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Single Nucleotide Polymorphisms in Tumor Necrosis Factor-α and Susceptibility to HIV Infection in Local Population of Lahore Pakistan

Shahid Nawaz[1], Tahir yaqub[1]*, Arfan Ahmad[1], Asif Nadeem[2] Nadia Mukhtar[1], Firnas Ata Ur Rehman[1], Saima Yaqub[1], Furqan Shahid[1], Muzaffar Ali[1]

[1] *Department of Microbiology University of Veterinary and Animal sciences Lahore, Pakistan*
[2] *Institute of Biochemistry and Biotechnology UVAS Lahore, Pakistan*

Abstract

A single nucleotide polymorphism (SNP) is observed at -308 position of the promoter region of tumor necrosis factor (TNF- α) gene due to which TNF is categorized into TNF1 and TNF2 allele. TNF2 allele is associated with higher concentration of TNF- α which in turn is associated with HIV infection. In order to know the association between TNF2 and HIV infection n =75 HIV positive samples and n=15 HIV negative samples were observed for TNF polymorphism. It was found that among the infected patients 53 patients had TNF2.The total percentage of the patients and controls having TNF2 allele was found to be 63.34.%. Chi square value was significant showing that there is a strong correlation between HIV susceptibility and TNF SNPs (-308) of the promoter region.

Key words: HIV, TNF-α, polymorphism, TNF-1, TNF-2, -308 region

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**Corresponding Author:*
Tahiryaqub@uvas.edu.pk

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1. INTRODUCTION

Cytokines have been involved in human immunodeficiency virus (HIV) transmission. The genes coding for cytokines production are thought to be highly conserved. Any disturbance in regulation of these genes leads to the variation in cytokine release which in turn can affect the pathogenesis of a given disease [1].

HIV infection alters regulation of cytokines in result there is decrease in secretion of some cytokines while increase in others. There is a general decrease in type 1 T helper cells cytokines while an increase in Type 2 T helper cells cytokines. TNF-α is a pro inflammatory cytokine that mediates inflammation. In case of HIV high production of TNF-α facilitate progression of disease simply aiding in HIV replication and bystander TH

cell apoptosis. Several types of cells are involved in the production of TNF-α but mainly it is secreted by macrophages. The TNF-α gene is located at (6 p 21.1-21.3) adjacent to the major histocompatibility factor (MHC). Regulation at transcriptional and post transcriptional level control the production of TNF [2].

According to Wilson et al. 1992, a bi-allelic transition from G to A occurs at position 308 in promoter region of TNF-α. Based on this transition there are two types of TNF alleles: -308 G is known as TNF 1 and -308A is known as TNF 2 alleles. The less common TNF 2 allele is associated with the MHC haplotypes HLA-A1, B8 and DR3. Given haplotypes of MHC are strongly associated with high production of TNF [3].

HLA (human leukocyte antigen) is a gene complex that encodes major histocompatibility complex (MHC). There are considerable evidences that support the vision that HLA haplotypes correlates with HIV infection leading to AIDS [4,5].

Although still it is not confirmed that what is the exact mechanism of this association and whether any causative relationship exists between the two factors or not. However, it is shown that HLA alleles may act as markers for other alleles of the related cytokines which may impact HIV pathogenesis. It was reported that TNF- α high producer allele (TNF2) was associated with HLA-DR3 and DR4 [6]. Immune responses mediated by HLA class I molecules are imprinting mutations in HIV-1, which in turn affects HIV-1 diversity. Intra- and inter-ethnic studies have shown reproducible HLA class I allele, haplotype and supertype associations with HIV-1 infection and the development of AIDS (HIV/AIDS)[7]. HLA-associated polymorphisms were identified in HIV-1 Gag, Pol and Nef in a cohort study. About 282 codons commonly mutating under HLA-associated immune pressures were identified in these three proteins. The greatest density of associations was observed in Nef protein confirming the extensive impact of immune selection on HIV evolution and diversity [8].

One of the ways where TNF-α can change the context of the HIV infection is that it enhances HIV-1 viral replication in vitro [9]. A group of scientists described the effects of TNF-α can be attributed to the activation of a transcription factor nuclear factor KB [10].so genetic differences in TNF - α gene may affect virus load and hence can change the course context of the disease progression [11].

TNF- α is diverse in its functions.it is associated with several pathological conditions such as auto immune disorder, cancer development inflammation etc [12].

In another study De Pablo-Bernal and co-workers showed that there is a significant increase in level of TNF-α and naïve HIV cases reported the [13].In another instance, cytokines profile of HIV-1 subtype C infected untreated patients revealed elevated levels of several cytokines including TNF-alpha [14]. Patients infected with HIV showing specific polymorphism in the TNF-α (-308) and (-238) regions are susceptible to HIV related dementia [15]. It can also affect the disease progression of HIV infection as well [16].

Cell lines have been used to understand the underlying mechanism of TNF- alpha and its role in HIV infection.one study reported that monocytic and lymphoid cell lines which were chronically infected with HIV-1 in vitro upon TNF-alpha stimulus has resulted in significant increase of viral growth and its transcription [17]. There are several binding sites for the host factors on the 5' long terminal repeat of the viral genome. One of those host factors which actually bind to the 5' end is NF-kB.NF-kB is thought to be activated by the TNF-alpha and hence in this way TNF-alpha may have some impact on the HIV infection and disease progression [18].

