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Genetic polymorphisms of Hiv-1 resistance-conferring polymorphisms (Sdf1-3′A, Ccr2-64i and Ccr5-Δ32) in Population of Madhya Pradesh

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Abstract
The Human Immunodeficiency Virus (HIV) which cause Acquired Immune Deficiency Syndrome (AIDS) in Human. Among retroviruses, human immune deficiency virus (HIV) remains major global health issue; it infects millions of people worldwide annually. A total of 50 blood samples were collected and after blood sample collection, DNA was extracted. Extracted DNA samples were used for the Genetic assessment of selected genes of Chemokine Receptors (Chemokine Receptor 5 and Chemokine Receptor 2) and one ligand (SDF1). SDF1 is a ligand of CXCR4 receptor. For the polymorphism study selected primers were used for PCR of Chemokine Receptor 5, Chemokine Receptor2 and Stromal derived factor 1. Amplified PCR products were subjected to Restriction digestion using established protocols, Finally RFLP data of Chemokine Receptor 5, Chemokine Receptor 2 and Stromal derived factor 1, were further validated using random samples by DNA sequencing. Progression rate of HIV-1 virus after infection in slower in those individuals who carry the mutant form of genes such as CCR5, CCR2 and SDF1. These mutations have originated outside of India, Therefore the frequency of minor alleles for studied genes were reported very less (CCR5 = 0,CCR2=0.08, SDF1=0.16) in the present investigation. The present study concludes that if caste population will get infecting from the virus, the infection will be severe with rapid replication in caste population of Indian State M.P.

Keywords: Chemokine receptors, HIV-1 resistant polymorphisms, caste population, gene

Introduction
The Human Immunodeficiency Virus (HIV) which cause Acquired Immune Deficiency Syndrome (AIDS) in Human. Among retroviruses, human immune deficiency virus (HIV) remains major global health issue; it infects millions of people worldwide annually**.** India also got secured third position in HIV pandemic in the world (UNAIDS, 2017). According to NACO the prevalence of HIV in India recorded around 0.26% during 2015. The five states have marked for the high prevalence of HIV1, namely Manipur, Mizoram, Nagaland, Karnataka and Andhra Pradesh, Some states in the north also found with high incidence of HIV. Around 2.2% of female sex workers are living with HIV in India. HIV virus belongs to retroviridae family which is a RNA virus's family which needs reverse transcriptase enzymes for replication [1]. HIV has two major classes HIV-1 and HIV2, where HIV2 restricted in Africa whereas HIV1 is distributed in whole world. HIV-1 has different subtypes viz A,B,C,D,E,F,G,H,I,J and K. (D1) HIV selectively target immune cells; T cells (CD4 T helper cell) and reside there for a long time, as a result, native immune surveillance mechanism fail to identify infection and pathogen entry [2]. The unique ability of HIV to bypass immune surveillance completely relies on glycoprotein mimic ligand for CCR-5 (early stage) and CXCR in advance stage [3].

The chemokines play crucial role during HIV infection [4]. Chemokines are small protein molecule associated with various physiological events precisely in immune modulation via chemokine receptors. The discovery of the CCR5 delta 32 (Δ32) allele demonstrated the crucial role ccr5 co-receptor during HIV entry in the cell 32-base pair (bp) deletion (open reading frame) in the CCR5 gene, this cause the production of truncated protein, as its empirically proved the mutated proteins are not able to insert into the membrane of the cell. Hence, it confers resistance to HIV in the individual who is carrying homozygous alleles (32-base pair (bp) deletion) and slow progression of disease progression in heterozygotes [5].Screening of the entire CCR2 gene has revealed a G→A transition at DNA position 190 resulting in a change of valine to isoleucine at amino acid position 64 (CCR2- V64I)[6]. Earlier, studies suggested the CCR2-V64I polymorphism negatively regulate the CCR5 and CXCR4

Molecules at the cell surface, thereby decreasing the binding sites for HIV-1 virus. Stromal-derived factor-1 (SDF-1) is ligand of CXCR4 with numerous distinctive characteristics. The Polymorphism of SDF1-3′A reported in conserved sequence of 3′ UTR (untranslated region), which has some protective effect against HIV1 virus because variant of SDF generally has some down regulation impact on CXCR4, thus inhibit the infection of T-tropic virus. SDF1 is present on chromosome no. 3 and acts as a chemokine ligand for CXCR4 [7].The single gene of chemokine family has capability to change the susceptibility rate toward disease but the combine impact of polymorphism of CCR5, CCR2, SDF-1 and CCR5 promoter are associated with delayed development of the AIDS in HIV-1 infected patients, Hence these mutant allele play crucial role during pathogenesis of HIV1. Hence, in present investigation we are trying to get prevalence of the protective allele frequency in caste population of Madhya Pradesh, India.

