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The Frequency and Effects of *CCR5 Delta 32* Allele in Gondar Population

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Abstract

HIV is the virus that causes AIDS. Its infection occurs by binding to CD4+ receptor and chemokine receptor 5 (CCR5). Recent studies have shown that reasons for progression and non-progression are multi factorial and may involve genetic, virological and immunological factors that influence HIV disease progression in various ways.

Chemokine receptors act as important co receptors mediating the entry of the human immunodeficiency virus type 1 (HIV-1) into susceptible cells. The Δ32 mutation at the CCR5 locus is a well-studied example of natural selection acting in humans. Homozygous carriers of the Δ32 mutation are resistant to HIV-1.

The aim of the present study was to assess the frequency of CCR5-Δ32 (Chemokine receptor *delta* 32 allele) in HIV-1 untreated individuals who visited Gondar university teaching hospital.

3 ml blood samples of fifty HIV-1 untreated seropositive individual and equal numbers of age and sex match seronegative individual who are exposed and uninfected were collected using EDTA coated vacutenous tubes. DNA from 1 ml blood samples was isolated using phenol-chloroform method.

Specially designed primers, both forward and reverse was used to amplify the alleles of CCR5 using PCR (Polymerase chain reaction). Desirable data regarding study subjects were collected by using specifically designed questioner. SPSS 16 and EPI-info version 3.2 were used to analyze the data.

The frequency of the homozygous CCR5 mutant allele was zero in the study population and there was statistically no significance difference between the frequency of the heterozygous allele among cases and controls. There was no homozygous mutant allele both in cases and controls in the present study. This may be due to small sample size and thus another study on the same population with large sample size is warranted to further confirm the result.

Keywords: CCR5 Δ32; CD4; CXCR4; Disease progression; HIV-1

Introduction

Background information

HIV (Human Immunodeficiency Virus) is the virus that causes AIDS. This virus may be passed from one person to another with infected blood, semen, or vaginal secretions come in contact with an uninfected person's broken skin or mucous membranes. In addition, infected pregnant women can pass HIV to their baby during pregnancy or delivery, as well as through breast-feeding [1].

Acquired immunodeficiency syndrome (AIDS) is a progressive deterioration of the immune status of the individual. It is characterized by the progressive depletion of the CD4 T lymphocyte, which represents a major target of viral infection by the causative human immunodeficiency virus (HIV).The continuing rise in the population of people living with HIV reflects the combined effects of continued high rates of new HIV infections and the beneficial impact of antiretroviral therapy [2].

United Nations Joint Programme on HIV/AIDS indicated on the Global AIDS epidemic 2012 reports that despite variances between the rise and decline among infected people across the world, globally 34.0 million [31.4 million–35.9 million] people were living with HIV at the end of 2011. An estimated 0.8% of adults aged 15–49 years worldwide are living with HIV, although the burden of the epidemic continues to vary considerably between countries and regions. Sub-Saharan Africa remains most severely affected, with nearly 1 in every 20 adults (4.9%) living with HIV and accounting for 69% of the people living with HIV worldwide. Although the regional prevalence of HIV infection is nearly 25 times higher in sub-Saharan Africa than in Asia, almost 5 million people are living with HIV in South, South-East and East Asia combined. After sub-Saharan Africa, the region's most heavily affected are the Caribbean and Eastern Europe and Central Asia, where 1.0% of adults were living with HIV in 2011 [3].

The Federal Democratic Republic of Ethiopia joined UN Member States in June 2006 at the UN General Assembly to issue the Political Declaration on HIV/AIDS, which included a commitment to move towards the goal of universal access to HIV prevention, treatment, care and support by 2010. Since that commitment was made, Ethiopia has updated its planning framework with ambitious targets to achieve universal access, and it has launched a "Millennium AIDS Campaign that has catalyzed more rapid scale up of key prevention and treatment programs. Data obtained in 2005 from the Demographic and Health Survey (DHS) indicate that the epidemic may be less severe, less generalized and more heterogeneous than previously believed.

