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IL-8 Alterations in HIV-1 Infected Children With Disease Progression

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Abstract: Disease progression in HIV-1 infected children is faster than in adults. Less than 5% of the infected children maintain stable CD4 counts beyond 7 years of infection and are termed long-term nonprogressors (LTNPs). Delineating the host immune response in antiretroviral naïve (ART) and treated HIV-1 infected children at different disease stages will help in understanding the immunopathogenesis of the disease.

A total of 79 asymptomatic, perinatally HIV-1 infected children (50 ART naïve and 29 ART treated) and 8 seronegative donors were recruited in this study. T- and B-cell activation PCR arrays were performed from the cDNA, using total RNA extracted from the peripheral blood mononuclear cells (PBMCs) of 14 HIV-1 infected children at different stages of the disease. The differentially expressed genes were identified. Quantitative RT-PCR was performed for the (interleukin-8) IL-8 gene and its transcriptional mediators, that is, SHP2, GRB2, and IL-8R (IL-8 receptor/CXCR1). Plasma levels of IL-8 were measured by flow cytometry.

Gene array data revealed a higher expression of IL-8 in the ART naïve HIV-1 infected progressors and in ART nonresponders than LTNPs and ART responders, respectively. Quantitative RT-PCR analysis demonstrated a significant higher expression of IL-8 ($P < 0.001$), its receptor CXCR1 ($P = 0.03$) and the upstream signaling molecule SHP2 ($P = 0.04$) in the progressors versus LTNPs. Plasma levels of IL-8 were significantly higher in progressors versus LTNPs ($P < 0.001$), and ART nonresponders versus ART responders ($P < 0.001$). A significant negative correlation of plasma levels of IL-8 with CD4 counts (cells/μL) was observed in HIV-1 infected ART naïve subjects ($r = -0.488$; $P < 0.001$), while the IL-8 levels positively correlated with viral load in the ART treated children ($r = 0.5494$; $P < 0.001$). ART naïve progressors on follow up demonstrated a significant reduction in the mRNA expression ($P = 0.05$) and plasma levels of IL-8 ($P = 0.05$) post 6 months of ART initiation suggesting the beneficial role of ART therapy in reducing inflammation in infected children.

Our data suggest that IL-8 may serve as a potential prognostic marker in adjunct with CD4 counts to monitor disease progression in the HIV-1 infected children and the efficacy of ART.

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Abbreviations: AIDS = acquired immune deficiency syndrome, ART = antiretroviral therapy, BCL-2 = B-cell lymphoma 2, CCL3 = chemokine (C-C motif) ligand 3, CCR5 = C-C chemokine receptor type 5, CD40 = cluster of differentiation 40, cDNA = complementary DNA, CXCR1 = C-X-C motif chemokine receptor 1, GAPDH = glyceraldehyde 3-phosphate dehydrogenase, GRB2 = growth factor receptor-bound protein 2, HIV-1 = human immunodeficiency virus-1, IFN-α = interferon alpha, IL-8 = interleukin 8, LTNPs = long-term nonprogressors, MCP-1 = monocyte chemoattractant protein-1, PBMCs = peripheral blood mononuclear cells, RAG-1 = recombination activating gene 1, RNA = ribonucleic acid, RT-PCR = reverse transcription polymerase chain reaction, SHP2 = SRC homology phosphatase 2, sIL-2R = soluble IL-2 receptor, TNF-α = tumor necrosis factor-alpha.

INTRODUCTION

A number of host and viral factors contribute to the extensive variability in susceptibility to HIV-1 infection and disease progression.[1–3] Among those infected with HIV-1, 5% to 15% adults,[4–7] and <5% children,[8,9] who are antiretroviral therapy (ART) naïve maintain a stable CD4 count (>500 cells/μL) for more than 7 years and are termed long-term nonprogressors (LTNPs). Slower disease progression in HIV-1 infection has been shown to be associated with some of the host factors like the 32 base pair deletion of the coreceptor CCR5,[10,11] and the presence of specific HLA (human leukocyte antigen) class I alleles (HLA-B57 and HLA-B27).[11,12] However, not all the LTNPs have protective alleles, thereby suggesting contribution of immune factors in the maintenance of T- and B-cell homeostasis and effective antiviral immunity. In perinatal HIV infection, the immune responses of the infected children may depend on a number of factors, one of them being the maternal viral load. Thus the host genetic and immune responses to HIV-1 in infants in early life are complex. Understanding the various immune parameters involved in the pathogenesis, such as chemokines and their receptors, will help us identify critical