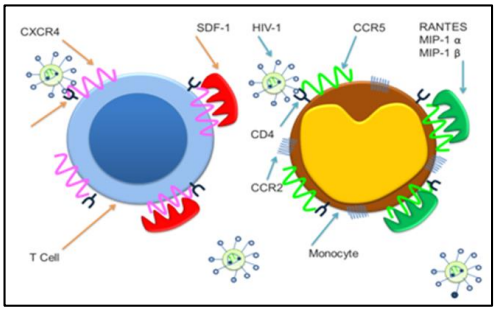

Fig 1: The mode of interaction of HIV1 with different receptors of cells

Material and Methods

Collection of samples

50 blood samples have been collected from caste population of Madhya Pradesh. Isolation of DNA from Blood samples were performed using phenol-chloroform DNA extraction method [8]. Quantitation and quality check of DNA by spectrophotometry. After the extraction of DNA PCR were performed the details of protocol which was used for the amplification of desired genes (CCR2, CCR5 and SDF1) is given below.

CCR5 gene was amplified using CCR5 primer set (Fw primer: 5'-GCT GTGTC CAT GCT GTG TTT-3' Rv primer: 5'-CAA CCT GTT AGA GCT ACT GCA ATT-3'

PCR conditions as follows:

S. No.	Treatments	Temperature	Duration
1	Predenaturation	95^{0}C	5 min
2	Denaturation	95^{0}C	30 sec
3	Annealing	60.5^{0}C	30 sec
4	Primer extension	72^{0}C	30 sec
5	Final extension	72^{0}C	7 min

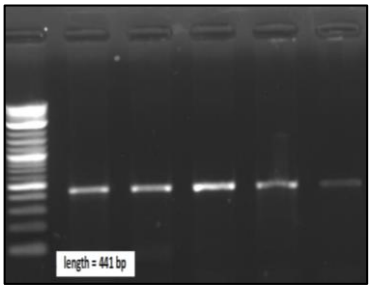

Fig 2: CCR5 gene fragment 441 bp amplification by PCR

CCR2 gene: Samples are amplified using CCR2 primer set (Fw primer TTGTGGGCA ACATGATGG, Rv primer CTGTGAATAATTTGCACATTGC [9]

PCR conditions as follows:

S. No.	Treatments	Temperature	Duration
1	Predenaturation	95^{0}C	5 min
2	Denaturation	95^{0}C	30 sec
3	Annealing	60.5^{0}C	30 sec
4	Primer extension	72^{0}C	30 sec
5	Final extension	72^{0}C	7 min

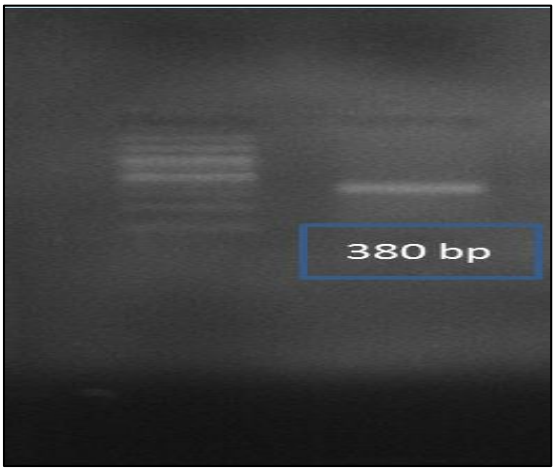

Fig 3: CCR2 gene desired gene fragment 380 bp amplification

SDF1 gene: Samples are amplified using SDF1 primer (SDF1 FW 5 CAGTCAACCTG GGCAAAGCC 3, SDF1 RV 5 AGCTTTGGTCCTGAGAGTCC3) [10].

PCR conditions as follows:

S. No.	Treatments	Temperature	Duration
1	Predenaturation	95^{0}C	5 min
2	Denaturation	95^{0}C	30 sec
3	Annealing	60.0^{0}C	30 sec
4	Primer extension	72^{0}C	30 sec
5	Final extension	72^{0}C	7 min

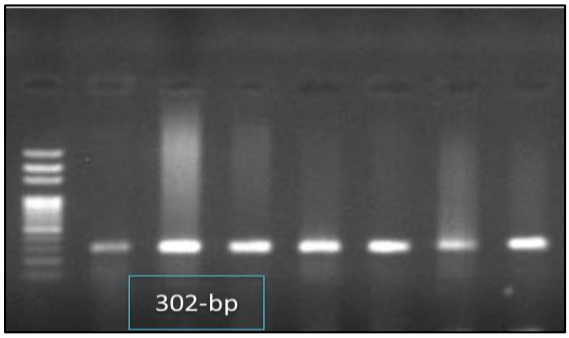

Fig 4: SDF-1 gene fragment 302bp amplification

All PCR products subjected to be used for electrophoresis. The pictures of electrophoresis were aforementioned.