A careful assessment of data gathered over the last six years suggests that the epidemic has stabilized, with adult HIV prevalence estimated at 2.2% in 2003/04 and 2.1% in 2006/07. The rural epidemic appears to be relatively widespread but heterogeneous, with most regions having a relatively low prevalence of HIV, but a few demonstrating adult prevalence greater than 5%. In general, HIV incidence is leveling off after declining over the last few years (1996-2001) [4]. Country HIV prevalence estimates hinge on assumptions such as representativeness of surveillance systems. Until recently, Ethiopia had a national HIV prevalence estimate of 9.3% among persons aged 15–49 years and ranked third in terms of total persons infected. In 2002, this estimate has been adjusted to 6.4% [5].

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The natural history of HIV-1 infection varies considerably from one individual to another, with some individuals progressing to AIDS rapidly after primary infection, while others remain clinically asymptomatic with no evidence of immune dysfunction. Recent studies have shown that reasons for progression and non-progression are multi factorial and may involve genetic, virological and immunological factors that influence HIV disease progression in various ways. The virus has varied impacts on the immune cells. These include direct viral killing of the infected cells (microphages and microglia), apoptosis and killing of the CD4+ T-cells [6].

Human immunodeficiency virus-1 infection

Human Immune deficiency Virus-1 (HIV-1) invades immune cells. Because macrophage and T-cell possess CD4 proteins that are why the virus easily invades them [7] .It was the time since 1984 CD4 is the components of HIV-1 receptor it was discovered but not to be sufficient to allow HIV-1 to infect cells [8].

For attachment and subsequent entry of the virus to the target cells requires both the CD4 proteins and one of the main co receptors (*CCR5* and *CXCR4*) or minor co receptor like *CCR2* and *CCR3* [9]. The main step in HIV-1 replication cycle is attachment and entry into target cells and this is mediated by binding of viral glycoprotein gp 120 to CD4 proteins expressed on target cells [10].

Viral replication

The first step in the reproductive cycle of HIV is the virus attaching to a susceptible host cell. This interaction is mediated through the host-cell CD4 antigen, which serves as a receptor for the virus by binding the gp120 glycoprotein on the outer envelope of HIV. T helper cells are the main target for HIV infection, because they express high numbers of CD4 molecules on their cell surface and bind the virus with high affinity [11]. Other cells such as macrophages, monocytes, dendritic cells, Langerhans cells and microglial brain cells can also be infected with HIV, because they have some surface CD4. HIV viruses that preferentially infect T cells are known as *T-tropic* or *X4* strains, while those strains that can infect both macrophages and T cells are called *M-tropic* or *R5* strains. Entry of HIV into the host cells to which it has attached requires an additional binding step involving co receptors that promote fusion of the HIV envelope with the plasma cell membrane. These co receptors belong to a family of proteins known as chemokine receptors, whose main function is to direct white blood cells to sites of inflammation. The chemokine receptor *CXCR4* is required for HIV to enter T lymphocytes, while the chemokine receptor *CCR5* is required for entry into macrophages. Binding of the co receptors allows for HIV entry by inducing a conformational change in the gp41 glycoprotein, which mediates fusion of the virus to the cell membrane. After fusion occurs, the viral particle is taken into the cell and uncoating of the particle exposes the viral genome [12].

Action of the enzyme reverse transcriptase produces complementary DNA from the viral RNA. This DNA becomes integrated into the host cell's genome and is called a provirus. The provirus can remain in a latent state for a long period of time, during which viral replication does not occur. Eventually, expression of the viral genesis induced when the infected host cell is activated by binding to antigen or by exposure to cytokines. Viral DNA within the cell nucleus is then transcribed into genomic RNA and messenger RNA (mRNA), which are transported to the cytoplasm. Translation of mRNA occurs, with production of viral proteins and assembly of viral particles. The intact virions bud out from the host cell membrane and acquire their envelope during the process. These viruses can then proceed to infect additional host cells. Viral replication occurs to the greatest extent in antigen-activated T helper cells. Because viral replication occurs very rapidly, and the reverse transcriptase enzyme lacks proofreading activity, genetic mutations commonly occur, producing distinct isolates that exhibit antigenic variation. These isolates can vary in their susceptibility to the host's immune responses [13].

Despite multiple sexual exposures to HIV-1 virus, some individuals remain HIV-1 seronegative. The mechanisms underlying this resistance remain still unclear, although a multi factorial pathogenesis can be hypothesized. Also, several genetic factors have been related to HIV-1 as the etiologic agent of AIDS, the chemokine receptor *CCR5* was identified as one of the major co receptors for macrophage tropic virus, the key pathogenic strains *in vivo* [14].