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components of protective immunity and the factors involved in rapid disease progression in the infected children.[13]

During disease progression in HIV-1, a complex set of interactions between viral and host factors takes place leading to a chronic immune activation state.[14,15] Recent observations suggest that inflammation is a crucial contributing factor, in addition to T cell activation, to predict disease progression and mortality.[16–19] HIV-1 triggers several immunological changes like decreased CD4 T cells, polyclonal B cell activation and high levels of proinflammatory cytokines.[20] A panel of plasma inflammatory biomarkers consisting of CXCL9, CXCL10, sIL-2R, and sCD14 has been suggested to serve as a surrogate prognostic marker to monitor immune activation in both viremic and aviremic HIV patients on ART.[21] In a study conducted by Stacey et al[22] the increase in plasma viremia in acute HIV-1 infection was found to be associated with elevations in plasma levels of multiple cytokines and chemokines such as IFNα, IL-15, TNFα, and MCP-1, more slowly initiated elevations in levels of IL-6, IL-8, IL-18; and a late-peaking increase in levels of the immunoregulatory cytokine IL-10.

The introduction of ART, by reducing viral load and increasing the CD4 count, has dramatically improved survival in HIV-1 infected individuals. However, despite ART, there is incomplete immune reconstitution due to the persistent immune activation, although at a lower level.[23–28] Furthermore, some patients cannot tolerate therapy, due to cytotoxicity caused by the available ART drugs,[29,30] and in others, nonadherence to the treatment regimen or development of resistance to the drugs constitutes a limiting factor in controlling the infection.[31,32] In a recent study, we have reported mutations in the reverse transcriptase and protease genes of the virus from both antiretroviral naïve (ART) and treated HIV-1 infected children.[33] The investigations for immunology-based therapies, new viral or cellular targets, that in combination with ART,[32,34] would bring the infection under control are underway. Profiling the cytokines and their receptors will plausibly enable identification of factors that could complement the information from viral load and CD4 counts in the analysis of the disease status,[35] thus aiding in better management of the disease and therapeutic interventions.[36]

Both, humoral and cell-mediated immune responses contribute to the protective immunity in LTNPs and cytokines are integral components of the immune response.[37,38] It is important to evaluate the modulation of cytokine responses in HIV-1 infection as cytokines are not only crucial for cell-mediated immunity,[38–40] but can also stimulate HIV-1 replication in latently infected cells.[38,41] The role of immune activation at different stages of HIV-1 infection and disease progression in ART naïve and treated children has not been investigated so far. In HIV-1 infected children, persistent higher viremia and limited immunological memory contribute to faster disease progression than in adults,[9,42] thereby warranting therapeutic interventions at an early age. This study is aimed to assess the expression of various immune response genes as a correlate of disease progression in HIV-1 infected children.

METHODS

Subjects

The study was approved by the Institute Ethics Committee (IEC/NP-269/2012 and RP-39/2012). A total of 79 HIV-1 infected children (50 ART naïve and 29 ART treated) and 8 seronegative donors were recruited in this study. After obtaining written informed consent from parents/guardians, blood samples (2–4 mL) of HIV-1 infected children and seronegative donors, scheduled for unrelated surgeries were collected in Ethylenediaminetetraacetic acid (EDTA) vacutainers from the Pediatrics Out Patient Department (OPD) and Pediatrics Surgery OPD, respectively, at the All India Institute of Medical Sciences (AIIMS), New Delhi. The HIV-1 infected children recruited in the study were documented to have acquired the infection through perinatal mode of transmission. The HIV-1 infected ART naïve children were grouped into 24 LTNPs and 26 progressors; the ART treated children were sub-grouped into 17 ART responders and 12 ART nonresponders. All the infected children were asymptomatic at the time of recruitment. Children with coinfections like respiratory, gut infections or tuberculosis were excluded from the study. HIV-1 infected asymptomatic ART naïve children with >7 years of infection, with CD4 counts ≥500 cells/μL at the time of recruitment, and stable for the last 18 months, were categorized as LTNPs. Progressors were defined as infected children (age <5 years) with CD4 count ≤25% and children (age >5 years) with CD4 counts ≤500 cells/μL or declining for 18 months before recruitment.[43–45] Majority of the progressors in this study were below 5 years of age (rapid progressors), while in only 3 children, the onset of disease progression was at a later age (>5–15 years) (slow progressors). Due to limited number of slow progressors, we have clubbed both rapid and slow progressors under the category of progressors. ART responders were defined as infected children on ART for at least 6 months, with controlled viremia, that is, ≤2000 viral RNA copies/mL. ART nonresponders were defined as children on ART for at least 6 months, with uncontrolled viremia, that is, ≥2000 viral RNA copies/mL.[7] Fifteen of the 26 ART progressors were followed up post 6 months initiation of ART during the span of this study, while a few of the infected children were lost to follow up.

