APOBEC3G Variant (rs6001417) CG and GG Genotypes and their protective feature against HIV-1 Infection in Pakistani Dwelled Community

Qaisar Ali\(^2\), Arshad Jamal\(^{1,2}\), Sajjad Ullah\(^2\), Ahmed Bilal Waqar*\(^2\)

Abstract

Background: APOBEC3G (Apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3G) gene is one of the genetic host factors, have been linked with HIV-1 AIDS predisposing and protection in different residence populations. The investigation of genetic marker (APOBEC3G) variant (rs6001417) CC, CG and GG genotypes in Pakistan.

Methods: The extraction of DNA, the DNA Rapid Salting-out method was used. Then the observed DNA with electrophoresis technique referred for quantitative real-time PCR to identify the APOBEC3G variant rs6001417 genotypes and Taq Man genotyping.

Results: Three genotypes of rs6001417 (CC, CG and GG) were compared both in HIV-1 infected patients and healthy control groups (p=0.73, p=0.007, p=0.01 respectively). The rs6001417 CG and GG genotype demonstrated a significant involvement in both the healthy and infected individuals and portraying possible protective effect against HIV-1 infection with predictive value of 36.43% and 13.57% respectively.

Conclusion: APOBEC3G (rs6001417) CG and GG genotypes may have a protective feature in the progression of HIV-1 infection and we may use this as a preliminary predictive marker in the country for HIV-1 infected individuals as well.
Introduction

HIV-1 infection is one of the global health problems, affecting almost 37.9 as million human population as of 2019 according to UNAIDS worldwide, with 3.1 million new cases reported every year [1-3, 40]. In recent past, HIV infection is highly prevalent in Sub-Saharan countries [4], however in Pakistan, currently it is one of the leading causes of morbidity and mortality as it hits approximately 160,000 infected individuals according to UN. Mostly HIV infection is being transmitted via contaminated blood and sexual contacts [5,6].

Immune related genetic mechanism can participate with the immune system while excluding a specific type of antigen from the body [7-11]. Recent findings portray that, both the host and viral genetics may have a substantial influence in the disease progression and protection [12-14]. In case of homozygous allelic variant of CCR5 protein has a substantial contribution in against the HIV infection [15,16]. Also, the ethnic background and DNA sequence similarity has a great role in the susceptibility and protection of a disease [17,19]. The study attracts researchers globally to study the genetic role in the progression of HIV infection.

In recent times, several studies have demonstrated that multiple host factors have influenced the pathogenesis of HIV-1/AIDS condition. These factors includes; APOBEC3G, Chemokine Receptor 5 (CCR-5), Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN), Tripartite motif 5a (TRIM5a), Tetherin, and (SAM-domain HD-domain containing protein) SAMHD1 [5,20-23]. These are antagonized by accessory viral proteins [24,25].

Moreover, Apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3G (APOBEC3G) is an effective factor inside the host, which interferes with HIV-1 [22]. Virion infectivity factor (vif) which is structural part of HIV-1 is able to counteract APOBEC3G antiviral activity by targeting it for degradation in proteasomes [26,27]. Vif proteins derived from subtypes A, B, CRF01_AE, and CRF_02AG showed non-significant but what-some differential anti-APOBEC3G activity levels based on infectivity profiles while subtype C was highly significant [28,29]. The APOBEC3G protein was incorporated into newly synthesized viral particles, in the absence of the virion infectivity factor (vif), and deamination of cytosine (C) to uracil (U) made viral DNA mutated. APOBEC3G polymorphisms, such as (H186R) rs8177832, are supposed to be related with HIV-1 subtype B and C pathogenesis in different ethnic groups [7,30], however this association is not found in other populations [31-33]. These previous studies did not take the Circulating Recombinant Forms of HIV-1 into consideration, nor examine the effect of APOBEC3G polymorphisms in Asian Pakistani ethnic groups. The present study was conducted to understand and make clear the role of rs6001417, variants of APOBEC3G in HIV-1 infection in Pakistani population.

Methods

Study population

A total of 240 subjects (100 patients and 140 healthy persons) were included in this study. Samples were collected from different HIV centers of Pakistan and processed at Imperial Diagnostics and Research Center, Lahore.

Sample collection, HIV-1 testing

Intravenous blood samples collected from HIV-infected subjects were genotyped using Real Time PCR Quantitative kit (SYBR GreenER) 100-rxn according to manufacturer’s instructions.

