.3
IL-2 + 160	N = 121		N = 172		N = 45		*N* = 28		N = 88		N = 112		N = 60	
G	192	79.3	295	85.8	81	90	58	85.3	149	84.7	192	85.7	103	85
T	50	20.7	49	14.2	9	10	10	14.7	27	15.3	32	14.3	17	15
IL-4-1098	N = 117		*N* = 161		N = 47		*N* = 28		N = 86		*N* = 101		N = 60	
G	37	15.8	48	14.9	16	17	10	17.9	22	12.8	37	18.3	11	9.2
T	197	84.2	274	85.1	78	83	46	82.1	150	87.2	165	81.7	109	90.8
IL-4-590	N = 117		N = 161		N = 47		N = 28		N = 86		N = 101		N = 60	
C	201	85.8	233	72.4	70	74.5	38	67.9	125	72.7	144	71.3	89	74.2
T	33	14.2[b]	89	27.6[b]	24	25.5	18	32.1	47	27.3	58	28.7	31	25.8
IL-4-33	N = 117		N = 161		N = 47		N = 28		N = 86		N = 101		N = 60	
C	201	85.8	234	72.7	70	74.5	38	67.9	126	73.3	145	71.8	89	74.2
T	33	14.2[c]	88	27.3[c]	24	25.5	18	32.1	46	26.7	57	28.2	31	25.8
IL-6-174	N = 117		N = 161		N = 47		*N* = 29		*N* = 85		N = 101		N = 60	
C	43	18.1	28	8.7	10	10.6	9	15.5	9	5.3	21	10.4	7	5.8
G	195	81.9[d]	294	91.3[d]	84	89.4	49	84.5	161	94.7	181	89.6	113	94.2
IL-6 nt565	N = 117		N = 161		N = 47		N = 29		N = 85		N = 101		N = 60	
A	34	14.7	29	9.1	12	12.8	9	15.5	8	4.7	21	10.4	8	6.7
G	200	85.3	293	90.9	82	87.2	49	84.5	162	95.3	181	89.6	112	93.3
IL-10-1082	N = 123		*N* = 164		N = 48		N = 28		N = 88		*N* = 104		N = 60	
A	189	76.8	246	75	77	80.2	43	76.8	126	71.6	144	69.2	86	71.7
G	57	23.2	82	25	19	19.8	13	23.2	50	28.4	64	30.8	34	28.3
IL-10-819	N = 123		N = 164		N = 48		N = 28		N = 88		N = 104		N = 60	
C	141	57.4	170	51.8	50	52	31	55.4	89	50.6	119	57.2	66	55
T	105	42.6	158	48.2	46	48	25	44.6	87	49.4	89	42.8	54	45
IL-10-590	N = 123		N = 164		N = 48		N = 28		N = 88		N = 104		N = 60	
A	105	42.6	159	48.5	46	48	25	44.6	88	50	84	40.4	51	42.5
C	141	57.4	169	51.5	50	52	31	55.4	88	50	124	59.6	69	57.5

[a] IFN-γ +874 T = Total HIV+ vs Healthy (31.5% vs 43%), $\chi 2 = 7.75$, $p = .005$.
[b] IL4–590 T = Total HIV+ vs Healthy (27.6% vs 14.2%), $\chi 2 = 13.7$, $p = .0001$.
[c] IL4–33 T = Total HIV+ vs Healthy (27.3% vs 14.2%), $\chi 2 = 13.1$, $p = .0004$.
[d] IL6–174G = Total HIV+ vs Healthy (91.3% vs 81.9%), $\chi 2 = 10.0$, $p = .002$.

[e] IL2–330G = CDC-C vs CDC-A (37.5% vs 51.1%), $\chi 2 = 3.9$, $p = .046$.
[f] IL1α-889T = Asymptomatic vs Symptomatic (B + C) (32.1% vs17.5%), $\chi 2 = 9.2$, p = .002.
[g] TGFβ codon25 = Symptomatic (B + C) vs Asymptomatic (93.8% vs 86.7%), $\chi 2 = 3.9$, $p = .04$.