The ART regimen for the infected children on treatment included a combination of 2 nucleoside reverse transcriptase inhibitors (NRTIs)—lamivudine with zidovudine or stavudine and 1 nonnucleoside reverse transcriptase inhibitor (NNRTI)—nevirapine or ffavirenz, as per the national guidelines.[46]

CD4 Count and Viral Load Measurement

The plasma viral load was determined by quantitative reverse transcriptase polymerase chain reaction (quantitative RT-PCR) (Roche COBAS TaqMan HIV-1 v2.0; Roche Diagnostics, Indianapolis, IN), according to the manufacturer's instructions. The lower detection limit of the assay was 47 HIV-1 copies/mL. The $CD4^+$ T cell counts were assessed by flow cytometric analysis (BD Biosciences, Sparks, MD) at the Department of Microbiology, AIIMS.

Isolation of PBMCs, RNA, and cDNA Synthesis

Blood (2–4 mL) drawn from the HIV-1 infected children and seronegative donors was centrifuged within 30 minutes at 3000 rpm for 10 minutes. The separated plasma samples were stored frozen at −80°C for assessment of cytokine levels. PBMCs were isolated using the Ficoll (Histopaque) gradient centrifugation method. RNA was extracted using RNeasy kit (Qiagen Ltd, Crawley, UK), quantified by nanospectrophotometry NanoDrop, ND-1000, (Thermo Scientific, Wilmington, DE, USA). The cDNA was prepared using 500 ng RNA by Reverse Transcriptase (Genei, Bangalore, India) in the presence of RNAse inhibitor (Genei) and Random Hexamer Primers (Genei).

TABLE 1. List of Primers

Primers	Forward Primer	Reverse Primer
GAPDH	5′-GACAAGCTTCCCGTTCTCAG-3′	5′-GAGTCAACGGATTTGGTCGTCGT-3′
IL-8	5′-TTCAGAGACAGCAGAGCACA-3′	5′-AGCACTCCTTGGCAAAACTG-3′
IL-8R (CXCR1)	5′-TGAGCCCCGAATCTGACATT-3′	5′-CAGCTGGCAGGTTGATGTTT-3′
GRB2	5′-GCTTCCGCCGGGGAGATTTT-3′	5′-GGGGAAACATGCCGGTCTGC-3′
SHP2	5′-CTGGTGTGGAGGCAGAAAAC-3′	5′–GGACCAACTCAGCCAAAGTG-3′

CXCR1 = C-X-C motif chemokine receptor 1, GAPDH = glyceraldehyde 3-phosphate dehydrogenase, GRB2 = growth factor receptor-bound protein 2, IL-8 = interleukin-8, SHP2 = SRC homology phosphatase 2.

T- and B-Cell Activation PCR Array

Quantitative RT-PCR array was performed for T- and B-cell activation genes and for various cytokines using Qiagen T and B cell activation array kit (Qiagen Ltd, cat no.-PAHS-053Z). Reactions were set up using the cDNA from HIV-1 infected children, of the following categories; 5 LTNPs, 3 progressors, 3 ART responders, 3 ART nonresponders, and 3 HIV-1 seronegative donors. The array data were analyzed using the software (RT Profiler PCR Array Data analysis version 3.5).

Gene Expression Analysis by Real-Time PCR

The array data were validated by performing quantitative RT-PCR analysis of the selected differentially expressed genes. Gene expression profiling of SHP2 and GRB2 involved in the induction of IL-8 and IL-8R (CXCR1) was done by qRT-PCR using specific primers (Table 1), Taq Polymerase (Invitrogen, Thermo Scientific, Wilmington, DE, USA) and Sybr Green dye (Bio Rad CFX96 Touch™ Real-Time PCR Detection System-California, USA). Fold expression was calculated by the $2^{-\Delta\Delta CT}$ method.