DNA extraction and genotyping

The DNA was extracted from blood by using the “DNA Rapid Salting-out” procedure which has characterized by Miller et al.[34]. The extracted DNA was stored at -20°C till further processing. The concept of SNP was based on A3G variant rs6001417 along with the defense in contact with HIV-1 infection [30]. The rs6001417 in regard to A3G was genotyped by applying common SYBR GreenER SNP assay on the Fast Real-Time PCR Systems (Applied Biosystem Step One ™). PCR amplification was performed using the reverse and forward primers respectively Table 1. Every reaction was consisted of absolute amount of 25 µl, containing 10x PCR buffer 2.5 µl, Taq polymerase 0.5 µl, d-NTPs 1 µl, each primer 1 µl, genonic DNA 1 µl. The processing started by denaturation at 95°C, 30s of annealing at 55°C and 30s of extension at 68°C, in 35 cycles. The final extension was, at 72°C for 7 minutes. After electrophoresis over a 2 percent agarose gel with 0.5 ug/ml ethidium bromide, the amplified product was examined on UV light.

<table>
<thead>
<tr>
<th>Variant’s name</th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
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<tbody>
<tr>
<td>rs6001417</td>
<td>CGGTTGCCACCATGAGATC</td>
<td>GTTACAGTCAGGCGACCT</td>
</tr>
</tbody>
</table>

Table 1: Primer of APO-BEC-3G variant rs6001417

Ethical considerations

The study was approved from the Institutional Ethical Committee of Imperial College of Business Studies and written consent from each participant was obtained.

Statistical analysis

The epidemiological data were recorded on a pre-designed form and handled within excel software. All calculations were performed by applying SPSS software version 20.0 statistical package. Data is expressed as Mean ± S.D and calibrations like diagnostic aspects has been calculated. Categorical variable was analyzed with X² test. Hardy Weinberg equilibrium is also applied for Allele frequency. Contrasts to genotype placement of the groups were determined through the X² trial. P<0.05 is considered as statistically significant.

Results

A total of 240 individuals were included into the study. Study population consisted of 100 HIV-1 infected patients and 140 healthy controls. Gender wise distribution, age of the cases and healthy groups have been defined in Table 2. The mean age of the study population was 39.21±11.7 in the healthy control group.
and 35.94±9.84 in the HIV-1 cases group. We found gender and age comparable variables among the two groups (P> 0.05). Genotype frequencies of the one APOBEC3G loci (rs6001417) for both the cases and controls were given correspondingly in Table 2.

1. Association between APOBEC3G variants and HIV-1 status
We analyzed and compared SNP rs6001417 genotypes (CC, CG and GG) between cases and control groups by using P-value of > 0.05, as shown in Table 2. The electrophoresis pattern of APOBEC3G (rs6001417) CC, CG, and GG genotypes was shown in Figure 1.

1.1 APOBEC3G (rs6001417)
The genotype frequencies of APOBEC3G (rs6001417) CC, CG, and GG genotypes were 27.50%, 11.20%, 2.90% in the patient with HIV-1 group and 29.20%, 21.20%, 7.90% in the control group, respectively as shown in Table 1. Individuals account for HIV-1 infection has lower frequencies of the APOBEC3G (rs6001417) CC, GG genotypes than healthy individuals while CC in HIV-1 patients. Chi-square analysis provided information that rs6001417 CG and GG genotypes (51.21% vs 27 (11.20%); p=0.007 and 19 (7.90%) vs 07 (2.90%); p=0.19) reflect a significant variation between the two groups as shown in Figure 2. This analysis showed that subjects account for rs6001417 CG and GG genotype displays a protective role toward the HIV-1 infection.

2. Association of gender with protective and predisposing attaining APOBEC3G variants genotypes
We found rs6001417 CC,CG and GG genotype frequencies and distribution as 42.10%, 20.00%, 07.10% and 14.60%, 12.50%, 3.80% in both male and female gender, respectively and had found comparable (P< 0.05) Table 2, Figure 3.
APOBEC3G Variant (rs6001417) CG and GG Genotypes and their protective feature against HIV-1 Infection in Pakistani Dwelled Community

population. Moreover, (rs6001417) CC genotype was more common in male than female in HIV-1 studied population and statistically significant (42.10% vs 14.60%; p < 0.001) as shown in Table 2. These findings showed the maximum contribution by male gender towards the CC genotype which is being insignificant in both the HIV-1 cases and control groups. These comparisons were noticed in the HIV-1 studied population only.

3. Predictive value of APOBEC3G (rs6001417) CG and GG genotypes

We have already discussed the protective rs6001417 CG, and GG genotypes for HIV-1 infection. In addition, we examined predictive value of these two genotypes as well. Both the rs6001417 CG and GG genotypes were found to be the protective genotypes, we also calculated positive predictive value (PPV) which was 36.43% and 13.57%, respectively as shown in Table 3.

