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ORIGINAL RESEARCH

Genetic Variation in Toll-Like Receptors (TLRs) 2, 4, and 9 Influences HIV Disease Progression Toward Active TB and AIDS

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Background: Toll-like receptors (TLRs) are identified as one of the key components of the innate immune system. The objective of this study was to explore the influence of genetic variability in these TLRs on human immunodeficiency virus (HIV) disease progression with and without tuberculosis (TB) co-infection.

Materials and Methods: This prospective, cross-sectional, and longitudinal study included 373 HIV-positive patients without TB infection. This study aimed to examine the genetic variation in TLRs (TLR2, TLR4, and TLR9) between patients with HIV-1 infection and those who progressed to active TB during the two years of follow-up.

Results: During the two year follow-up of 373 positive patients, 98 patients progressed to active TB/AIDS (acquired immunodeficiency syndrome). When comparing 98 HIV patients who developed active TB/AIDS to 275 HIV patients who did not, it was discovered that the frequency of the A allele in TLR9 was considerably higher (p <0.001) in HIV patients progressed to active TB/AIDS. Ninety eight HIV individuals who advanced to active TB/AIDS showed a significantly higher frequency of the AA genotype in TLR9 than did in HIV patients who had no TB/AIDS (p <0.001).

Conclusion: The increased association of the AA genotype of TLR9 in HIV patients who progressed to active TB during follow-up suggests that HIV-positive patients with the AA genotype of TLR9 have increased susceptibility towards TB during the disease progression.

Keywords: toll-like receptors, human immunodeficiency virus, tuberculosis, acquired immunodeficiency syndrome, immune system

Introduction

An estimated 4197 people died of acquired immunodeficiency syndrome (AIDS) in India in 2021. The Annual New Infection (ANI) count in India is estimated at 6297 in 2021.[1,2] Tuberculosis (TB) remains one of the deadliest communicable diseases in the world.[3] It has been demonstrated that human immunodeficiency virus (HIV) positive subjects to exhibit variable susceptibility to TB infection and disease.[4,5]

To reduce the mortality and morbidity associated with HIV-TB co-infection or avert TB development among HIV-positive patients, it is important to investigate the factors responsible for generating this inter-individual variability among HIV-positive hosts for TB infection. Studies have demonstrated a significant correlation between the elements of the immune system, susceptibility to various infections, and disease progression. It is well known that the host immune response contributes to host–pathogen interaction/host–virus crosstalk.[6,7]

A genetic mutation, also known as genetic polymorphism, is defined as a change in the nucleotide sequence at a specific locus of the genome that occurs among individuals, groups, or populations. The most common genetic polymorphism in humans is a single nucleotide polymorphism (SNP), which arises from nucleotide base substitutions between purines (A, G) or between pyrimidines (C, T). Detailed analysis of human deoxyribonucleic acid (DNA) revealed that SNPs are primarily

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concentrated in specific regions of the genome, which encode proteins involved in host defense mechanisms, compared with other genes encoding body proteins/enzymes. The occurrence of genetic polymorphisms or variations in the DNA sequence confers variability in susceptibility to various infectious diseases.[8,9]

Numerous studies have shown that the innate immune response is crucial for the etiology, progression, and susceptibility to TB of HIV disease.[10–13] The key component of the innate immune system, toll-like receptors (TLRs), are responsible for activating the signaling pathway that regulates HIV replication and disease progression.[14]

In other words, the latent and chronic stages of HIV infection are dependent on TLR-mediated pathways.[15] Increasing evidence has indicated that mutations in TLRs and their associated signal transduction molecules may impair or modulate a host's ability to respond to pathogens.[16]

It has been demonstrated that TLR2, TLR4, and TLR9 (in particular), which were initially known to recognize bacterial products, are involved in the detection of viral components.[17–19] However, it was noted that the majority of these genetic studies were carried out in cohorts of Western European ancestors, and very few studies have investigated the role of TLR mutations in susceptibility to HIV in the Indian population. Studies have revealed that TLR polymorphisms are associated with an increased risk of bacterial and viral infections. Several SNPs in various TLR genes, such as TLR2, TLR4, TLR5, TLR6, TLR9, and TLR10, have been associated with disease susceptibility.[20–22] Therefore, a prospective longitudinal study involving HIV-positive patients from the North Indian population who were ethnically homogeneous and naive to antiretroviral therapy (ART) was conducted.