A mutant allele of the βchemokine receptors gene *CCR5* bearing a 32 BP deletion (denoted Δ*CCR5)* which protects cell invasions by the primary transmitting strains of HIV-1 has recently been characterized .The denoted Δ*32* mutation at the *CCR5* locus is a well -studied -example of natural selection acting in humans .The mutation is found principally in Europe and Western Asia, with higher frequencies generally in north Europe [15].

Homozygous carriers of Δ32 mutations are resistance to HIV-1 infection because the mutation prevents functional expression of the *CCR5* chemokine receptors normally used by HIV-1 to enter CD4T cells. More recently, additional polymorphisms in the *CCR5* promoter have been identified, some of which appear to have clinical relevance to HIV-1 disease. It is plausible that these promoter mutations may also affect the level of expression of CCR5, which is known to vary even among individuals with wild-type *CCR5*-coding allele. To date, however, increased promoter activity has been demonstrated only for the 59029-A allele [16]. Polymorphisms with single nucleotide changes have been described at position 59029, 59353, 59356 and 59402. In one study, a CCR5 promoter variant haplotype with a specific combination of polymorphism (defined as p1 with 59353-C 59356-C,and 59402-A) was associated with acceleration of disease progression [17]. In contrast, the 59353-C polymorphism was associated with delayed progression to immune suppression but not to AIDS. The effects of these 2 polymorphisms were analyzed in a recent study that confirmed an association between 59029-A and more rapid disease progression and conclude that 59353-C homozygous individuals were significantly under represented among long term non progresses [18].

Significance and the anticipated output

Several natural polymorphisms in the genes for human *CXCR4* and *CCRS* chemokine receptor have been associated with HIV-1 progression to AIDS [19].

As far as our knowledge goes this is the first study in Ethiopia, therefore, this study will fill that gap.

Objective of the Study

General objectives

The general objective of this study was to compare the frequency of *CCR5* allele among people who visited Gondar university teaching hospital and other non Ethiopian population.

Specific objectives

The specific objectives of this study were:

- Determined and compare the frequency of the alleles among cases and controls.

- Determined the role of *CCR5-Δ32* allele in response to disease progression
- Analyzed the number of exposed and uninfected individuals who visited Gondar University teaching hospital, if any

The *CCR5 delta32* variant and AIDS

A common genetic variant in the coding region of the *CCR5* structural gene involves a 32 base pair deletion (*CCR5-_32*) that shifts the open reading frame to create a truncated protein. This protein fails to reach the cell surface in individuals homozygous for the variant [20].

The pathogenic mechanisms that underlie HIV-1 infection are complex and highly variable and depend on the interplay between numerous viral and host factors [21].

When HIV binds to host cells, it uses the CD4 receptor on the surface of host immune cells together with a co receptor, mainly the *CCR5* and *CXCR4* chemokine receptors. *CCR5* is a host–cell chemokine receptor, which is also used as a co receptor by R5 strains of HIV that are generally acquired during sexual transmissions .Homozygous mutations for this 32 bp deletion offer almost complete protection from HIV infection and heterozygous mutations are associated with lower pre-AIDS viral load and delayed progressions to AIDS. *CCRSΔ32* generally is found in population of Europe decent, with allelic frequencies ranging from 0 to 0.29. Africa and Asian population studied outside the united states or Europe appear to be short of the CCR5Δ32 allele, with allelic frequencies less than 0.29 [22].

Genetic association analysis of over 10,000 individuals at risk for HIV infection has shown that *CCR5-_32/_32* homozygote's completely resist infection by primary R5-tropic HIV strains [23], although there are a few reports of homozygote's who have become infected with the later stage X4 strains, likely because the virus has surpassed the requirement for *CCR5* by utilizing CXCR4 instead [24]. It may be important that rare homozygote's who harbor X4 strains have a lower HIV viral load (i.e., concentration in blood) than do *CCR5+/_32* or*CCR5+/+* individuals infected with R5 or R5X4 viruses [25]. This differential may reflect limits on the ability of *CXCR4+* cells to replicate HIV maximally *in vivo.* It could explain, at least partially, why R5 viruses are so favored in early infection, estimated at 90–95% of primary infections [26].