Measurement of Plasma Levels of IL-8

Plasma levels of IL-8 were measured using Cytometric Bead Flex Set (BD Biosciences, Sparks, MD, USA) in the HIV-1 infected children and seronegative donors. Briefly, 50 μL of beads coated with capture anti IL-8 antibodies (capture beads), were mixed with 50 μL of sample and incubated for 1 hour in dark at room temperature. After 1 hour, 50 μL of phycoerythrin conjugated detection antibody was added to this sample-bead mixture, incubated for 2 hours in the dark at room temperature. The samples were washed with 1 mL of wash buffer (300 g, 5 minutes) and were then acquired on the FACS Accuri C6 and analyzed using FCAP Array Software (BD Biosciences, Sparks, MD, USA). Calibration curves were constructed using serial dilutions of cytokine standard provided with the kit. Instrument calibration was done (BD FACS Accuri C6) with SPHERO 8-peak Rainbow Particles to ensure accuracy/validity of the data.

In order to evaluate the changes in the relative mRNA expression and plasma levels of IL-8 with time, we followed up 15 ART progressors post 6 months' initiation of ART.

Statistical Analysis

Fold changes in the expression of T- and B-cell genes in the HIV-1 infected children of different groups were quantitated by qRT-PCR array and analyzed using RT Profiler PCR Array Data analysis version 3.5). Relative mRNA expression of the P38, SHP2, GRB2, IL-8, CXCR1 genes, and plasma levels of IL-8 were calculated between the different groups using the Mann–Whitney *U* test (GraphPad Software, San Diego, CA). Results are given as median and interquartile ranges. Spearman Rank test was used to evaluate the correlation between plasma levels of IL-8 and immune parameters in the infected children. *P*-values <0.05 were considered significant. For analysis of paired samples of HIV-1 infected children before and after initiation of ART, paired *t* test was used to calculate the relative IL-8 mRNA expression, and alterations in plasma levels of IL-8.

RESULTS

A total of 79 asymptomatic, perinatally HIV-1 infected children, including 50 ART naïve, 29 ART treated, and 8 healthy seronegative donors were recruited for the study. The median age of LTNPs was 8 years, while that of progressors was

TABLE 2. Demographic and Clinical Profile of the HIV-1 Infected Children

Parameter	LTNPs (n = 24)	Progressors (n = 26)	*P*	ART Responders (n = 17)	ART Nonresponders (n = 12)	*P*
Age (y), median (range)	8 (7–18)	2 (0.6–15)	–	12 (5–16)	11.5 (7.5–14)	–
CD4 count cells/μL, median (range)	791 (500–1918)	494 (130–1689)	0.013	861.5 (309–1439)	417 (101–1690)	0.0726
Viral load RNA copies/mL, median (range)	16900 (37–2.6x10^5)	824000 (8200–1x10^7)	0.0002	423.5 (39.30–2090)	48600 (6140–1.8x10^5)	<0.0001
Duration of ART in treated children (y) median (range)	N/A	N/A	–	2.95 (1–10.5)	6 (1–6.8)	0.2466

ART = antiretroviral therapy, LTNPs = long-term nonprogressors, N/A = not applicable, n = number of subjects, RNA = ribonucleic acid, y = years.

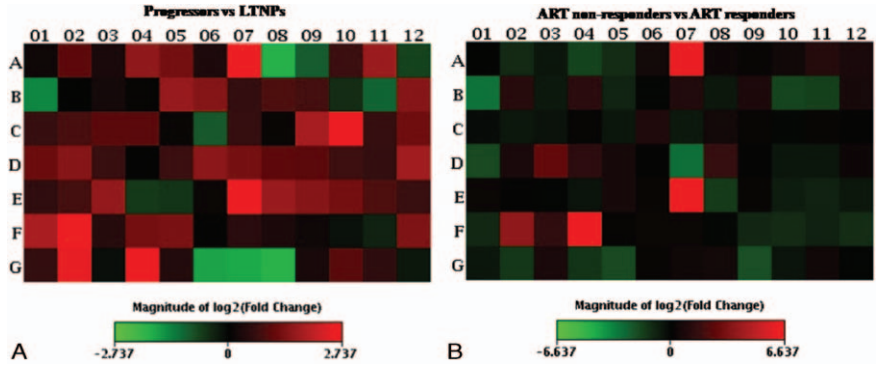

FIGURE 1. Visualization of log2 (fold change) of HIV-1-specific qRT-PCR array of T and B cell activation genes. F4 represents relative mRNA expression of IL-8 gene in (A), progressors (n = 3) vs long-term nonprogressors (LTNPs) (n = 5) (B), ART nonresponders (n = 3) vs ART responders (n = 3).

2 years. The CD4 counts were significantly lower and viral load was significantly higher in progressors than LTNPs. Among the ART treated group, ART non-responders showed lower CD4 counts and significantly higher viral load than ART responders. The demographic profile of the HIV-1 infected children is summarized in Table 2.