This study aimed to examine the genetic variation in TLRs (TLR2, TLR4, and TLR9) between patients with HIV-1 infection and those who developed TB during the two years of follow-up. Two years of follow-up was carried out for 373 HIV patients. Ninety eight HIV positive patients were progressed to active TB/AIDS during follow-up..

Three SNPs (rs121917864 in TLR2, rs4986790 in TLR4, and rs352140 in TLR9) were examined in 373 HIV positive patients which consist of 275 HIV patients (no TB/AIDS) and HIV patients who advanced to active TB/AIDS (n = 98).

Materials and Methods

Study Setting and Design

The institutional ethics committee of the All India Institute of Medical Sciences (AIIMS), located in New Delhi, approved the study protocol. All study participants provided written informed consent before enrollment in the study.

Study Population

The goal of the current study was to look at genetic polymorphism in TLRs (TLR2, TLR4, and TLR9) in HIV-positive patients. The study population comprised HIV-positive individuals without tuberculosis infection or disease. The Infectious Diseases (ID) Clinic and ART Clinic, which are supported by the Ministry of Health and Family Welfare, Government of India, AIIMS, New Delhi, were the sources from which all HIV-positive individuals were selected.

HIV Patients (n = 373)

This group included patients who were HIV-seropositive. HIV-1 infection was documented according to the recommendations of the World Health Organization (WHO) and adopted by the National AIDS Control Organization (NACO) Government of India. These patients were tuberculin skin test (TST) negative (induration <5 mm with five Tuberculin Units; purified protein derivative [PPD], Span Diagnostics Limited, Surat, India). All the patients underwent rigorous clinical, microbiological, and radiological investigations to rule out the presence of active TB The group included patients who were HIV-seropositive. HIV-1 infection was documented according to the recommendations of the World Health Organization (WHO) and adopted by the National AIDS Control Organization (NACO) Government of India. These patients were tuberculin skin test (TST) negative (induration < 5 mm with five Tuberculin Units; purified protein derivative [PPD], Span Diagnostics Limited, Surat, India). All the patients underwent rigorous clinical, microbiological, and radiological investigations to rule out the presence of active TB. Figure 1 shows the patient allocations, which were as follows: first, HIV+ (n = 275), and second, HIV patients who acquire tuberculosis (n = 98) (Figure 1).