IL-4, IL-6, IL-10), the Heidelberg PCR-SSP kit was used to detect true haplotypes. Distribution of haplotypes among total HIV+ subjects, different CDC categories and HIV positive subjects co-infected with TB are presented in Table 4. The most frequent haplotypes for TGF-β1 were TG (50.4%) and CG (42.1%) while the TGF-β1 TC haplotype was absent in the healthy population. Haplotype GG of TNF-α was the predominant type in healthy and HIV individuals (> 85%) followed by AG (6–7%) and GA (6–7%) haplotypes. The high producer haplotype AA was found at a low frequency (~1%) in the study. The IL2 haplotypes were distributed in the healthy controls as 54.1% GG, followed by TG (25.6%), TT (19.8%), with the smallest frequency being of GT haplotype (0.5%). A significantly decreased haplotype frequency of TG was observed in symptomatic (CDC-B + C) group of subjects (19.5%) as compared to asymptomatic subjects (39%, $\chi^2 = 17$; $p = .0004$). The most frequent IL4 haplotype was TCC (71.4%), followed by GCC (15.3%) and TTT (13.3%) in the healthy Indian control population. An over-representation of TTT haplotype was seen in total HIV + subjects (27%) as compared to healthy controls (13.3%, $\chi^2 = 15.59$; $p = .0007$). Further a decreased frequency of TCC haplotype was observed in HIV + cohort (58.1%) as compared to controls (71.4%, $\chi^2 = 9.6$; $p = .002$). Comparative analysis revealed no significant variation between dually infected patients and HIV + subjects without TB. Analysis of haplotypes showed that the most frequent IL-6 haplotype was GG (82%), followed by CA (13.8%) and CG (4.2%) in the healthy controls. The distribution of haplotype frequencies in total HIV + subjects was found to be GG (90.1%), CA (7.8%), GA (1.2%) and CG (0.9%). The IL-10 gene SNPs appeared in three predominant haplotypes, namely ATA (42.7%), ACC (34.5%) and GCC (22.8%), in healthy controls. However, a high producer haplotype GCC was found to be more frequent in symptomatic group (26%) as compared to the asymptomatic group of patients (19.8%) but was not significant. However the haplotype combination GCC/ATA was found at significantly increased frequency in HIV + subjects (35.4%) as compared to healthy controls (21.1%; $\chi^2 = 5.7$; $p = .017$) and also among the HIV + subjects without TB (43.3%) versus HIV +, TB + subjects (21.6%; $\chi^2 = 6.8$; $p = .009$).

4. Discussion

Cytokines are important immunomodulatory molecules in immune response against microorganisms and also have an important role in the setting of disorders affecting the immune system. The various SNPs in cytokine genes have been extensively studied in different population as well as in relation to disease. Some of these polymorphisms affect cytokine gene transcription and expression. The polymorphisms of cytokine genes are potentially important as genetic predictors of disease susceptibility and clinical outcome or as a tool for anthropological studies (Singh and Arora, 2015; Rodrigues et al., 2017; Tsiara et al., 2018).

The current study attempted to understand the effect of genetic variations in cytokine genes on progression of HIV/AIDS. Our study cohort showed concordance with the NACO report indicating heterosexual contact (75%) as the predominant mode of HIV transmission in India, followed by blood transfusion and blood product infusion (8%), mother to child transmission (3%), injecting drug abuse (2%) and others (12%). Further, *M. tuberculosis* infection was found to be the main (40%) opportunistic infection in this cohort which is in accordance with other published reports (Swaminathan et al., 2000; Low et al., 2016). The high incidence of HIV/TB in Indian HIV + subjects suggests that apart from other factors, tuberculosis might be one of the leading causes of rapid progression of HIV disease in India.

Gradual decline in absolute $CD4^+$T cells has been an important biomarker of progression of HIV infection. $CD4^+$T cells serve as major targets of HIV and have been recommended to be analyzed every 3–6 months in each HIV-infected patient (De Gruttola et al., 1994). As per the Center for Disease Control and Prevention (CDC), there are 3 categories of HIV-infected cases based on the clinical conditions and $CD4^+$T cell counts (CDC, 1993), in which, Category 1 includes patients with CD4 T cell counts ≥500 cells/μl; Category 2 includes patients with 200–499 cells/ul counts and Category 3 covers patients with < 200 CD4 T cells/ul. Along with these 3 ranges of $CD4^+$T cell counts, 3 clinical categories (A, B & C) and 9 mutually exclusive categories of HIV-patients has been defined by the CDC (CDC, 1993). Although this system seems complicated, it allows the clinicians to classify the severity of HIV infection and is significant in AIDS surveillance (CDC, 1992).