Following the categorization of the infected children into the different groups, 5 LTNPs, 3 progressors, 3 ART

TABLE 3. List of Genes Differentially Expressed in T and B Cell Activation PCR Array (A)

Genes Upregulated	Genes Downregulated	Genes Upregulated	Genes Downregulated
AICDA	CCR1	CCL3	AICDA
BCL2	CD1D	CCR4	BCL2
BLM	CD40	CD2	BLM
CCL3	TLR1	CD274	CD1D
CCR4	TLR2	EGR1	CD4
CD276	TLR4	FAS	CD40
CD28		IFNG	CXCR5
CD40LG		IL-1β	ICOSLG
CD7		IL6	IL2
CD80		IL7	IL5
CSF2		IL8	MICB
CX3CL1			MS4A1
CXCR4			NOS2
CXCR5			RAG1
DPP4			SOCS1
FOXP3			TGFB1
ICOSLG			TLR6
IFNG			
IL10			
IL12β			
IL13			
IL1β			
IL2			
IL2RA			
IL3			
IL5			
IL6			
IL8			
IRF4			
NOS2			
RAG1			
SOCS1			

Progressors (n = 3) vs LTNPs (n = 5) (B), ART nonresponders (n = 3) versus ART responders (n = 3).
(A) Progressors vs LTNPs (B) ART nonresponders vs ART responders.
ART = antiretroviral therapy, LTNPs = long-term nonprogressors.

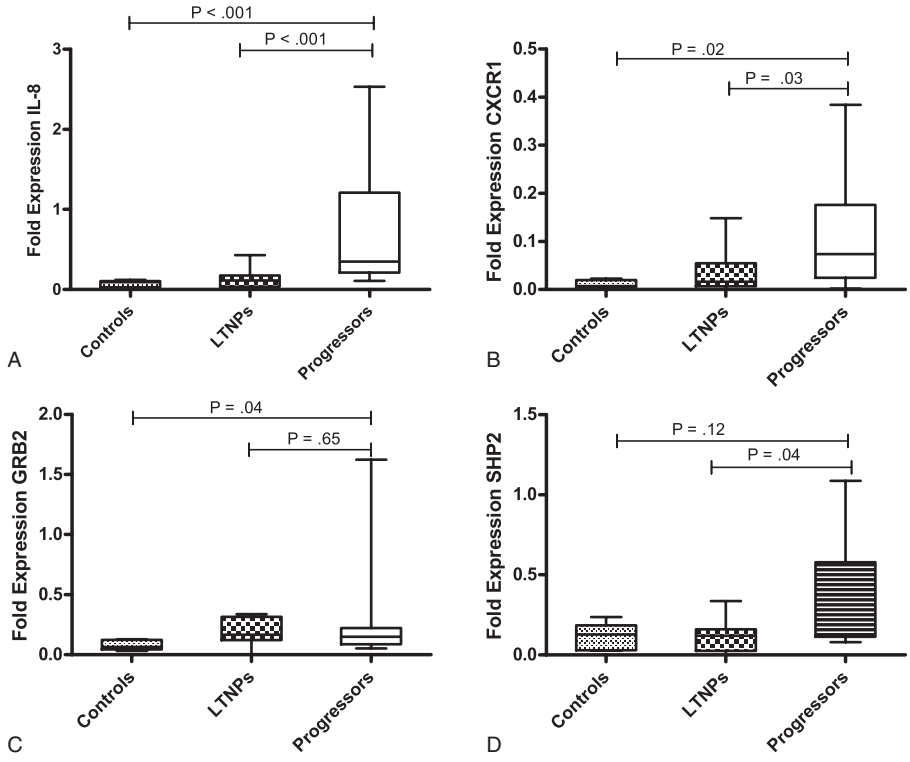

FIGURE 2. Relative mRNA expression of (A), IL-8 (B), CXCR1 (C), GRB2, (D), SHP2, in PBMCs of HIV infected ART naïve children at different stages of the disease, that is, long-term nonprogressors (LTNPs) (n = 12) vs progressors (n = 16). Lines indicate the median, boxes interquartile ranges, and whiskers indicate the minimum–maximum.

responders, and 3 ART nonresponders were analyzed for the expression of immune response genes by qRT-PCR arrays (Figure 1). The array layout is given in Fig. S1, http://links.lww.com/MD/A988 and the differentially expressed genes in the ART naïve and ART treated groups of HIV-1 infected children are provided in Table 3.