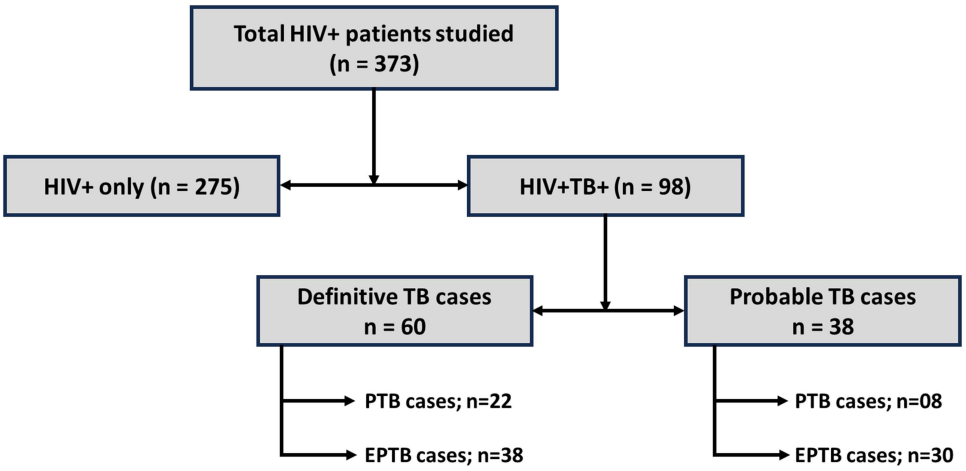

Figure 1 Shematic diagram of study design.
Abbreviations: HIV, human immunodeficiency virus; TB, tuberculosis; PTB, pulmonary tuberculosis; EPTB, Extrapulmonary tuberculosis.

Exclusion Criteria of the Study

The exclusion criteria were as follows: (i) subjects with a history of TB; (ii) patients with multi-drug resistant tuberculosis (MDR-TB); (iii) pregnant and lactating females; (iv) study subjects with hepatitis B or C positivity; (v) presence of secondary immunodeficiency states among study subjects, such as organ transplantation, diabetes mellitus, malignancy, and treatment with corticosteroids (on detailed history and laboratory investigations); (vi) patients requiring surgical intervention; (vii) terminally ill patients according to the clinician's judgment; and (viii) patients who had difficulty in follow-up.

Study Procedure

The demographic and clinical information of all recruited subjects was captured using a standardized questionnaire. Baseline body weight and height were measured in all subjects along with checking of BCG (Bacille Calmette Guerin) scars. Data on smoking, alcoholism, substance abuse, and family history of TB were also collected.

Biological Samples

A 4–6 mL whole venous blood sample was obtained from all HIV-positive patients at baseline for CD4 cell count and plasma viral load (PVL) estimations. Approximately 6–8 mL of peripheral venous blood was obtained aseptically in ethylenediaminetetraacetic acid (EDTA) vials from all study subjects for molecular studies and genomic DNA extraction.

Follow-Up and Treatment of Recruited Patients

HIV positive patients were longitudinally followed up for 2 years to study HIV disease progression. During the 2-year follow-up period, participants were asked to visit the hospital every 6 months (6, 12, 18, and 24 months). Patients who did not return for scheduled follow-up visits were contacted via telephone. At every follow-up visit, patients were meticulously examined for the presence of active TB. Immunological (CD4 cell count) and plasma viral load (PVL) at baseline and at the end of 2 years were also assessed in these patients during the follow-up period.

According to the Revised National Tuberculosis Control Programme (RNTCP) guidelines, Ministry of Health and Family Welfare, Government of India, anti - tuberculosis treatment (ATT) was initially (thrice weekly intermittently) administered from the respective Directly observed treatment, short-course (DOTS) center to HIV+ patients with a CD4 cell count of <250 cells/μL who were diagnosed with tuberculosis disease. This was in accordance with the 2007 NACO guidelines.

Molecular Techniques and Protocols

DNA Extraction

A DNA blood maxi kit (Qiagen, Hilden, Germany) was used to extract genomic DNA from peripheral blood. Each centrifugation step was performed between 15°C and 25°C. This procedure was performed in accordance with the manufacturer's guidelines.

Determination of DNA Concentration

Quantification of genomic DNA was carried out using optical density (OD) measurement of 1 µL of DNA at a wavelength of 260 nm and 280 nm using an ultraviolet (UV) nanophotometer. The pure DNA preparation gave a OD 260/OD 280 ratio of 1.8:2. The OD value at 260 nm was used to calculate the DNA concentration of DNA considering that 1 OD at 260 nm is equivalent to 50 µg DNA.

DNA was subjected to electrophoresis on 0.8% agarose gel stained with ethidium bromide (EtBr). For daily use, DNA was stored at 4°C and, for long-term storage, it was preserved at –80°C.