In our study, the median $CD4^+$T cell counts of the total HIV infected patients was 346 cells/μl (range; 21–2180 cells/μl). These values are appreciably lower than the normal Indian reference 666 cells/μl (range; 304–2095) among healthy population (Amatya et al., 2004). This suggests that HIV infected patients in India are immuno-compromised and thus more susceptible to opportunistic infections (OIs). Among all the OIs, tuberculosis exhibited maximum occurence at 40% in our study, and in others as well at 47% (Vajpayee et al., 2003) in Indian population. A meta-analysis reported tuberculosis and bacterial pneumonia as the major incident and prevalent infections in childhood HIV cases (B-Lajoie et al., 2016). The HIV and TB interaction is synergistic and bi-directional and leads to exaggerated HIV progression in co-infected cases due to enhanced activation of systemic immune responses (Vanham et al., 1996; Sharma et al., 2005). In contrast to other opportunistic infections, active TB is encountered irrespective of the $CD4^+$T cell counts (Havlir and Barnes, 1999), rather it depends on the level of HIV infection-led immunosuppression. TB infection seems to worsen the HIV infection even in the HIV patients with > 200 cells/μl CD4 cells. In our study, TB co-infection led to a marked decline in CD4 levels 145 cells/μl (40–410) as compared to the non-TB HIV patients (460 cells/μl (40–2180)) (Table 1). This is in concordance with the previous reports demonstrating low CD4 counts (189 cells/μl) in HIV + TB infected Indian population (Vajpayee et al., 2003).

Along with decline in CD4 T cells, an unflagging rise in CD8 T-cells is observed during progression of HIV infection. CD8+ T-cells recognize and cause lysis of the viral infected cells. In an attempt to survive, the virus, through its high mutation rate, escapes $CD8^+$T-cell mediated viral clearance by down-regulating the MHC-I expression on infected cells. This leads to disruption of CD8+ T-cell signaling. In addition, inappropriate functioning of $CD4^+$T-cells and antigen presenting cells (required for appropriate maturation of $CD8^+$T-cells), further leads to decline in circulating pool of effector and memory $CD8^+$T-cells; thus, disrupting effective $CD8^+$T-cell function. It has been reported that the $CD8^+$T cells remain elevated during HIV infection. Co-infection of TB significantly increases these cell counts in comparison to only HIV-infected patients (Mbow et al., 2013). In concordance to this, the median $CD8^+$T cell counts were found to be highest [1180 (321–2854) cells] in the symptomatic HIV-TB co-infected patients as compared to the asymptomatic HIV-infected patients [1035 (450–4330) cells] in our study (Table 1). The CD8 T cells presumably respond to the rise in viral load and that the CD8 levels may serve as useful marker for virological failure in HIV-infected patients after onset of combination anti-retroviral therapy (Ku et al., 2016).

It is well-known that the rate of transition from one stage to another during the course of HIV infection is characterized by changes in the cytokine network balance (TH1 to TH2 shift). The alterations in the cytokine balance during HIV infection depends on the individual profile of cytokine production predetermined by the functioning of the genes encoding the immune-modulators. A balanced Type 1/Type 2 response

Table 3
Cytokine genotypic frequencies among healthy and HIV+ subjects.