The present study is undertaken to enquire about polymorphism in the promoter region of TNF-alpha specially to check the association of TNF2 (-308/A) allele with susceptibility to HIV infection. The study is also important in the context that it is the first ever study regarding TNF-alpha polymorphism in HIV infected person of the local population of Pakistan.

2. MATERIALS AND METHODS

The study was designed to check the possible single nucleotide polymorphism (SNPs) in the promoter region of TNF-α gene at position-308. The samples were collected and processed in the IPH (institute of public health) Lahore Pakistan with the collaboration of PACP (Punjab Aids control program). Samples were

categorized into two groups. Group A contained positive samples n=75, while group B contained negative (control) samples n=15.

Samples were collected through syringes. Blood was drawn from the patients through 3 ml syringes. Blood was then transferred to the vacutainers containing anticoagulant Ethylenediaminetetraacetic acid (EDTA) 1.8 mg per ml of blood.

Samples were screened by using recommended immune chromatographic technique (ICT). the ICT used was from Alere Determine™ HIV–1/2 Ag/Ab Combo. The test is specific for the simultaneous detection of HIV-1/2 antigen (p24) and HIV1/2 antibodies if present in the test sample.

2.1 Genomic DNA extraction from whole blood sample

DNA was extracted from the whole blood samples using commercially available kit (Favor pep blood genomic DNA mini extraction kit in accordance with the manufacturers' protocol. Briefly cells were lysed using proteinase K. Ethanol was added then. This step was then followed by washing step and at the end elusion buffer was added to elute the total DNA. From ethanol to addition of elusion buffer every step was followed by centrifugation.

2.2 Polymerase chain reaction

The extracted DNA was processed in the polymerase chain reaction in the following way. Commercially available kit DreamTaq Green PCR Master Mix was used for this purpose. For polymerase chain reaction 100ng(5ul) DNA was added to the total of 30ul reaction mixture. The reaction mixture contained 15ul of the dream taq master mix, 1ul (uM)of each forward and reverse primer and 08ul of the water. The primer used for TNF-α amplification was forward primer; 5'-AGGCAATAGGTTTTGA reverse primer 5'-TCCTCCCTGCTCCGATTC-3'. Cyclic conditions for the amplification of -308 region of TNF-alpha were as follows. Initial denaturation for 5 minutes at 95 ºC, denaturation at 94 ºC for 30 seconds, annealing at 60 ºC for 30 seconds, extension at 72 ºC for 30 seconds, final extension at 72 ºC for 10 minutes. The cycles were run in a thermocycler machine (Biorad cycler). For observation of the PCR products 3% agarose gel was made The PCR products were observed in 3% agarose gel.

2.3 Sequencing

The amplified products were sent for sequencing to the First base laboratories Malaysia. The products were processed through sanger sequencing. results were analyzed though NCBI blast. sequence similarities and differences were observed using NCBI BLAST

2.4 Statistical analysis

For statistical analysis SPSS software version 16 was used. At the 95 % of confidence interval chi square tests of the results were performed in order to determine the P value. P value shows determines the statistical significance of the relationship which is tested.

3. RESULTS AND DISCUSSIONS

3.1 Results of polymerase chain reaction (PCR

Bio-Rad thermo cycler was used for the PCR. A product of 107 base pairs was obtained after amplifying the -308 region of the TNF-alpha gene. Results were observed using UV tans illuminator and Gel doc.

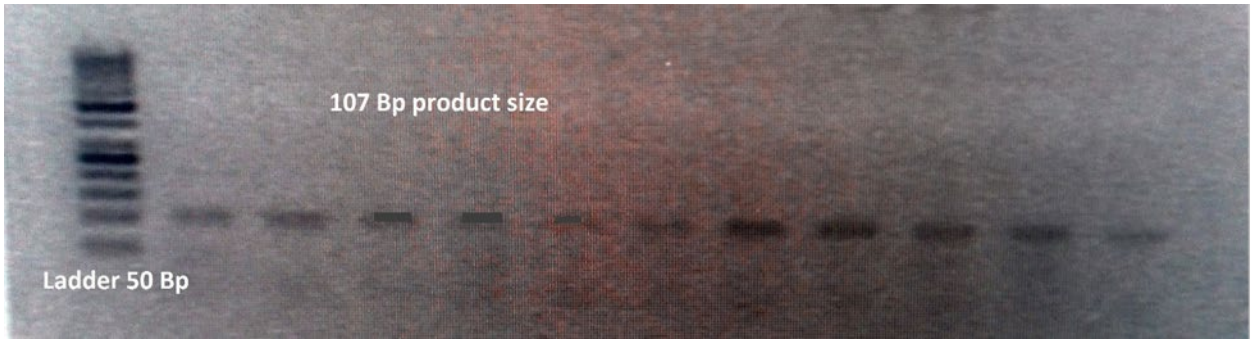

Fig .1. depicting the 50 bp ladder and the amplified 107 bp product

3.2 Results of sequencing

Overall, out of 75 HIV positive samples n=53 samples were having TNF-2 (-308/A) allele. The rest n=22were found to be TNF-1 (-308/G) allele. While in group B HIV negative samples out of n=15 HIV negative samples only n=4 had TNF-2(-308/A) allele while n=11 had TNF-1(-308/G) allele. Sequencing results were analyzed through NCBI BLAST by aligning the query with pre-assembled data of -308 TNF-alpha sequence with accession number of rs 1800629. Some of the aligned blast results are shown below.