Alterations in T- and B-Cell Activation Genes Array in ART Naïve Progressors Versus LTNPs and ART Nonresponders Versus ART Responders

A >2-fold upregulation was observed in a number of genes, predominantly those encoding proinflammatory cytokines such as IL-8, IL-1β, CCL3, and IL-6 in the 3 progressors versus 5 LTNPs and 3 ART responders versus 3 ART nonresponders. Downregulation of expression of CD1d and CD40 was observed in progressors and ART nonresponders. Among the B-cell response genes, a lower expression of BCL-2 and RAG-1 was observed in ART non-responders versus ART responders (Table 3).

IL-8 Upregulation in ART Naïve Progressors Versus LTNPs and ART Nonresponders Versus ART Responders in T- and B-Cell Activation Array

The expression of IL-8 was 2.4-fold higher in HIV-1 infected 3 progressors as compared to 5 LTNPs (Figure 1A) and 85.4-fold higher in 3 HIV-1 infected ART nonresponders than in the 3 ART responders (Figure 1B).

Higher Expression of IL-8, IL-8R, and the Mediators of IL-8 in ART Naïve Progressors Versus LTNPs Confirmed by qRT-PCR

The differential expression of the IL-8 gene observed between the different study groups was validated by qRT-

TABLE 4. Plasma IL-8 Levels in Different Categories of HIV-1 Infected Children

Plasma IL-8 Levels (pg/mL)	Controls (n = 8)	Progressors (n = 26)	LTNPs (n = 24)	ART Nonresponders (n = 12)	ART Responders (n = 17)
Median (range)	9.88 (2.72–19.88)	46.24 (5.48–557.7)	5.34 (1.47–48.96)	22.90 (1.8–181.8)	4.05 (0.58–18.90)

ART = antiretroviral therapy, IL-8 = interleukin 8, LTNPs = long-term nonprogressors, n = number of subjects.

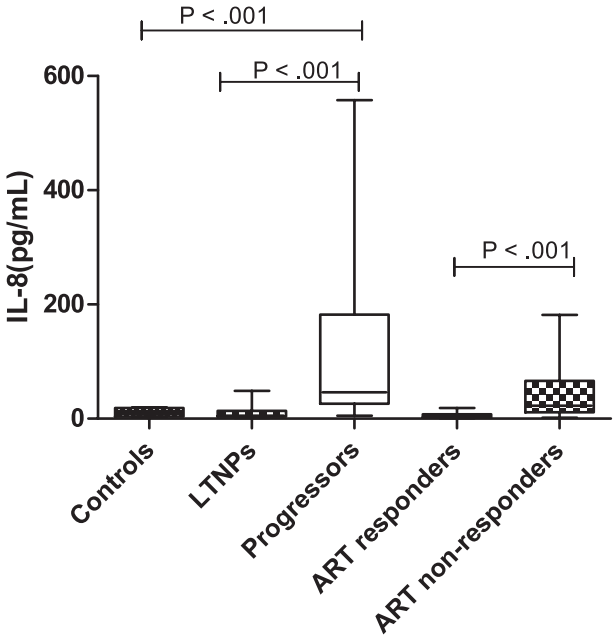

FIGURE 3. Plasma levels of IL-8 (pg/mL) in HIV infected children at different stages of the disease, that is, HIV infected ART naïve, long-term nonprogressors (LTNPs) (n = 24) versus progressors (n = 26) and ART responders (n = 17) versus ART nonresponders (n = 12). Lines indicate the median, boxes interquartile ranges, and whiskers indicate the minimum–maximum.

PCR analysis of IL-8, its receptor CXCR1 (IL-8R), and the upstream signaling molecules, that is, SHP2 and GRB2 in 12 LTNPs and 16 progressors, including the samples analyzed by gene arrays. Relative mRNA expression of IL-8 ($P < 0.001$), CXCR1 ($P = 0.03$) and SHP2 ($P = 0.04$) were significantly higher in progressors compared to LTNPs (Figure 2 A, B, and D). The expression of GRB2 was comparable between the progressors and LTNPs ($P = 0.65$) (Figure 2C).

High Levels of Plasma IL-8 in Antiretroviral Naïve Progressors Versus LTNPs and ART Nonresponders Versus ART Responders

The plasma levels of IL-8, as determined by CBA Flex set, were found to be significantly higher in 26 progressors versus 24 LTNPs ($P < 0.001$) and 12 ART nonresponders versus 17 ART responders ($P < 0.001$) (Figure 2). Median plasma levels of IL-8 were 46.24, 5.34, 22.9, and 4.05 pg/mL, in progressors, LTNPs, ART nonresponders, ART responders, respectively. The median plasma IL-8 levels in the LTNPs and ART responders were comparable to that in the control samples (Table 4, Figure 3).