Locus	Healthy		Total HIV		Asymptomatic		Symptomatic				HIV + TB-		HIV + TB+	
					(CDC-A)		CDC-B		CDC-C					
	n+	F (%)	n+	F (%)	n+	F(%)	n+	F(%)	n+	F	n+	F(%)	n + (60)	F(%)
IL1α-889T	N = 130		N = 196		N = 56		N = 46		N = 94		N = 136		N = 60	
C:C	79	60.8	131	66.8	31	55.4	39	84.8	61	64.9	94	69.2	37	61.7
C:T	38	29.2	45	23	14	25	5	10.9	26	27.7	27	19.8	18	30
T:T	13	10	20	10.2	11	19.6[d]	2	4.3[d]	7	7.4[d]	15	11	5	8.3
IL1β-511	N = 130		N = 197		N = 55		N = 47		N = 95		N = 137		N = 60	
C:C	19	14.6	34	17.3	9	16.4	8	17	17	17.9	24	17.5	10	16.7
C:T	59	45.4	83	42.1	21	38.2	19	40.5	43	45.3	54	39.4	29	48.3
T:T	52	40	80	40.6	25	45.4	20	42.5	35	36.8	59	43.1	21	35
IL1β + 3962	N = 130		N = 194		N = 55		N = 45		N = 94		N = 134		N = 60	
C:C	95	73.1	139	71.7	37	67.3	31	68.9	71	75.5	92	68.6	47	78.3
C:T	31	23.8	47	24.2	15	27.3	14	31.1	18	19.2	35	26.1	12	20
T:T	4	3.1	8	4.1	3	5.4	0	0	5	5.3	7	5.3	1	1.7
IL1R pstl1970	N = 130		N = 195		N = 55		N = 46		N = 94		N = 135		N = 60	
C:C	44	33.8	68	34.9	19	34.5	14	30.5	35	37.2	44	32.6	24	40
C:T	63	48.5	85	43.6	27	49.1	22	47.8	36	38.3	64	47.4	21	35
T:T	23	17.7	42	21.5	9	16.4	10	21.7	23	24.5	27	20	15	25
IL1RA mspa111100	N = 130		N = 175		N = 51		N = 38		N = 86		N = 115		N = 60	
C:C	3	2.3	5	2.8	2	3.9	0	0	3	3.5	4	3.5	1	1.6
C:T	28	21.5	41	23.4	13	25.5	7	18.4	21	24.4	25	21.7	16	26.7
T:T	99	76.2	129	73.8	36	70.6	31	81.6	62	72.1	86	74.8	43	71.7
IL-4Ra-1902	N = 130		N = 188		N = 51		N = 42		N = 95		N = 128		N = 60	
A:A	68	52.3	118	62.8	33	64.7	27	64.3	58	61.1	84	65.6	34	56.7
A:G	50	38.5	56	29.8	15	29.4	12	28.6	29	30.5	33	25.8	23	38.3
G:G	12	9.2	14	7.4	3	5.9	3	7.1	8	8.4	11	8.6	3	5
IL-12B-1188	N = 123		N = 174		N = 48		N = 36		N = 90		N = 114		N = 60	
A:A	60	48.8	87	50	26	54.2	21	58.3	40	44.4	58	50.9	29	48.3
A:C	49	39.8	76	43.7	19	39.6	13	36.1	44	48.9	50	43.9	26	43.3
C:C	14	11.4	11	6.3	3	6.2	2	5.6	6	6.7	6	5.2	5	8.4
γ-IFN + 874	N = 127		N = 173		N = 39		N = 36		N = 91		N = 113		N = 60	
A:A	44	34.6	86	49.7	18	46	17	47.2	41	45	54	47.8	32	53.3
A:T	57	44.9	65	37.6	13	33	16	44.4	36	39.6	44	38.9	21	35
T:T	26	20.5	22	12.7	8	21	3	8.4	14	15.4	15	13.3	7	11.7
TGFβ1Cdn10	N = 126		N = 177		N = 49		N = 37		N = 91		N = 117		N = 60	
C:C	32	25.4	33	18.6	9	18.4	6	16.2	18	19.8	24	20.5	9	15
C:T	61	48.4[b]	109	61.6[b]	32	65.3	25	67.6	52	57.1	65	55.6[f]	44	73.3[f]
T:T	33	26.2	35	19.8	8	16.3	6	16.2	21	23.1	28	23.9	7	11.7
TGFβ1Cdn 25	N = 126		N = 177		N = 49		N = 37		N = 91		N = 117		N = 60	
C:C	1	0.8	1	0.6	1	2	0	0	0	0	1	0.9	0	0
C:G	18	14.3	27	15.2	6	12.2	6	16.2	10	11	22	18.8	5	8.3
G:G	107	84.9	149	84.2	42	85.8	31	83.8	81	89	94	80.3	55	91.7
TNFα-308	N = 128		N = 175		N = 48		N = 37		N = 90		N = 115		N = 60	
A:A	3	2.3	2	1.2	0	0	1	2.7	1	1.1	2	1.7	0	0
A:G	16	12.5	25	14.3	11	22.9	2	5.4	12	13.3	15	13	10	16.7
G:G	109	85.2	148	84.5	37	77.1	34	91.9	77	85.6	98	85.3	50	83.3
TNFα-238	N = 128		N = 175		N = 48		N = 37		N = 90		N = 115		N = 60	
A:A	1	0.8	2	1.1	1	2	0	0	1	1.1	1	0.9	1	1.7
A:G	16	12.5	18	10.3	1	2[e]	4	10.8	13	14.5[e]	13	11.3	5	8.3
G:G	111	86.7	155	88.6	46	96	33	89.2	76	84.4	101	87.8	54	90
IL-2-330	N = 121		N = 172		N = 45		N = 34		N = 88		N = 112		N = 60	
G:G	35	28.9	63	36.6	10	22.2	18	52.9	37	42	53	47.4	24	40
G:T	61	50.4	77	44.8	25	55.6[i]	11	32.4[i]	36	41[i]	37	33	26	43.3
T:T	25	20.7	32	18.6	10	22.2	5	14.7	15	17	22	19.6	10	16.7
IL-2 + 160	N = 121		N = 172		N = 45		N = 34		N = 88		N = 112		N = 60	
G:G	79	65.3	127	73.8	25	56	25	73.5	64	72.7	83	74.1	44	73.3
G:T	34	28.1	41	23.8	18	40[j]	8	23.5[j]	21	23.9[j]	26	23.2	15	25
T:T	8	6.6	4	2.4	2	4	1	3	3	3.4	3	2.7	1	1.7
IL-4-1098	N = 117		N = 161		N = 47		N = 28		N = 86		N = 101		N = 60	
G:G	4	3.4	8	5	3	6.4	2	7.2	3	3.5	8	7.9	0	0
G:T	31	26.5	32	19.9	10	21.3	6	21.4	16	18.6	21	20.8	11	18.3
T:T	82	70.1	121	75.1	34	72.3	20	71.4	67	77.9	72	71.3	49	81.7
IL-4-590	N = 117		N = 161		N = 47		N = 28		N = 86		N = 101		N = 60	
C:C	85	72.6	92	57.2	30	63.8	13	46.5	49	57	59	58.4	33	55
C:T	31	26.5	49	30.4	10	21.3	12	42.8	27	31.4	26	25.7	23	38.3
T:T	1	0.9[a]	20	12.4[a]	7	14.9	3	10.7	10	11.6	16	15.9	4	6.7
IL-4-33	N = 117		*N* = 161		*N* = 47		N = 28		N = 86		N = 101		*N* = 60	
C:C	85	72.6	93	57.8	30	63.8	13	46.5	50	58.2	60	59.4	33	55
C:T	31	26.5	48	29.8	10	21.3	12	42.8	26	30.2	25	24.8	23	38.3
T:T	1	0.9	20	12.4	7	14.9	3	10.7	10	11.6	16	15.8	4	6.7
IL-6-174	*N* = 119		N = 161		N = 47		N = 29		N = 85		N = 101		N = 60	
C:C	8	6.7	1	0.6	0	0	0	0	1	1.2	0	0	1	1.7