<table>
<thead>
<tr>
<th>APOBEC3G variants</th>
<th>PPV</th>
<th>NPV</th>
<th>Specificity</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs6001417 CG</td>
<td>36.43%</td>
<td>27.00%</td>
<td>23.28%</td>
<td>41.13%</td>
</tr>
<tr>
<td>rs6001417 GG</td>
<td>13.57%</td>
<td>7.00%</td>
<td>5.47%</td>
<td>16.96%</td>
</tr>
</tbody>
</table>

Table 3: Prediction of APOBEC3G rs6001417 genotypes in HIV-1 infection

NOTE. According to this table, the protective rs6001417 CG and GG genotypes analyzed for positive and negative predictive value through Medcalc online calculator towards HIV-1 infection.

Discussion

Continual exposure to HIV infection does not certainly result in AIDS occurrence [35]. Multiple genetic and immune factors help in HIV acquirement, pathogenesis and AIDS progression at various stages of HIV life-cycle. So, HIV infection activates multiple intrinsic host factors that confer resistance to HIV pathogenesis, though the most important one is intrinsic inhibition to HIV infection by APOBEC3G genetic host factor [24,36]. APOBEC3G single nucleotide polymorphisms (SNPs) are of particular importance and its twenty-nine SNPs have been studied in American [37] and European [38] cohorts to reveal its influence on AIDS development and progression. We examined the frequency distribution of the variants rs6001417 of APOBEC3G gene in the population of Pakistan. Moreover, we studied the APOBEC3G gene polymorphism without the fact of that virion infectivity factor (vif), as it degrades the HIV-1 virus along with APOBEC3G gene [26,27].

The most common genotype was CC of APOBEC3G variants (rs6001417) followed by CG, GG, in the whole studied population. Furthermore, it was observed, that APOBEC3G rs6001417 CG and GG genotypes provide significant protection with 36.43% and 13.57% positive predictive value (PPV) respectively, towards HIV-1 infection. We have already reported predictive value in the treatment of HCV infection recently in which IL28B rs12979860 CT predicted 81.56% [39]. In a recently reported study from Pakistan, has shown that rs8177832 AA genotype has a predisposing role whereas, rs8177832 AG genotype portrayed a protective prediction against HIV-1 disease [40]. Recently a study conducted in Burkina Faso revealed that APOBEC3G GGT haplotypes for rs6001417 variants have an influential outcome by providing protection against HIV infection in comparison to other haplotypes. The results of the same study also demonstrated that individuals with haplotypes GGC has an increase of two- to five-folds in susceptibility against the HIV infecton [41]. However, French cohort study showed a comparable association of APOBEC3G genetic variation, H186R, with the disease progression [31]. Interestingly, Teegwinde Rebeca Compaore et.al reported that rs6001417 GG and CG genotypes predicted protection and predisposing factor against the HIV-1 respectively, which is in line with our study that also showed both rs6001417 GG and CG genotypes with protective feature [42].

Moreover, in the recent past APOBEC3G was also studied with HBV infection, though a Moroccan based study where 179 chronic infected individuals along with 216 control were involved concluded with comparable results after testing hypothesis [43]. But, this variation of the results might be due to the distinct level of population and genetic makeup. This gentic variation was noted as higher as 37%, 3% and 5% in African Americans, European Americans and europian, respectively [7]. Similarly, Single Nucleotide polymorphisms of APOBEC3G docking proteins such as Vif and CUL5 can also help in the progression of the disease [44].

According to our studied population, the effect of APOBEC3G rs6001417 CC (42.10% vs 14.60%; P = <0.001) genotype with in gender as it found frequently in males compare to female. Therefore, the notion that the rs6001417 CT genotype may be supported by the male in term of protection or predisposing against HIV-1 infection is not supported by our results because we found this CT genotype insignificant (neither protective nor predisposing). Interestingly, we reported gender association in recent past with IL28B rs12979860 CT genotype against the spontaneous clearance of HCV infection, in which female gender supported CT genotype in term of spontaneous clearance of HCV infection [45]. To the best of our knowledge, no one has studied or reported the gender interaction with APOBEC3G gene polymorphism and HIV-1 infection. The limitations of this interaction, need further investigations as the sample size of our study was not enough and need further investigation in order to clarify the association.

APOBEC3G polymorphisms variants (rs6001417) CG and GG genotypes may play a vital role at biological level in the interaction of HIV-1 susceptibility to the host, however, extra efforts are required on a larger cohort of patients to elucidate the association.

Conflict of Interest Statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

Author Contributions

All authors contributed equally in preparation and publication of this manuscript.
References