Genotyping for the TLR Polymorphisms

The TLR genotypes for TLR2, TLR4, and TLR9 were assessed by polymerase chain reaction –restriction fragment length polymorphism (PCR-RFLP) analysis using an Eppendorf thermocycler for PCR amplification (Eppendorf AG 22331, Hamburg, Germany). Specific primers purified by polyacrylamide gel electrophoresis (PAGE) were obtained from Bio Basic Inc. (East Markham, Canada), while restriction enzymes and other reagents were procured from New England BioLabs.

TLR2 Polymorphism

TLR2 gene polymorphisms were determined by PCR followed by RFLP. The forward (5'-CCCCTTCAAGTT GTGGCTTCATAAG-3') and reverse (5'AGTCCAGTTCAT ACTTGCACCAC-3') primers used to genotype the TLR2 SNP were based on a previous report.[23] Primers were suspended in TE solution, and stocks were made of 100 pmol/µL concentration. All the PCRs were performed in a reaction volume of 25 µL, containing 2.5 µL of 10 × PCR buffer, 2 µL of 25 mM $MgCl_2$, 0.2 µL of 25 mM dNTPs, 0.75 µL of each primer (10 pmol/µL), 1.5 U of *Taq* polymerase (from Bangalore Genei) and 2 µL of DNA (50–100 ng/µL) depending on quality. The PCR reactions were run under the following thermal conditions: 15 minutes of initial denaturation at 95°C; 35 cycles of 30 seconds each at 94°C, 65°C, and 72°C; and 10 minutes of final extension at 72°C.

After amplification, restriction digestion was performed in 20 µL of reaction mixture with 15 µL amplicon and 5 U of MwoI restriction enzyme incubated at 60°C overnight. The amplification products were electrophoresed on a 5% polyacrylamide gel (PAGE) in Tris-borate-EDTA buffer. The gel was stained with ethidium bromide, visualized, and photographed using a UV transilluminator (Multi-Image™ UV transilluminator, alpha Innotech, Johannesburg 1715, South Africa).

In Arg677Trp genotyping, restriction digestion of the wild-type homozygous genotype (CC) resulted in two restriction fragments (130 and 22 bp) and three bands (152, 130, and 22 bp) in heterozygotes. Mutant homozygotes (TT) were not cut by MwoI restriction and therefore retained a single band (152 bp).

TLR4 Polymorphism

TLR4 gene polymorphisms were determined using PCR followed by RFLP. The forward (5'GATTAGCAT ACTTAGACTACTACCTCCATG-3') and reverse (5'GATCAACTTCTGAAAAAGCATTCCCAC-3') primers used to genotype the TLR4 SNP were based on a previous report.[24] Primers were suspended in TE solution, and stocks were made of 100 pmol/µL concentration. All PCRs were performed in a reaction volume of 25 µL, containing 2.5 µL of 10× PCR buffer, 1 µL DNA at a concentration of 100 ng/µL and 10 pmol of each primer with Taq polymerase (0.5 U of *Taq* polymerase. PCR reactions were run under the following thermal conditions: 4 minutes of initial denaturation at 95°C; 30 cycles of 30 seconds each at 95°C, 55°C, and 72°C; and 7 minutes of final extension at 72°C.

After amplification, restriction digestion was performed with 15 µL aliquots of the amplified product, which were digested overnight with the 5 U of restriction enzyme *NcoI* and electrophoresed on a 3% agarose gel to identify the TLR4 alleles on the basis of their respective allele sizes.

TLR9 Polymorphism

TLR9 gene polymorphisms were determined using PCR followed by RFLP. The forward (5'-CAAGTCCAG CCAGATCAAA-3') and reverse (5'-GCTAGACCTGTCCCACAATAA-3') primers used to genotype the TLR9 SNP were based on a previous report.[25] PCRs were performed in 20 µL of a reaction mixture containing 2 µL of genomic DNA with a concentration of 50 ng/µL, 2 U of *Taq* polymerase and 0.5 µL (10 pico mole) of each primer. PCR amplification involved 45 cycles of denaturation (95°C for 30 seconds), annealing (55°C for 30 seconds), and elongation (72°C for 30 seconds). The initial denaturation was performed at 95°C for 5 minutes.