(*continued on next page*)

Table 3 (*continued*)

Locus	Healthy		Total HIV		Asymptomatic (CDC-A)		Symptomatic CDC-B		Symptomatic CDC-C		HIV + TB-		HIV + TB+	
	n+	F (%)	n+	F (%)	n+	F(%)	n+	F(%)	n+	F	n+	F(%)	n + (60)	F(%)
C:G	29	24.4	26	16.2	10	21.3	9	31	7	8.2	21	20.8	5	8.3
G:G	82	68.9[c]	134	83.2[c]	37	78.7[k]	20	69	77	90.6[k]	80	79.2	54	90
IL-6 nt565	N = 119		N = 161		N = 47		N = 29		N = 85		N = 101		N = 60	
A:A	6	5.1	1	0.6	0	0	0	0	1	1.2	0	0	1	1.7
A:G	25	21	27	16.8	12	25.5	9	31	6	7	21	20.8	6	10
G:G	88	73.9	133	82.6	35	74.5[l]	20	69	78	91.8[l]	80	79.2	53	88.3
IL-10-1082	N = 123		N = 164		N = 48		N = 28		N = 88		N = 104		N = 60	
A:A	73	59.3	87	53	31	64.5	16	57.1	40	45.4	48	46.2	39	65
A:G	43	35	72	44	15	31.3[h]	11	39.3[h]	46	52.3[h]	52	50[g]	20	33.3[g]
G:G	7	5.7	5	3	2	4.2	1	3.6	2	2.3	4	3.8	1	1.7
IL-10-819	N = 123		N = 164		N = 48		N = 28		N = 88		N = 104		N = 60	
C:C	35	28.5	38	23.2	13	27	7	25	18	20.5	29	27.9	9	15
C:T	71	57.7	94	57.3	24	50	17	60.7	53	60.2	56	53.8	38	63.3
T:T	17	13.8	32	19.5	11	23	4	14.3	17	19.3	19	18.3	13	21.7
IL-10-590	N = 123		N = 164		N = 48		N = 28		N = 88		N = 104		N = 60	
A:A	17	13.8	33	20.1	11	23	4	14.3	18	20.5	15	14.4	18	30
A:C	71	57.7	93	56.7	24	50	17	60.7	52	59	59	56.7	34	57
C:C	35	28.5	38	23.2	13	27	7	25	18	20.5	30	28.9	8	13.3