Plasma Levels of IL-8 Correlated Positively With CD4 Counts and Inversely With Viremia

Plasma levels of IL-8 showed significant negative correlation with CD4 count (cells/μL) in HIV-1 infected 50 ART naïve subjects ($r = -0.488$; $P < 0.001$) (Figure 4 A). Among the 29 ART treated children, the plasma levels of IL-8 positively correlated with viral load ($r = 0.5494$; $P < 0.001$) (Figure 4B).

Reduced Plasma Levels and Relative mRNA Expression of IL-8 in Progressors, 6 Months Post-ART

On following up 15 ART naïve progressors, we observed a significant reduction in the plasma levels of IL-8 ($P = 0.05$), after 6 months of initiation of ART (Figure 5A). Similarly, a significant reduction in the relative mRNA expression of IL-8 ($P = 0.05$) was found in 8 children post 6 months of ART as compared to their ART naïve status (Figure 5B).

DISCUSSION

According to the recent guidelines of the National AIDS Control Organization (NACO), India,[47] all children <24 months, tested to be seropositive for HIV-1 should be started on ART, irrespective of clinical or immunological stage. Therefore, the present existing cohort of LTNPs and ART-naïve progressors included in this study are potential candidates to study the immunopathogenesis of HIV. The perinatally infected children recruited here comprised of ART naïve and treated children that have been systematically followed up since the year 2009. Hence we have identified 24 potential LTNPs demonstrating stable CD4 counts for at least more than 7 years of infection. Majority of the ART naïve progressors included here (23 of the 26 progressors) were younger than the LTNPs as expected. As is also well documented in literature that disease progression is faster in children than adults,[42] most of the progressors in the ART naïve group progressed rapidly within 5 years of infection. To the best of our knowledge, this is the first systematic study conducted in HIV-1 infected children wherein we assessed the array of T- and B-cell activation genes and evaluated the alterations in the modulated genes at different stages of disease progression.

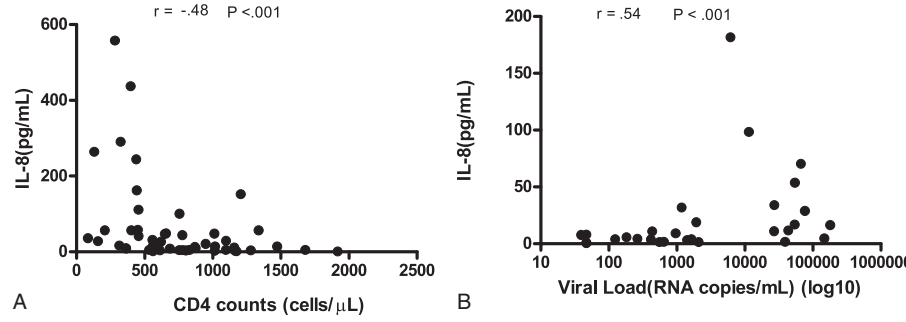

FIGURE 4. (A) Correlation of CD4 counts with plasma levels of IL-8 in ART naïve (n = 50) HIV-1 infected children. (B) correlation of viral load with plasma levels of IL-8 in ART treated (n = 29) HIV-1 infected children.

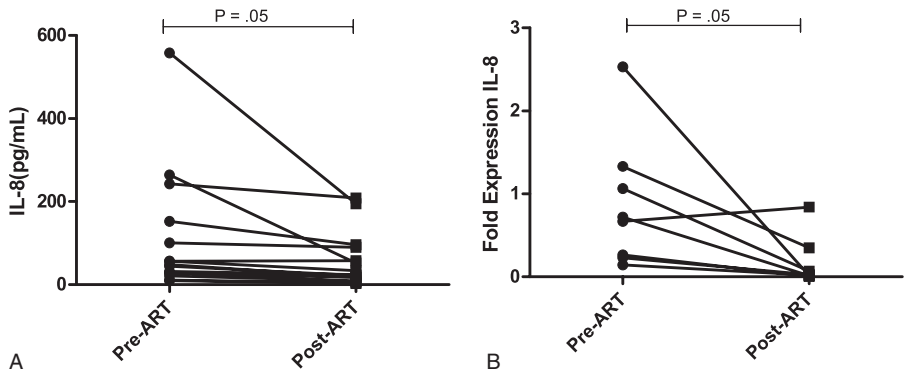

FIGURE 5. (A), Plasma levels of IL-8 (pg/ml) in progressors (n = 15) (B), relative mRNA expression in progressors (n = 8), pre- and postinitiation of ART.