Following amplification, a 10-µL aliquot of the amplified product was subjected to restriction digestion using 5 U of the restriction enzyme BstuI for an overnight period at 60°C. The amplified product was electrophoresed in a 3% agarose gel to determine TLR9 alleles based on allele size.

Statistical Analysis

One-way analysis of variance was used to compare baseline characteristics such as age and body mass index (BMI) between the groups. Data are shown as the median (interquartile range) = mean standard deviation/mean. Qualitative traits, including genotypic and allelic frequencies, were compared using the Chi-square test. Stata 12.0 (Stata Corporation, College Station, Texas, USA) was used for all the analyses. For statistical significance, a two-sided *p*-value of 0.05 was used.

Results

An analysis was performed to compare the baseline characteristics of HIV patients who had no TB or AIDS and those who developed active TB or AIDS. Table 1 shows the baseline characteristics of 275 HIV patients (no TB/AIDS), and those who progressed to active TB/AIDS (n = 98).

The mean BMI of 275 HIV patients (20.34 ± 2.36 kg/m^2) at baseline was slightly higher than that of 98 HIV patients who progressed to active TB/AIDS (18.23 ± 2.14 kg/m^2). The baseline CD4 cell count of 275 HIV patients (no TB/AIDS), [421 (329–634) cells/mm^3)] was higher than that of 98 HIV patients who progressed to active TB/AIDS [353 (267–436) cells/mm^3], The PVL of HIV patients who progressed to active TB/AIDS [4.67 (3.65–6.18) $\log_{10}$ copies/mL] was higher than baseline PVL of 275 HIV patients with no TB/AIDS [3.42 (3.59–4.98) $\log_{10}$ copies/mL].

Of the 98 HIV patients who progressed to active TB/AIDS, 30 (30.61%) developed PTB, whereas the remaining 68 (69.39%) developed extrapulmonary tuberculosis (EPTB). Of the 98 patients, 60 (61.22) had a definitive diagnosis of TB and 38 (38.78%) had probable TB (Table 1).

Of the 60 definitive cases, 22 had PTB, and 38 had EPTB. Of the 22 definitive PTB patients, 14 were *M.tb* sputum smear positive and 8 were sputum smear and culture positive. Of the 38 EPTB cases, 23 had PE and 15 had LNTB. All PE cases were positive for Mycobacterium *M.tb* smear, culture, or PCR. Patients with LNTB were diagnosed using FNAC.

Of the 38 probable cases, 8 were PTB (contrast-enhanced computed tomography (CECT) chest suggestive of TB), and 30 patients had EPTB. Of the 30 EPTB patients, 17 had LNTB (CECT chest or CECT abdomen suggestive of TB), and 13 had DTB (CECT chest and abdomen suggestive of TB).

Examination of TLRs allelic and genotypic frequencies in HIV individuals (no TB/AIDS) and HIV individuals who developed active TB/AIDS was carried out.

The frequency of the TLR9 allele was significantly higher in 98 HIV patients (62.75%) who developed active TB/AIDS than in 275 HIV patients (no TB/AIDS) (42.91%; $p < 0.001$).

A significant increased frequency of 'AA' genotype (54.10%) in TLR9 was observed in 98 HIV individuals who advanced to active TB/AIDS as compared to 19.64% in HIV positive patients (no TB/AIDS) ($p < 0.001$). However, the frequency of the AG genotype was significantly decreased in 98 HIV individuals who advanced to active TB/AIDS

Table 1 Baseline characteristics of HIV patients (no TB/AIDS), HIV patients who progressed to active TB/AIDS