[a] IL4–590TT = Total HIV vs healthy (12.4% vs 0.9%), $\chi 2 = 11$, $p = .001$.
[b] TGFβ Cdn 10 CT = Total HIV+ vs healthy controls, (61.6% vs 48.4%), $\chi 2 = 4.66$, $p = .031$.
[c] IL-6-174GG = Total HIV+ vs healthy controls (83.2% vs 68.9%), $\chi 2 = 7.169$, $p = .007$.
[d] IL1αTT = CDC-A vs CDC-B + C (19.6% vs 6.2%), $\chi 2 = 6.0$, p = .01.
[e] TNFα-238 = CDC-C vs CDC-A (14.5% vs 2.0%), $\chi 2 = 10$, p = .005.
[f] TGFβ Cdn 10 CT = HIV + TB+ vs HIV + TB-ve (73.3% vs 55.6%), $\chi 2 = 4.5$, $p = .03$.
[g] IL10–1082AG = HIV + TB+ vs HIV + TB-ve (33.3% vs 50.0%), $\chi 2 = 3.9$, $p = .05$.
[h] IL10–1082AG = symptomatic vs asymptomatic (49.1% vs 31.3%), $\chi 2 = 4.2$, $p = .04$.
[i] IL2–330GT = asymptomatic vs symptomatic (55.6% vs 36.7%), $\chi 2 = 6.0$, $p = .01$.
[j] IL2 + 160GT = symptomatic vs asymptomatic (23.7% vs 40.0%),$\chi 2 = 5.6$,$p = .02$.
[k] IL6–174GG = CDC-C vs CDC-A (90.6% vs 78.7%), $\chi 2 = 5.0$, $p = .04$.
[l] IL6-nt565GG = CDC-C vs CDC-A (91.8% vs 74.5%), $\chi 2 = 5.9$, $p = .014$.

was found to be associated with long-term non-progressive HIV-1 infection (Imami et al., 2002). The current study was undertaken to investigate whether there are associations between cytokine gene polymorphism profile and the risk of acquisition of HIV infection in the Indian population where incidence of HIV is very high.

We have observed a significant association of a few pro-inflammatory (IL2, IFN-γ, TNF-α) and anti-inflammatory cytokine gene variants (IL4, IL10) with the risk to acquire the HIV infection and development of AIDS related illness in this study.

IL-1α is a potent pro-inflammatory cytokine known to enhance HIV-1 production through NF-*k*B-mediated transactivation of the viral long terminal repeat (Osborn et al., 1989). Elevated levels of IL-1 have been earlier reported in AIDS patients (Vicenzi et al., 1997) and also correlated with predicting viremia in AIDS patients (Price et al., 2004). It has been reported that IL1α-889 T is a high producer allele (Parks et al., 2004). We did not find any difference in its frequency between healthy controls and total HIV infected cohort. However, we observed an overrepresentation of the T allele and homozygous TT genotype in the asymptomatic category of HIV patients which needs to be further evaluated in clinically well categorized naïve ART patients.

In case of γ-IFN, a notable decrease in the frequency of high producer +874 T allele and its TT genotype was observed in the total HIV + cohort as well as symptomatic group of patients. Therefore, it can be postulated that the genotypes favoring low γ-IFN levels are more prevalent in investigated cohort and thus may ultimately lead to an up-regulation or upsurge of TH2 milieu in these patients. Decreased frequency of IFN-γ high producer genotype (+874TT) has also been reported in Korean HIV infected population (Kang et al., 2006). In a recent study, the presence of IFN + 874A allele was found to confer susceptibility to HIV-1 infection along with reduced levels of $CD4^+$ T lymphocytes (Freitas et al., 2015). The protective influence of γ-IFN was also suggested earlier in a study carried out on Nairobian prostitutes who remained uninfected but had high levels of T cells that produced γ-IFN (Trivedi et al., 2001). Our data also suggests that low occurrence of the IFN- γ + 874 T variant in the Indian HIV infected individuals might lead to the low level of IFN- γ cytokine and thus favour to the TH2 milieu.

A marginally significant increase in the frequency of IL2–330 G (high producer) allele was observed in the CDC-A category of patients. This suggests that genetically predetermined high IL2- production (Th1) capacity of this asymptomatic group of infected subjects, might be able to mount an effective cell mediated immune response and contribute in maintaining the asymptomatic status of infection. Inter-individualistic differences have been seen in the production of IL2 and some studies have shown a depressed production of IL2 in AIDS patients (Clerici and Shearer, 1994; Cohen et al., 1997; Vicenzi et al., 1997).

