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RESEARCH ARTICLE

Correlation of Apolipoprotein B mRNA-editing Enzyme, Catalytic Polypeptide-like 3G Genetic Variant rs8177832 with HIV-1 Predisposition in Pakistani Population

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Abstract: ***Background*:** Human immunodeficiency virus (HIV) infection is a global health burden which ultimately results in acquired immune deficiency syndrome (AIDS). There are multiple host factors which are capable of limiting HIV-1 replication. One of the most important host factors which inhibit HIV-1 DNA synthesis is the apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3G (APOBEC3G). Any genetic variation of this important host factor may influence the host susceptibility to viral infection.

***Objective*:** The aim of the current study was to evaluate any correlation of APOBEC3G genetic variation rs8177832 with HIV-1 infection.

***Methods*:** The study involved 142 healthy control and 100 HIV-1 infected subjects. The genetic variation rs8177832 of all studied subjects was determined by allele-specific polymerase chain reaction (AS-PCR).

***Results*:** The results showed that the distribution of rs8177832 genotypes AA, AG and GG in healthy subjects and HIV-1 subjects was; 42.253%, 42.957%, 14.788% and 66%, 27%, 7% respectively. Statistical analyses of data showed that there was a significant variation in rs8177832 genotype AA in healthy control and HIV-1 infected subjects (42.257% *vs* 66%; p-value<0.001).

***Conclusion*:** Thus it was concluded that APOBEC3G rs8177832 AA genotype contributes in genetic predisposition to HIV-1 infection in Pakistani population.

Keywords: HIV, pathogenesis, SNPs, Correlation, APOBEC3G, DC-SIGN.

1. INTRODUCTION

HIV-1 infection is a worldwide dilemma which has currently infected more than 37 million people with an annual addition of 3.1 million new cases [1-3]. HIV infection is the most common infection in Sub-Saharan countries [4]. In Pakistan, it is estimated that 83,468 people are suffering from HIV-1 infection. The most common routes of HIV transmissions are blood and sexual contacts [5, 6].

There has always been an adaptive struggle for existence between host and pathogens. The development by the host an arsenal of strategies to fight against infectious agents at the immune level has put a selective pressure on the pathogens which leads to the evolution of novel mechanisms to escape the host immune surveillance [7-11]. With the advent of human genetic era, it is shown by a number of studies that both viral and host genetic factors are critically involved in genetic predisposition or protection against infectious agents [12-14]. Now it has been cleared that individuals with homozygous Δ32 allelic variants of the CCR5 protein are strongly, although not completely resistant to HIV infection. Truncated CCR5 does not act as a co-receptor for binding of HIV virion with the host cell, hence providing protection against HIV infection [15, 16]. Moreover, the ethnic differences also participate in host predisposition or protection against viral infection [17-19]. Thus these genetic and ethnic differences and their influence on pathogenesis have encouraged the researchers to explore other genetic and ethnic factors that may influence HIV infection or disease progression. Recently, it has been demonstrated that a number of host factors are involved in disease development/increase HIV-1/AIDS condition. These host factors involve; APOBEC3G, Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN), Chemokine Receptor 5 (CCR-5), Tripartite motif 5a, (TRIM5a), Tetherin, and (SAM-domain HD-domain containing protein) SAMHD1 [5, 20-23]. These host factors are antagonized by accessory viral proteins [24, 25].

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APOBEC3G gene is believed to possess potent antiviral activity and interrupts HIV infection. It is responsible for hypermutation in viral DNA, eventually leading to the inhibition of HIV-1 DNA synthesis [22]. It causes deamination of cytosine (C) to uracil (U) in viral DNA and attenuates its integration into the host genome. The accessory HIV-1 protein that counteracts APOBEC3G is the viral infectivity (Vif) protein. It targets APOBEC3G for proteasomal degradation. This importance of APOBEC3G gene draws the attention of several researchers to identify APOBEC3G variants that may possibly escape degradation by Vif and contribute in host resistance to HIV infection. Moreover, it has been noticed that APOBEC3G also reduces viral reverse transcription. It is hypothesized that the dU in viral DNA is taken as anomalous which may ultimately lead to viral DNA degradation even before its integration into the host genome [26]. This might be occurred by the removal of uracil residues by uracil-DNA glycosylases (UDGs) which may further lead to apurinic/ apyrimidinic (AP) endonuclease-mediated degradation [26, 27]. In addition to viral restriction through induction of hypermutations in viral DNA, APOBEC3G also interferes the annealing and removal of tRNA, initiation of DNA synthesis and its elongation and strand transfer reaction [28-40]. APOBEC3 is also counteracted by viral protein R (Vpr). Vpr interacts with uracil glycosylase and diminishes its incorporation in the DNA strand. However, this process results in the activation of DNA-damage response and the expression of natural killer cell ligands. Thus, APOBEC3 is involved in the recognition of infected cells by natural killer cells [41].