Variables	HIV patients (no TB/AIDS)* (n = 275)	HIV patients who progressed to active TB/AIDS (n = 98) (EPTB = 68, PTB = 30)
BMI (kg/m2)	20.34 ± 2.36	18.23 ± 2.14
CD4 cell count (cells/ mm^3) Median (IQR)	421 (329–634)	353 (267–436)
Plasma viral load (log_{10} copies/mL) Median (IQR)	3.42 (3.59–4.98)	4.67 (3.65–6.18)
Details of TB diagnosis		**Definitive = 60** **PTB (n = 22)** *M.tb* sputum smear +ve but culture –ve = 14 *M.tb* sputum smear and culture +ve = 8 **EPTB (n = 38)** LNTB = 15 (FNAC proven = 15) PE = 23 (*Mtb* smear +ve but culture –ve = 15) (*Mtb* PCR +ve = 8) **Probable = 38** **PTB (n = 08)** (CECT chest suggestive of TB = 08) **EPTB (n = 30)** LNTB = 17 (CECT chest suggestive of TB = 9) (CECT abdomen suggestive of TB = 8) DTB = 13 CECT chest + abdomen suggestive of TB=13

Notes: Age and BMI are presented as mean ± SD, and CD_4 cell count and plasma viral load are presented as median and interquartile range (IQR).
Abbreviations: HIV, human immunodeficiency virus; AIDS, acquired immunodeficiency syndrome; TB, tuberculosis; PTB, pulmonary tuberculosis; *Mtb*, Mycobacterium tuberculosis; ART, antiretroviral treatment; BMI, body mass index; EPTB, extrapulmonary tuberculosis; LNTB, lymph node tuberculosis; DTB, disseminated tuberculosis; PE, pleural effusion; FNAC, fine needle aspiration cytology; PCR, polymerase chain reaction; CECT, contrast-enhanced computed tomography; ND, not done.

(13.25%) compared with 42.54% in HIV patients without TB/AIDS (p <0.001). No significant difference in the GG genotype was observed between the two groups (Table 2).

Compared to 275 HIV patients (no TB/AIDS), the frequency of the TLR9 "A" allele was found to be significantly higher in 98 HIV+ patients who progressed to active TB/AIDS (62.75%, p <0.001). These findings suggest that the 'A' allele of TLR9 might be playing an important role in acquisition of TB among HIV patients and the 'G' allele was protective against serious infections like TB in HIV patients. When comparing the TLR2 and TLR4 allelic frequencies between the two groups, no discernible differences were found.

There was a notable variation in the frequency of the 'AA' genotype of TLR9 between HIV patients who developed active TB/AIDS (54.10%) and HIV patients who did not have TB/AIDS (19.64%), as indicated by an OR of 0.21 with a 95% confidence interval of 0.06–0.37. On the other hand, 'AG' and 'GG' genotype frequencies were lower in HIV patients who developed active TB/AIDS (13.25% and 32.65%) versus 42.54% and 37.82%, respectively, in HIV positive patients (no TB/AIDS) (OR = 0.38 with 95% CI = 0.17–0.21).

These observations led to the conclusion that the 'AA' genotype of TLR9 might be associated with the susceptibility of TB among HIV patients and responsible for disease progression while 'AG' and 'GG' genotypes of TLR9 might be

Table 2 Comparison of TLR2, 4, and 9 allelic and genotypic frequencies between 275 HIV patients (no TB/AIDS) and 98 HIV patients who progressed to active TB/AIDS