Studies have shown that both TNF-α and lymphotoxin (TNF-β) are potent inducers of HIV replication. Their variants influence the activation of the transcription factor NF-*k*B (Duh et al., 1989) and increased levels of TNF-α have been observed in AIDS patients (Brinkman et al., 1997; Delgado et al., 2003). An increase in high producer TNF-α −238 AG heterozygote was observed in the symptomatic category (CDC-B + C) of patients as compared to the asymptomatic CDC-A, indicating that AG genotype might be associated with increased HIV replication and development of AIDS defining symptoms in these symptomatic category of patients. However, a study in 2013 reported the association of TNF-α −238 AG genotype in HIV infected long term non-progressors in Caucasians (Nasi et al., 2013).

An increased frequency of IL4–590 T (high producer) allele and IL4–590TT genotype in total HIV+ cohort similarly suggests that this polymorphism could be a risk factor for susceptibility to HIV infection. Further, higher proportion of IL4-CT heterozygote in symptomatic

Table 4
Haplotypic frequencies among healthy controls and HIV + subjects.

LOCUS	Healthy		Total HIV		Asymptomatic (CDC-A)		Symptomatic CDC-B		Symptomatic CDC-C		HIV + TB-		HIV + TB +	
	n +	F (%)	n +	F (%)	n +	F(%)	n +	F(%)	n +	F	n +	F(%)	n + (60)	F(%)
TGFβ1Haplotype	N = 126		N = 177		N = 49		*N* = 37		N = 91		N = 117		N = 60	
CC	19	7.5	27	7.6	12	12.2	6	8.1	9	4.9	23	9.8	4	3.3
CG	106	42.1	148	41.8	38	38.8	31	41.9	79	43.5	90	38.5	58	48.3
TC	0	0	3	0.9	1	1	1	1.3	1	0.5	2	0.9	1	0.9
TG	127	50.4	176	49.7	47	48	36	48.7	93	51.1	119	50.8	57	47.5
TNFα Haplotype	N = 127		N = 175		N = 48		N = 37		N = 90		N = 115		N = 60	
AA	2	0.7	1	0.3	0	0	0	0	1	0.6	1	0.5	0	0
AG	20	7.4	24	6.9	9	9.4	3	4	12	6.7	17	7.4	7	5.9
GA	16	6.2	25	7.1	5	5.2	5	6.8	15	8.3	15	6.5	10	8.3
GG	216	85.7	300	85.7	82	85.4	66	89.2	152	84.4	197	85.6	103	85.8
IL-2Haplotype	N = 121		N = 172		N = 45		N = 34		N = 88		N = 112		N = 60	
GG	131	54.1	216	62.8	46	51	47	69.1	109	61.9	142	63.4	74	61.7
GT	1	0.5	1	0	0	0	0	0	1	0.6	1	0.5	0	0
TG	62	25.6	79	23	35	39[c]	11	16.2[c]	40	22.7[c]	50	22.3	29	24.2
TT	48	19.8	48	14.2	9	10	10	14.7	26	14.8	31	13.8	17	14.1
IL-4Haplotype	N = 117		N = 161		N = 47		N = 28		N = 86		N = 101		N = 60	
GCC	34	15.3	47	14.6	16	17.1	10	17.9	21	12.2	36	17	11	9.2
GCT	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GTC	0	0	1	0.3	0	0	0	0	1	0.6	1	0.5	0	0
GTT	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TCC	160	71.4[b]	187	58.1[b]	54	57.4	28	50	105	61	111	55.5	78	65
TCT	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TTC	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TTT	30	13.3[a]	87	27.0[a]	24	25.5	18	32.1	45	26.2	56	27	31	25.8
IL-6Haplotype	N = 117		N = 161		N = 47		N = 29		N = 85		N = 101		N = 60	
CA	32	13.8	25	7.8	10	10.6	9	15.5	6	3.5	19	9.4	6	5
CG	10	4.2	3	0.9	0	0	0	0	3	1.8	2	1	1	0.8
GA	0	0	4	1.2	2	2.1	0	0	2	1.2	2	1	2	1.6
GG	192	82	290	90.1	82	87.3	49	84.5	159	93.5	179	88.6	111	92.6
IL10Haplotype	N = 123		N = 164		N = 48		N = 28		N = 88		N = 104		N = 60	
ACA	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GTA	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GTC	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ACC	85	34.5	88	26.8	31	32.3	18	32.2	39	22.2	54	25.9	34	28.3
ATA	105	42.7	158	48.2	46	47.9	25	44.6	87	49.4	94	45.2	64	53.4
ATC	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GCC	56	22.8	82	25	19	19.8	13	23.2	50	28.4	60	28.9	22	18.3