The array represents the host immune response, with key genes encoding mediators of both innate and adaptive immunity. A change of >2-fold was taken into consideration. The expression of several genes, such as the proinflammatory cytokines were higher in progressors versus LTNPs and ART non-responders versus ART responders. IL-8, a proinflammatory cytokine, serves as a chemoattractant involved in the activation of neutrophils, basophils, and some subpopulation of lymphocytes.[48,49] During HIV-1 infection, IL-8 plays an important role in the recruitment of CD4-positive T cells to the lymph nodes, thus generating more targets for viral replication.[50] Elevated levels of IL-8 are reported in the serum of HIV-1 infected individuals and in the lymphoid tissue of patients with AIDS.[51] The precise role of IL-8 in disease progression needs to be established. Majority of the studies addressing the cytokine profile in HIV-1 infection are conducted in adults, with no information available in the infected children. This study focused on assessing alterations in IL-8 and its related signaling molecules in the infected children at different disease stages. A salient finding of our study is the several fold higher genotypic and phenotypic expression of IL-8, as observed from the PCR array and plasma levels, in the HIV-1 infected progressors and ART nonresponders as compared to LTNPs and ART responders, respectively. Our findings are in concordance with earlier studies demonstrating the in vitro induction of IL-8 by HIV-1 envelope glycoprotein gp120.[52] The high viremia in the progressors and ART nonresponders could be one of the factors contributing to the elevated expression and plasma levels of IL-8, in addition to a number of other host factors.[15] Moreover, we observed that high IL-8 levels correlates with lower number of CD4 T cells (in progressors) and high viremia (in ART nonresponders), the factors contributing to faster disease progression, irrespective of age. Further, the reduction in IL-8 levels in progressors who were initiated on ART treatment suggests the beneficial effects of antiretroviral therapy in reducing inflammation, which may plausibly be due to the effect of the therapy in controlling viremia.

The high expression of IL-8, IL-8R (CXCR1), and SHP2 along with elevated plasma levels of IL-8 in the progressors as compared to LTNPs implicates the involvement of the IL-8 pathway in the immunopathogenesis of HIV-1. The major biological effects of IL-8 are mediated via its receptor, rhodopsin-like guanine-protein-coupled receptors (GPCRs): CXCR1 (IL-8RA).[53,54] Also, our findings are in accordance with an earlier report implicating the role of SHP2, GRB2 as mediators of IL-8 induction in HIV and HCV infection.[55]

In vitro studies have demonstrated that IL-8 stimulates HIV-1 replication in monocyte derived macrophages (MDM) and T lymphocytes.[32] Currently, an IL-8-specific monoclonal antibody is in use in clinical trial of patients with psoriasis, a disease marked by chronic inflammation.[56] Antibodies that neutralize IL-8 activity, or block IL-8 receptors have been shown to inhibit HIV-1 replication in T-lymphocytes.[32]

In a study conducted by Roberts et al,[57] HIV infected adult participants were divided into low, medium, or high risk groups according to their risk scores calculated using CD4 counts by the β-coefficients of the Cox proportional-hazards model. However, there is wide variation in CD4 counts in children of different age groups, thereby warranting the need to identify additional immune based parameters, which along with CD4 counts can be useful for calculating the risk score for better prognosis of the disease. Cytokines are potential candidates to be considered for prognostic purposes.

One of the limitations of the present study is that we have focused on a single cytokine IL-8 and its related signaling molecules, although changes were observed in a number of proinflammatory cytokines such as IL-1β, CCL3, IL-6 and its receptor in the different study groups. These cytokines and their associated molecules could not be evaluated by qRT-PCR at the baseline or in the follow up samples due to limited sample availability from the children. The inflammatory responses in HIV-1 infection are mediated by a complex interaction between several cytokines. The role of other cytokines and immune response genes that promote inflammation and disease progression remain to be addressed. Further studies should be conducted in a larger sample size of HIV-1 infected children at various disease stages and in different populations to confirm our findings.

To conclude, our data suggest that IL-8 levels are indicative of the varying inflammatory status of the HIV-1 infected children at different stages of the disease. The study identifies IL-8 as one of the potential cytokines that may be used among a panel of cytokines that can be designed in future to evaluate disease progression in HIV-1 infected children. The role of inhibitors of IL-8 and its receptor CXCR1, to serve as candidates for therapeutic intervention, needs to be evaluated.

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