A limited capacity of *CXCR4+CCR5–* cells to replicate HIV may also explain the shift from X4 to R5 predominance observed in two *CCR5-+/+* individuals soon after primary infection, since an excess of virus production from *CCR5*+CD4+-activated lymphocytes would select *in vivo* for a preponderance of R5 HIV variants, although other explanations are also possible [27].

In the past, there were several attempts to control HIV-1 infection by means of allergenic stem cell transplantation without regard to the donor's *CCR5delta* 32 status, but these efforts were not successful. Host genetics impact HIV infection at two main points: (i) Cell virion fusion, mediated primary by the chemokine receptors *CCR5* and *CXCR4* and their natural ligands and (ii) the host immune response, mediated by human leukocyte antigen (HLA) molecules [28]. Although the median interval between HIV-1 infection and the development of the Acquired Immune Deficiency Syndrome(AIDS) in adults is 10 to 11 years, some infected persons rapidly progress to AIDS is less than 5 years. Still other remain asymptomatic without evidence of immunologic decline for more than 6 years .polymorphisms of the gene controlling these two pathways have been extensively studied and multiple genetic allele that have been found to correlated with either delayed or accelerated disease progression have been reported [29]. Studies of the effect of host genes on susceptibility to HIV infection were facilated by the identification of persons who were per-sistansely exposed to HIV but remain uninfected [30].

The recently characterized chemokine receptor gene *CCR5* and its most frequent mutation (Δ32) have become the objects of intense interest since their roles in the entry of HIV -1 into target cells were identified. Homozygous for a 32 bp deleted allele in the *CCR5* gene protects adults from HIV-1 infection following blood or sexual exposure .Several large studies of the effect *CCR5* genotypes on the course of HIV disease have been published [31]. Dean et al. examined the course of HIV (time to AIDS) in several U.S population with different exposure to HIV (homo sexual, intravenous drug user, persons with hemophilia) and found that persons with one copy of the deleted *CCR5* gene had a delayed approximately 2 years longer progressions to AIDS when compared with those with the homozygous wild type genotype [32]. Heterozygous for a *Δ32* deletion (*CCR5-wt/Δ32)* are not protected against HIV-1 infection but manifests slow progressions to HIV-1 and AIDS end points [33]. Some of the polymorphisms in the promoter region have been found to raise the risk of prenatal transmissions to HIV-1 [34]. A *G to A* substitution at position 180 affects the gene that codes for *CCR2*, a minor HIV-1 co-receptor. This mutation causes substitutions of isolecucine to valine at position 64 (designed as *CCR2-64I*) which eventually slows down progressions in adults [35]. Similar findings are reported [36] in Caucasian HIV-positive homosexual men. Another study of HIV–positive homosexual did not find such a striking effect of heterozygosis, although progressions to AIDS were again slowed [37]. Though the result was not similar to Caucasian population a study in Kenya has demonstrated that children with homozygous mutant allele were less exposed to vertical transmissions of HIV-1 from their mother. A study in South Africa demonstrated similar result [38].

Materials and Method

The study design was experimental and cross sectional.

Collection of sample

Peripheral blood samples were obtained from both cases and controls. For this case control study fifty blood samples from HIV-1sero positive individuals, age and sex matched exposed but seronegative individuals were taken from Gondar university teaching hospital from May, 2012–July, 2012.The blood samples of all this study subjects was collected with EDTA coated non-vacutenous tubes from these hospitals. Essential questioner regarding, age, sex, CD4 counts were filled by the concerned clinician. The blood samples were transported from these hospitals to Gondar University, department of biotechnology, molecular biology laboratory by ice cube box. The blood samples then were stored in deep freeze until processing.

Isolation of genomic DNA

Genomic DNA was isolated from EDTA anti-coagulated peripheral blood by phenol-chloroform extraction method.

Protocol

1. Frozen blood samples were thawed.
2. 1 ml of blood was added to 2.5 ml eppendorff. Then 1 ml of 0.8 M 1x saline sodium chloride buffer was added and content was mixed. The mixture was centrifuged at 10,000 rpm for 5 min in cooling centrifuge at 4°C.
3. 1 ml of the supernatant was discarded into disinfectant.
4. To the eppendorff 1 ml of 1x SSC buffer was added, it was

Vortexed and centrifuged as above for 5 min. Then all the supernatant was removed.