TLR Genotypes	HIV patients (no TB/AIDS) n = 275 (%)	HIV patients progressed to active TB/AIDS n = 98 (%)	*p*	OR (95% CI)
Genotype frequencies				
TLR2 C2180T (Arg677Trp)				
CC	07 (2.54)	2 (2.04)		1
CT	268 (97.46)	96 (97.96)	0.49	0.63 (0.07–15.12)
TT	0 (0)	0 (0)		
TLR4 Asp299Gly (A896G)				
AA	196 (71.27)	65 (66.32)		1
AG	79 (28.73)	33 (33.68)	0.29	0.60 (0.18–2.32)
GG	0 (0)	0 (0)		
TLR9 (1635A/G)				
AA	54 (19.64)	53 (54.10)		1
AG	117 (42.54)	13 (13.25)	<0.001	0.21 (0.06–0.37)
GG	104 (37.82)	32 (32.65)		0.38 (0.17–0.21)
Allele frequencies				
TLR2 C2180T (Arg677Trp)				
C	282 (51.28)	102 (52.04)	1.00	1
T	268 (48.72)	94 (47.96)		0.1 (0.58–1.32)
TLR4 Asp 299Gly (A896G)				
A	474 (86.18)	157 (80.11)	0.14	1
G	76 (13.82)	39 (19.89)		1.43 (0.79–2.74)
TLR9 (1635A/G)				
A	236 (42.91)	123 (62.75)	<0.001	1
G	314 (57.09)	73 (37.25)		0.39 (0.23–0.69)

Abbreviations: TLR, toll-like receptor; HIV, human immunodeficiency virus; TB, tuberculosis; AIDS, acquired immunodeficiency syndrome; OR, odds ratio; CI, confidence interval.

responsible for protecting the HIV patients from acquisition of TB infection. No significant differences were observed in the TLR2 and TLR4 genotypic frequencies between the two groups.

Discussion

Studies are limited to the association of TLR2, TLR4 and TLR9 polymorphism with HIV disease. A study conducted in Tanzania showed increased susceptibility to active tuberculosis in HIV-infected patients who carry the TLR4 Asp299Gly SNP.[4] In a Greek cohort study, the frequency of TLR4 SNP (Asp299Gly) was assessed in HIV-infected patients along with its association with increased susceptibility to severe infection in these patients.[26] A study conducted by Pine et al in 2009 demonstrated the potential role of the TLR4 Asp299Gly polymorphism with increased viral load in a white population.[27]

The associations between TLR9 and several autoimmune and infectious diseases have also been studied. Two recent studies have suggested a role for the TLR9 1635G polymorphism in the clinical course of HIV-1 infection. These studies have shown the possible role of innate immunity in HIV-1 infection.[10,25] Another study focusing on the association of HIV disease progression and the TLR9 1635G polymorphism demonstrated that a low viral load set point and slow progression in the seroincident cohort were linked to the 1635G allele. Previous studies showing the presence of TLR9 SNPs among

HIV-infected populations also suggest a possible role of this polymorphism in HIV disease pathogenesis.[25,26] A study demonstrating a significant association between TLR9 polymorphisms and transmission of HIV-1 infection from infected mothers to their children indicated the critical role of the innate immune response in perinatal HIV-1 infection.[27]

Previous studies showing the presence of TLR9 SNPs in HIV-infected populations also suggest a possible role for this polymorphism in HIV pathogenesis. A study demonstrating a significant association of TLR9 polymorphism with transmission of HIV-1 infection from infected mothers to their children indicates the critical role of innate immune response in perinatal HIV-1 infection.[27] Large cohort and candidate gene-based studies have highlighted the importance of host determinants comprising a number of immune response genes that contribute toward differential vulnerability of individuals to HIV/AIDS and TB.[28] Since variability in disease progression rate, susceptibility to OIs, development of associated complications and progression to profound immunodeficiency or AIDS is quite evident in HIV-positive individuals, it was, therefore, hypothesized that TLR polymorphism might influence disease progression in HIV-infected individuals. The current study was designed to explore common SNPs in TLRs (TLR2, TLR4, and TLR9) in HIV-positive individuals, and to assess the impact of genetic variability in TLRs on the course of HIV infection.

Conclusion

The increased association of 'AA' genotype of TLR9 with HIV patients, who progressed to active TB during follow-up, suggests that HIV-positive patients with the 'AA' genotype of TLR9 have increased susceptibility to active TB. These findings indicate the relevance of innate immunity in the course of HIV infection and contribute to the current understanding of HIV pathogenesis.

Disclosure

The authors report no conflicts of interest in this work.

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