Although till date, there is no reported variant of APOBEC3G which may escape proteosomal degradation by Vif protein, but *in vitro* studies have demonstrated a different expression level of APOBEC3G variants. The slight change in the expression level of APOBEC3G is significant in viral replication and disease progression [42]. It has been proposed that the APOBEC3G polymorphism rs8177832 (H186R) is associated with HIV-1 subtype B and C pathogenesis in some ethnic groups [7, 43]. The aim of the current study was to study the influence of APOBEC3G rs8177832 polymorphism for genetic predisposition or protection against HIV-1 infection in Pakistani population.

2. MATERIALS AND METHODS

2.1. Blood Sampling

The study was comprised 242 subjects involving 142 HIV-1 seronegative and 100 HIV-1 seropositive subjects. The study was approved by the Institutional Ethical Review Committee (IERC). HIV-1 patients were recruited from PACP (Punjab AIDS Control Program) Lahore. After written consent from the study participants, 5 ml blood was collected from each subject in EDTA containing tube. Plasma samples collected from whole blood were subjected to the detection of HIV-1 infection by using Alere determine HIV-1 or 2 test (Product code: 7D2346 CE Marked).

2.2. Amplification of Targeted APOBEC3G

DNA Extraction from the whole blood was performed by the non-enzymatic salting out method [44]. The quality of extracted DNA was analyzed on 1% agarose gel and quantitated by a Nanodrop spectrophotometer (EppendorfBiophotometer). DNA was stored at 4°C. The genetic variation of APOBEC3G rs8177832 (H186R) was determined by allele-specific polymerase chain reaction (AS-PCR) (Applied Biosystem). The primers sequences used were; forward primer 1; 5'-GAGCCTTGGAATAATCTGCCTA-3' forward primer 2; 5'-TTGAGCCTTGGAATAATCTGCG-3' and reverse primer; 5'-AGGGAGACCCTCACCTGAGAA-3'. The amplified product size was 79bp. Allele-specific primers work on the principle that polymerase enzyme requires perfect complementarity at 3' end. Two PCR tubes or reaction mixtures were used for each subject. Amplification of APOBEC3G in both tubes represented the heterozygous condition, while the amplification of a single set of primer indicated a homozygous condition for that particular allele. PCR amplification was carried out in 25ul mixture possessing 100-300 ng DNA. Cycling condition of PCR involved long denaturation at 95C° for 5 min followed by 35 cycles, each cycle comprised of denaturation at 95C° for 30; annealing at 55 °C for 30 sec and extension at 68 °C for 30 sec. The final extension of PCR was performed at 72 °C for 7 min.

2.3. Statistical Analyses

The data was statistically analyzed and interpreted by using SPSS 20.0. Hardy Weinberg Equilibrium was applied to determine the distribution of APOBEC3G SNPs both in patients and healthy controls. Pearson Chi-square test was used to determine any correlation of APOBEC3G rs8177832 genotypes with genetic predisposition or protection against HIV infection. A two-sided p-value <0.05 was considered to be statistically significant.

3. RESULTS

The cytosine deaminase APOBEC3G inhibits HIV-1 replication in the absence of HIV-1 accessory protein, HIV-1 Vif protein. It inhibits viral replication by inducing G ➞ A hypermutation in the newly synthesized negative strand of HIV-1. The aim of the current study was to analyze the effect of APOBEC3G rs8177832 (H186R) in HIV-1 infection by allele-specific polymerase chain reaction. There was no significant variation in gender distribution between the two studied groups. The frequency of male and female in seropositive and seronegative HIV-1 groups was; 54% and 46% *vs* 61% and 39% respectively. Similarly, the mean age of the two groups was also comparable. The mean age of HIV-1 seronegative and seropositive group was 36.44±7.49 and 35.22±6.58 respectively as shown in Table **1**.

The epidemiology of APOBEC3G rs8177832 genotypes; AA, AG, GG in healthy control and patient group was 42.253%, 42.957%, 14.788% and 66%, 27%, 7% respectively. Statistical analyses of data showed that there was significant variation of APOBEC3G rs8177832 AA genotypes between the healthy control and HIV-1 patients group (42.253% *vs* 66%; $p<0.001$) as shown in Fig. (**1**). The data showed that subjects with APOBEC3G rs8177832 AA genotypes are more susceptible to HIV-1 infection. We also noticed a significant variation in APOBEC3G, rs8177832 AG genotypes between the healthy control and HIV-1 patients group (42.957% *vs* 27%; $p<0.01$), but its p-value (0.014)

Table 1. Demographic profile of healthy controls and HIV-1 patients in Pakistani population.