5. 375 µl of 0.2 M sodium acetate was added to each pellet and briefly vortexed. It was followed by the addition of 250 µl of Sodium dodecyl sulphate and incubated for 1 h at 55°C.
6. 120 µl mixture of phenol (60 µl)/ chloroform (57.6 µl)/isoamyl alcohol (2.4 µl) was added to each eppendorff and then vortexed for 30 s. The sample was centrifuged for five minutes at 10,000 rpm.
7. The upper layer was carefully transferred to 1.5 ml microcentrifuge tube and 1 ml of cold 100% chilled ethanol was added. The mixture was gently mixed to precipitate the DNA.
8. The tube was kept at -20°C overnight and then centrifuged at 5000 rpm for 2 min.
9. The supernatant was drained and the pellet was dissolved in 100 µl of tris- EDTA.
10. It was stored at – 20°C after cheeking in 0.8% agarose gel.

Agarose gel electrophoresis

Reagents:

- Agarose
- 1x Tris-Acetate (TAE) buffer
- Bromophenol blue
- DNA marker (100 bp)

Protocol: The protocol for electrophoresis consisted of three steps:

Preparation of agarose gel:

1. 25 ml of (1x) TAE buffer was taken in a beaker.
2. Agarose (0.25 g) was added to this buffer.
3. The solution was heated to boil (using microwave oven) until all the gel particles were dissolved.

The solution was allowed to cool down to 50-60°C.

Casting of the horizontal agarose gel:

1. The gel was assembled to casting tray and the comb was positioned at its one end.
2. The agarose solution was poured into the gel tray and kept at room temperature.
3. The comb was carefully removed and the gel replaced in electrophoresis chamber.
4. The chamber was filled with TAE electrophoresis buffer until it reached 3-5 mm over the surface of the gel.

Loading and running DNA in agarose:

1. DNA (3 µl) was mixed with bromophenol blue in the ratio of 3:1 and loaded in the wells of the 0.8% agarose gel.
2. The cathode was connected to the well side of the unit and the anode to the other side.
3. The gel was run 60 V until, the bromophenol blue tracing dye migrated near to the other end.
4. The DNA was observed by staining the gel with ethidium bromide (0.5 µg/ml) and viewed under UV transilluminator.

CCR5 allele amplification from human blood genomic DNA by PCR

Polymerase chain reaction amplification of *CCR5* allele was performed on genomic DNA prepared from frozen peripheral blood mononuclear cells by phenol chloroform methods. Two primers encompassing the 32 bp deletion found in *CCR5 Δ32* were used :

The forward primer: TTCATTACACCTGCAGCTCTCATTTTC and the reverse primer: CTCACAGCCCTGTGCCTCTTCTTCTC, yielding respectively, 184 bp fragment for the wild–type allele (WT) and 152 bp fragment for the mutated allele. The PCR amplification was carried out in a reaction mixture of 25 µl containing genomic DNA, 5 Mm $MgCl_2$, 10 mM mixed dNTP (dATP, dCTP, dGTP and dTTP),0.3 units Taq polymerase, distilled water and 10x PCR buffer. Polymerase concentrations and primer sequence are available from the producer at Sigma Aldrich by ordering them based on the previous work of Micheline et al. and amplified using 96 well PCR thermo cycler.

The PCR amplification was carried out in a reaction mixture of 25 µl containing genomic DNA, 5 Mm $MgCl_2$, 10 mM mixed dNTP, 0.3 units Taq polymerase, distilled water and 10x PCR buffer.

The PCR condition for denaturation was set up of 95°C for 10 min, followed by 30 cycles of PCR were performed as follow: a denaturation step for 1 min at 93°C, annealing step for 1 min at 54°C and an extension step for 6 min at 72°C.

The presence of the wild allele (WT) and heterozygous allele were identified by using 100 bp DNA marker by 2% agarose gel electrophoresis (Figures 1 and 2).