[a] IL4TTT = Total HIV + vs healthy controls (27.0% vs 13.3%), $\chi 2 = 15.59$, $p = 0007$.
[b] IL4TCC = Total HIV + vs healthy controls (58.1% vs 71.4%),$\chi 2 = 9.6$, p = .002.
[c] IL2TG = symptomatic (B + C) vs asymptomatic (19.5% vs 39.0%), $\chi 2 = 17$, p = .0004.

group as well as dually infected (HIV + TB+) subjects indicated that this risk variant might also be involved in the development of AIDS related symptoms and acquisition of tuberculosis co-infection. IL4–590 T allele has been reported with susceptibility to TB in South India (Vidyarani et al., 2006). A study on Maharashtrian population has also shown a significant upsurge in the CXCR4 messenger RNA, its surface expression, as well as IL-4 mRNA and –589 T allele frequency (high IL-4 production) (Smolnikova and Konenkov, 2002; Biasin et al., 2003). These observations further strengthen the idea that development of AIDS is facilitated by IL4 levels (Nakayama et al., 2000). IL4 is a pleiotropic cytokine with various immune-modulating functions including induction of immunoglobulin E (IgE) production in B cells and downregulation of primary co-receptor CCR5, and up-regulation of CXCR4 (a coreceptor for HIV-1 SI variants) (Valentin et al., 1998). Thus the genetically predetermined high IL4 production in the studied subjects might facilitate rapid HIV entry and subsequently the clinical course of disease.

An increased occurrence of IL10–889 TT in asymptomatic patients, AG genotype at −1082 position of IL10 gene in symptomatic patients and GCC/ATA haplotype in HIV infected subjects was also observed in this study which suggest that these genotypes might influence the expression of IL10 cytokine levels and indirectly regulates the various effector immune functions in these patients which needs to be further evaluated in clinically well categorized longitudinal cohort studies. Nevertheless, the role of IL-10 in the pathogenesis of HIV-1 remain indecisive; possibly due to the associated factors, such as, host, virus, and study models in separate analyses. Various reports demonstrate that the alleles causing low IL-10 levels in the serum enhance the susceptibility to HIV-1 infection and accelerate progression to full-blown AIDS (Oleksyk et al., 2009; Shin et al., 2000). On the contrary, there are also counter-reports suggesting a shielding effect of the same alleles (−1082A and -592A) (Wang et al., 2004; Naicker et al., 2009).

For TGF-β, an association of codon 25 G (high producer) allele with symptomatic HIV infected subjects was observed. This study has shown that IL6–174GG homozygote, associated with increased IL6 production (Larcombe et al., 2005), were overrepresented among total HIV + patients and patients belonging to CDC-C category of HIV infected individuals. The latter observation again points towards the importance of Th2 type cytokines in development of AIDS. A recent south Indian study showed a trend towards a Th1 to Th2/Th0 shift in HIV clade C and clade A infected individuals (Ramalingam et al., 2005). We did not find any evidence for association of the IL-1β, IL1R, IL1RA, IL4Ra, IL12 genetic variants with HIV infection.

The genes which encode cytokines and their receptors are highly polymorphic and located within the critical promoter or other regulatory regions, affect levels of gene transcription resulting in inter-

individual variation in levels of cytokine production (Turner et al., 1997; Pravica et al., 1999; Hoffmann et al., 2002). The polymorphism (mostly single nucleotide polymorphisms (SNPs) or micro satellites) may either directly influence gene transcription or indirectly via tight linkage with other polymorphism occurring elsewhere in the cytokine gene (vanDeventer, 2000). The polymorphic nature of the cytokine genes may confer flexibility on the immune response with certain alleles promoting differential production of cytokines that may be responsible for the observed inter-individual differences in cytokine production and may be one of the possible mechanisms for the perturbation of the Th1/Th2 balance in the infectious diseases or any other disorders (Bidwell et al., 1999).

5. Conclusion

Or findings demonstrate that there are associations between certain cytokine gene polymorphisms and Human immunodeficiency virus (HIV-1) infection that may become valuable predictors for susceptibility and development of AIDS-related symptoms. Further longitudinal studies are required to investigate the independency of these polymorphisms involved in the acquisition of HIV infection in a larger sample size and correlate with levels of viremia and CD4 counts.

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