Results

Restriction enzyme digestion: After the confirmation of successful amplification of desired genes (CCR5, CCR2 and SDF1). The samples of CCR2 and SDF1 were further analyzed for SNP analysis using RFLP method. In case of CCR5, no need to digestion if there is 32 bp deletions it will appear in agarose gel directly after PCR.

RFLP of CCR2 gene: We amplify desired sequence of CCR2 Gene using PCR and provided treatment with Restriction Enzyme Fok I. For homozygous wild-type CCR2 gene, a single band at 380 bp was observed, for homozygous. CCR2-64I, there were 2 bands at 153 and 227 bp for mutant homozygous, and for heterozygous CCR2-64I, 3 bands at 380, 153, and 227 bp have been obtained after digestion with Fok I.

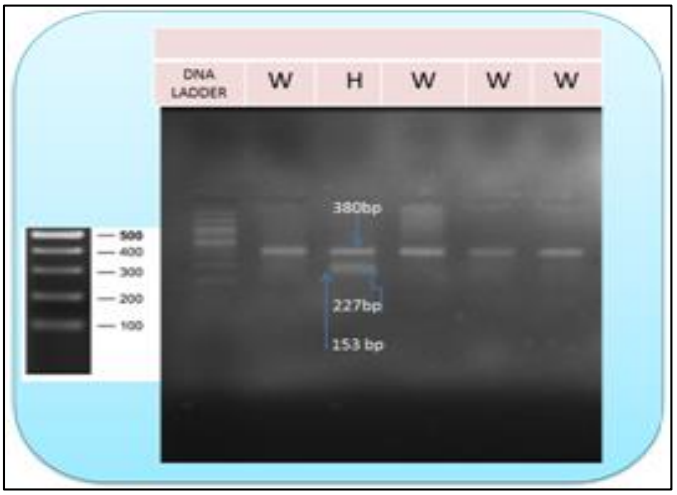

Fig 5: Picture showing restriction digestion of CCR2 PCR product using *Fok I* enzyme.

RFLP of SDF1 gene: In the fragment of SDF1 there is one single nucleotide polymorphisms (SNPs). The Msp I enzyme recognizes the following restriction site to produce blunt end fragments in SDF1 gene.In this RFLP of SDF1 Three patterns were observed: -A. 302-bp band (undigested) for the AA genotype**,** B. 302, 202and 100-bp fragments for the AG genotype**,** C. 202 and 100-bp bands for the GG genotype.

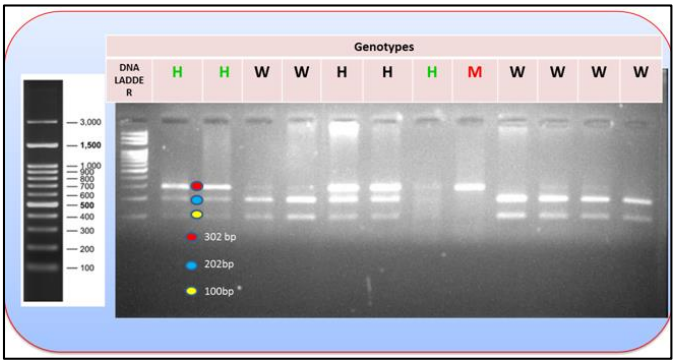

Fig 6: Picture showing restriction digestion of SDF1 gene PCR product using *MspI* enzyme

Sequencing: After RFLP**,** for further validation we sequenced random sample from each gene for Caste population of MP and found same results.