Statistical analysis

Essential questioner regarding, age, sex and CD4 count were prepared and filled by the concerned clinician. All these variables were computed using SPSS version 16. And the relative risk of each allele was computed by analyzing odds ratio and *p* value using EPI info version 3.2. (Center for disease control and prevention, Atlanta, USA) as per the recommendation of the World Health Organization.

Result

In the present study 50 HIV-1 untreated seropositive and equal number of exposed and un-infected individuals were included (Table 1).

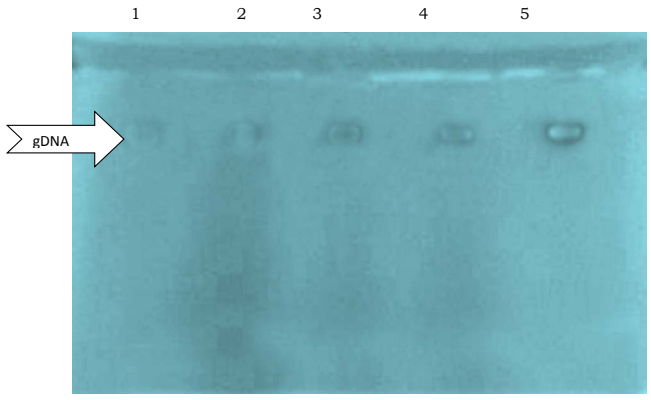

Figure 1: Agarose gel representative picture of genomic DNA extracted from cases blood Lan1-5 genomic DNA extracted from cases.

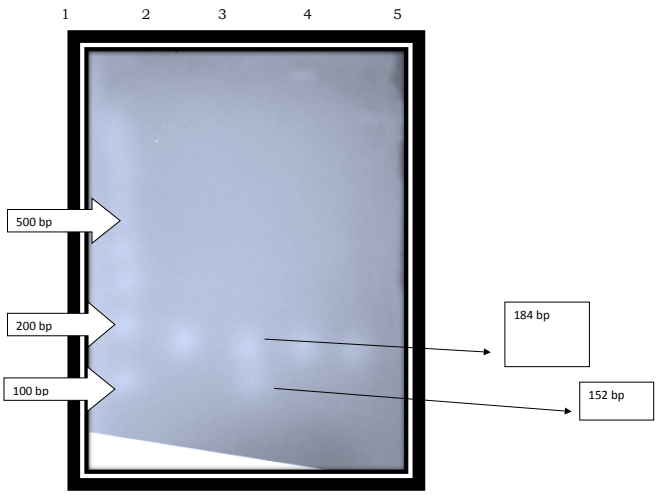

Figure 2: PCR amplified representative agarose gel picture of *CCR5* gene extracted from cases.
Lane 1. DNA Markers
Lane 2, 4 and 5. 184 bp
Lane 3. 152 and 184 bp

Age range		Control in n (%)	Cases in n (%)
17-23		21 (42%)	6 (12%)
24-30		13 (26%)	14 (28%)
31-37		7 (14%)	13 (26%)
38-44		8 (16%)	8 (16%)
45-51		1 (2%)	7 (14%)
52-58		0 (0%)	1 (2%)
59-65		0 (0%)	1 (2%)
Total		100%	100%
Sex	F	24 (48%)	25 (50%)
	M	26 (52%)	25 (50%)
Total		100%	100%

Table 1: Demographic characteristics of study subject by age range and sex.

As the above table indicated more HIV postive individual are with in the age range of 24-30 and the data shows 28% it is more number than others. Also, the table indicated more HIV positive individuals sex is equal in both male and sex that is in male 25% and also in female 25% but in HIV negative individuals were varied that was in female 24% and for male 26%.

Centers for Disease Controls classified CD4 as >500, 200-499 and <200 by these classification in our study we have seen the association between *CCR5 delta 32* allele and CD4 (Table 2).

In our case study individuals who have >500 CD4 have six wild type and 2 heterozygous, 200-499 have 24 (48%) wild type and 1 (2%) heterozygous and <200 have 17 (34%) wild type and 0 (0%) heterozygous.

The heterozygous *CCR5* genotype showed statistically reduced the progressions of HIV-1 with P value 0.041 that means individuals who have heterozygous allele can delay the progressions of the viruses (Table 3).