Score	Expect	Identities	Gaps	Strand
119 bits(64)	9e-24	66/67(99%)	0/67(0%)	Plus/Plus

```
Query  2    AGGCAATAGGTTTTGAGGGGCATGAGGACGGGGTTCAGCCTCCAGGGTCCTACACACAAA  61
            ||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct  506  AGGCAATAGGTTTTGAGGGGCATGGGGACGGGGTTCAGCCTCCAGGGTCCTACACACAAA  565

Query  62   TCAGTCA  68
            |||||||
Sbjct  566  TCAGTCA  572
```

Fig.2. Blast results for HIV positive sample showing TNF-2(-308/A) allele. Note the SNP which denotes TNF2. (Highlighted)

3.3 Statistical Analysis

Out of 75 HIV positive samples TNF2 was associated with 53 samples with the percentage of 70.67 %.in case of negative samples TNF 2 allele was found to be 4 out of 15 making the total percentage 26.67 %.chi square test was performed to check the independence and association of the TNF 2 with and TNF1 with HIV infection. At 95% confidence interval the chi square value was found to be 10.42. The p value was .0012. This result is significant at $p < .05$. this shows the actual association of TNF2 and its significant role in susceptibility to HIV infection.

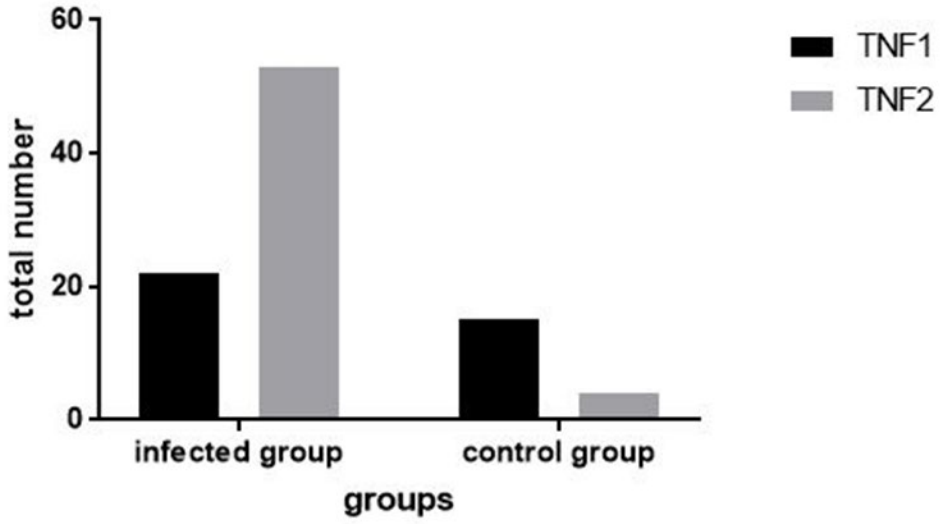

Fig.3. showing an overall representation of the study.

4. CONCLUSIONS

Among the two alleles studied, HIV seropositive patients, a significant association was observed for the TNF2 (rs1800629) only. This allele is responsible for the expression of TNF-alpha. Reports suggest that the TNF2 is associated with higher secretion of TNF-alpha while the other allele TNF1 is found to be associated with under secretion of TNF-alpha. The presence of TNF1 indicates a less susceptible condition [19].

It was found that among the infected patients 53 patients had TNF2.the total percentage of the patients having TNF2 allele was found to be 70.67% in infected individuals. While in those of non-infected individuals among 15 samples only 04 were having TNF2 making the percentage 26.67%. chi square value was 10.42. Which was determined using 95 % confidence interval the p value was found to be 0.0012.

We established the association of TNF-alpha with susceptibility to HIV infection. We can conclude based on our results that there is a strong proof of involvement of TNF1 in susceptibility to HIV infection. TNF1 can be considered as one of the major contributors in HIV infection. These findings can be used as a reference to study association of TNF polymorphism and its association with HIV infection. The study can be expanded by doing functional analysis of the effects of this SNP on gene expression.

The data is interesting in a fact that it provides insights to the peculiarity of TNF2 and its association with HIV infection. The study is novel in a sense that it is the first ever study regarding TNF SNPs in Pakistan.

The future implications of this study are possible by experimenting with TNF-alpha inhibitors in vitro and their possible impact on the disease progression.

The research can be helpful in designing new strategies to fight against HIV infection and the disease progression. The use of TNF inhibitors could be one possibility in this regard. Moreover, it is the first ever valuable data regarding SNPs of TNF-alpha in Pakistan.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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