Results and Discussion

The present investigation was mainly planned to provide baseline data on three genes which are involved in delaying of the progression of HIV1 virus in human immune cells in Caste population of Madhya Pradesh. A total of 50 blood samples were collected and after blood sample collection of DNA were extracted. Extracted DNA samples were used for the Genetic assessment of selected genes of Chemokine Receptors (Chemokine Receptor 5 and Chemokine Receptor2) and one ligand (SDF1). SDF1 is a ligand of CXCR4 receptor. For the polymorphism study selected primers were used for PCR of Chemokine Receptor 5, Chemokine Receptor2 and Stromal derived factor 1. Amplified PCR products were subjected to Restriction digestion using established protocols, Finally RFLP data of Chemokine Receptor 5, Chemokine Receptor 2 and Stromal derived factor 1, were further validated by random samples DNA sequencing. The entire selected gene's mutant allele (CCR5Δ32, CCR2-64I and SDF1-3'A) are somehow play protective role against for the HIV1 virus. These mutant forms cannot stop completely the viral entry in the immune cell, however these mutant forms considerably interrupt the virus entry in the cells resulting slow progression of the virus in the human tissues helper T cells (specifically CD4+ T cells), macrophages, and dendritic cells [11]. Genotype distribution of CCR5, CCR2 and SDF1 in the Caste populations of Madhya Pradesh is shown in table 1. As shown in table Caste with samples size 50 were studies for allelic variations. We have reported less variation in genotype in the studied group of population for CRC2 and SDF1 than CCR5. In the present study, we have not reported any number in CRC5 Δ 32 genotypes (mutant). The present population, CCR2 genotype with three major alleles GG, AG and AA had shown a distinct variation in caste population. Caste population χ^2test for all genes showing allele frequencies of the genes are in genetic equilibrium (CCR2, SDF1). It indicates there is good agreement between expected and observed allele frequencies of studied genes. We have also reported SDF1 variants (homozygous mutant) in Caste population. In case of CCR5 this locus lacked any variation, recorded completely monomorphic in studied population. The variation in genotype frequency of CCR2 and SDF1, SDF1 locus is showing more polymorphic than CCR2. Along with genotype distribution in given population allelic frequency of CCR 5, CCR2 and SDF1 were determined and shown in table 2. As a result, shown in table, minor allelic frequency of CCR 5 was reported 0 while two genes CCR2 and SDF1 had shown a significant variation in minor allele's distribution in the populations. In theof major allele was reported significant. Additionally, minor allele frequency of CCR2 and SDF1 frequency were also reported less in studied population, This signifies that less presence of minor protective alleles could be risk factors for HIV -1 infection and viral pathogenesis.

Table 1: Genotype distribution of CCR5, CCR2 and SDF alleles in Caste populations of Madhya Pradesh

Population	Sample no	CCR5 Genotype		CCR2 Genotype			χ^2 (H.-W.)	SDF1 Genotype			χ^2 (H.-W.)
		CCR5	CCR5/Δ32	GG	AG	AA		G	AG	AA	
Caste	50	50	0	42	8	0	P 0.714 NS	35	14	1	P 0.0499NS

Table 2: Allele frequencies distribution of CCR5, CCR2 and SDF1 in the present Caste populations of Madhya Pradesh

Population	n	CCR5 Allele frequencies		CCR2 Allele frequencies		SDF1 Allele frequencies	
		CCR5	CCR5Δ32	G Major allele	A Minor allele	G Major allele	A Minor allele
Caste	50	1	0	0.92	0.08	0.84	0.16

Table 3: Distribution of allele frequencies of CCR5, CCR2 and SDF1 Genes in populations of Madhya Pradesh and other states of Indian

S. No.	Sample No.	Population	Ccr5		Ccr2		Sdf1		Reference
			CCR5 Major allele	Δ32 Minor allele	G Major allele	A Minor allele	G Major allele	A Minor allele	
1	105	Caste MP	1	0	0.92	0.08	0.84	0.16	Present Study
2	150	Mixed Population Jammu and Kashmir	0.953	0.0460	-	-	-	-	Irtiza *et al*.,2011
3	518	Mixed Population Northeastern states: Manipur Nagaland, Mizoram, and Meghalaya	1	0.0	0.743	0.257	0.874	0.125	Sarkar *et al*., 2010
4	412	Mixed Population Tamil Nadu	-	-	0.91	0.088	0.75	0.25	Alagarasu *et al*.,2009
5	75	Punjab Mixed Population	-	-	0.86	0.14	0.85	0.15	Suresh *et al*.,2006
6	155	Andhra Pradesh Castes population	-	-	0.92	0.08	0.79	0.21	Ramana *et al*.,2001
7	45	Andhra Pradesh tribe Bagata	-	-	0.90	0.10	0.83	0.17	Ramana *et al*.,2001
8	35	Andhra Pradesh tribe Poroja	-	-	0.94	0.06	0.80	0.20	Ramana *et al*.,2001
9	42	Andhra Pradesh tribe Valmiki	-	-	0.97	0.03	0.76	0.24	Ramana *et al*.,2001
10	248	Muslims	-	-	0.888	0.112	0.69	0.31	Ramana *et al*.,2001

The allele frequencies of present caste were compared with earlier studied Indian population (Table 3) showed genetic similarity. We recorded significant less variation in CCr5, CCR2 and SDF1 gene in present studied Caste Population of MP, India. HIV-1 is more pathogenic and comparatively modern virus compared to the several other pathogens. Progression rate of HIV-1 virus after infection in slower in those individuals who carry the mutant form of genes such as *CCR5, CCR2* and *SDF1*. These mutational changes have originated outside India, Hence the frequency of mutant allele reported very less in Caste population of MP. Present study concludes that if caste population will get infecting from virus replication of HIV 1 virus will be high.

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