To facilitate rapid genetic screening for the *Δ32* allele in Gondar university hospital, a PCR-based assay was established using an oligonucleotide primer pair designed to identify *CCR5* genotypes without restriction enzyme analysis and it did not sequence simply we have ordered the specific primers to amplified the *CCR5 delta 32* allele from Sigma Aldrich.

CD4	*CCR5*wildtype allele n (%)	*CCR5* Heterozygous allele numbers	TOTAL
>500	6 (12%)	2 (4%)	8 (16%)
200-499	24 (48%)	1 (2%)	25 (50%)
<200	17 (34%)	0 (0%)	17 (34%)
Total	50 (100%)		

Table 2: Demographic characteristics of study subject by association of CD4 and CCR5 allele.

	Cases n (%)	Controls n (%)
Wild type	48 (96%)	49 (98%)
Heterozygous	2 (4%)	1 (2%)
Homozygous	0 (0%)	0 (0%)

Table 3: Genotype and allelic frequency of *CCR5* allele in cases and controls.

The frequency of homozygous mutant in cases and controls was zero. The percentage of heterozygous was 4% in cases and it was 2% in controls.

On the other hand the homozygous wild genotype was 98% and 96% in case and controls respectively.

Discussion

In the present study two groups of Ethiopian were genotyped for the *CCR5-Δ32* individuals that visited Gondar university teaching hospitals to assessed the host genetic susceptibility factors that predispose to rate of HIV/AIDS disease progression and resistance to develop AIDS in respect to a single gene, *CCR5* Δ32 and from the study *CCR5* Δ32 homozygous gene is totally absent in those population. But 32 bp deletion mutations among individuals of European Caucasians decent indicated that approximately 1 to 3% carries two defective alleles and 15 to 30% are heterozygous. Homozygous mutations for this 32 bp deletion offer almost complete protection from HIV infection, and heterozygous mutations are associated with lower pre-AIDS viral load and delayed progressions to AIDS [39].

However, in our studied population 0% two defective allele and 2% heterozygous allele in case was observed.

A north to south gradient in the *delta* 32 allele frequency has been reported across Europe, with the highest allele frequencies in the Finnish and other populations living around the Baltic Sea (10%–20% heterozygous;1% homozygous) and the lowest in Sardinia and Greece, where the frequency drops to almost zero. The mutation is also seen at very low frequencies in populations from Saudi Arabia, Syrian Arab Republic, Islamic Republic of Iran, Tunisia, Morocco, Cyprus (Greek), India, Pakistan and Asia. It is virtually absent in native populations from sub-Saharan Africa and Oceania [40].

Our results are consistent with reports in the medical literature because our result is close to what is being reported in the Multi-SNP analysis of *CCR5-CCR2* genes in Ethiopian Jews because those peoples are for a long time live in Gondar town which is in our study area and150 individuals from Uganda possessed the homozygous *Δ32 CCR5* was zero [41]. This study marks that the distribution of the homozygous *Δ32 CCR5* allele is similar to that of Uganda these indicated that our country Gondar and Uganda people have a little bit similar alleles because of the geographical location of those two country is nearby each other and both are located in Eastern Africa. Not only in the literature in Uganda and Gondar the distribution of the homozygous

mutated gene distribution is zero but also in most East Asian country the distribution of these homozygous mutated genes is zero [42]. Therefore, this shows that there may be genetic back ground similarity between Gondar and Asian country. On the other hand the result of this study may hint the genetic back ground differences among Gondar population and Europeans.

Conclusion and Recommendation

The HIV virus normally enters to a cell via its *CCR5*. But in people with receptors crippled by *CCR5 delta 32* mutations, entry of HIV by this means is blocked, providing immunity to AIDS for homozygous mutant carriers and greatly slowing progress of the disease in heterozygous carriers.

In many literature the researchers reported that the *CCR5–delta 32* mutation prevents HIV-1 and slow progressions of HIV-1. However, here in this study the frequency of homozygous *CCR5-delta 32* is totally absent and it can be concluded that the mutated gene in the present study is absent or rare. To exactly indicate the frequency of the mutated allele and its effect on HIV-1 large sample size with this population and other parts of the country shall be considered, has not significant difference between the frequency of the heterozygous allele among cases and controls in Gondar population. And individuals who have heterozygous allele have delayed the progressions of the virus.

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