Variable	Healthy Controls	HIV-1 Patients	p - value
Numbers	142	100	
Age	36.44 ± 7.49	35.22 ± 6.58	*N.S
Gender	84 male (61%) ; 58 (39%) female	54 male (54%);46 (46%) female	*N.S
APOBEC3G rs8177832 genotypes			
AA	60 (42.253%)	66 (66%)	**<0.001**
AG	61 (42.957%)	27 (27%)	0.014
GG	21 (14.788%)	7 (7%)	0.683

*N.S stands for non-significant

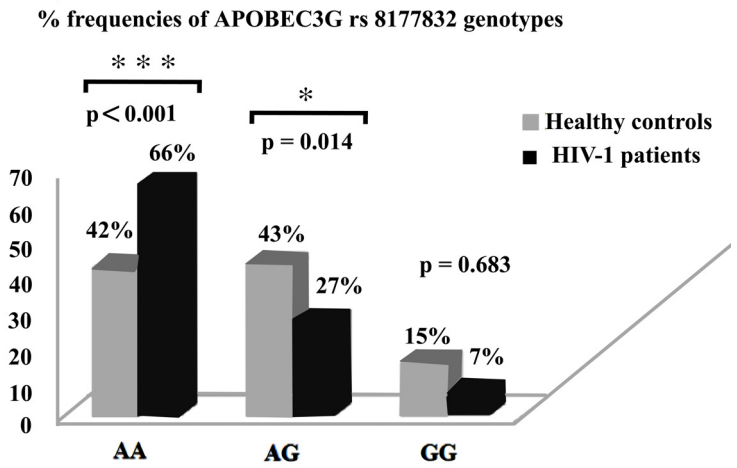

Fig. (1). Percentage frequencies of APOBEC3G genotypes: The percentage distribution of APOBEC3G rs8177832 AA, AG, GG genotypes in healthy controls and HIV-1 infected patients was 42 %, 43%, 15% and 66%, 27% and 7% respectively.

was not as much significant as AA genotype (0.0004) and may be attributed to the involvement of A allele.

4. DISCUSSION

Repeated exposure to HIV infection does not inevitably result in AIDS development [45]. There are multiple genetic and immune factors involved in HIV acquisition, pathogenesis and AIDS development at different stages of HIV life-cycle. Thus HIV infection leads to the activation of multiple intrinsic host factors which confer resistance to HIV pathogenesis [46]. One of the most important host factors that confer intrinsic block to HIV infection is APOBEC3G [24]. APOBEC3G single nucleotide polymorphisms (SNPs) are of particular importance and its twenty-nine SNPs have been studied in American [47] and European [48] cohorts to reveal its influence on AIDS development and progression.

CONCLUSION

In the current study, we investigated the distribution of APOBEC3G rs8177832 genotypes in healthy controls and HIV-1 infected subjects. Our results showed that there was a significant variation of APOBEC3G rs8177832 AA genotypes in the two studied groups (42.253% *vs* 66%; p<0.001). Its distribution in healthy control and HIV-1 infected subjects was 42.253% and 66%, respectively, with a p-value <0.001. Thus individuals with APOBEC3G rs8177832 AA genotypes are more genetically predisposed to get HIV-1 infection. Previously a report from Burkina Faso has shown that individuals with APOBEC3G GGT haplotypes for rs6001417, rs8177832 and rs35228531 genetic variants were providing protection against HIV infection as compared to other haplotypes. Furthermore, the results of the same study demonstrated that individuals with haplotypes GGC had almost two to fivefold higher risk of getting HIV infection [49]. However, a study from the French cohort showed no significant association of APOBEC3G genetic variation, H186R with the disease progression [50]. APOBEC3G also interacts with the hepatitis B virus infection. A study of 179 HBV chronic carriers and 216 healthy control subjects from Morocon population demonstrated that there was no significant difference in the frequencies of APOBEC3G H186R in the two studied groups [51]. The variation in the results of these studies might be attributed to differences in distributions of this genetic variation in different populations and ethnic diversities. A study of 3,073 subjects from six different cohorts demonstrated that the distribution of this APOBEC3G H186R in African Americans was 37% but rare in European American (<3%) and in Europeans (5%) [7].

Moreover, the SNPs of APOBEC3G interacting proteins such as Vif and CUL5 may also influence the disease progression [52].

The limitation of the current study involves that the expression level of APOBEC3G was not investigated and the sample size was small. Moreover, the other genetic variants and haplotypes of APOBEC3G genes were not explored.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Institutional Ethical Review Committee (IERC).

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All humans research procedures followed were in accordance with the standards set forth in the Declaration of Helsinki principles of 1975, as revised in 2008 (http://www.wma.net/en/20 activities/ 10ethics/10helsinki/).

CONSENT FOR PUBLICATION

Written informed consent was taken from all subjects before participation in this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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