The Distribution of CCR2-64I, SDF1-3'A and MCP1-2518 G/A Genes Polymorphism in a Specific High Risk Group from the Northeastern States West Bengal, and Gorkha Population in India

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Abstract We studied the prevalence and effects of host genetic polymorphisms for the three AIDS restriction genes (ARGs) namely CCR2-64I, SDF1-3'A and MCP1-2518 G/A for HIV infection and progression to AIDS using PCR–RFLP analysis on a total of 568 HIV seronegative serum samples collected from a specific high risk and young population hailing from the seven Northeastern states of India ($n = 346$), West Bengal ($n = 96$) and Gorkha population ($n = 101$). In addition, 181 HIV seropositive cases of which 92 inpatient cases in a large tertiary care hospital located at Kolkata were included in the study. HIV prevalence in our study group was 0.52 %. Four cases seroconverted, 25 cases progressed to AIDS and 05 died during the follow up period of 41 months. The genotype percentage of CCR2-64I, SDF1-3'A and MCP1-2518 G/A in the Northeastern states were 18.5, 40.3 and 54 % respectively in the seronegative population. Allele frequencies for SDF1-3'A in Northeastern states were significantly higher as compared to the Gorkha (21 %) and the North Indian population (24 %). Relative Hazard values were more than 0.9 for progression to AIDS and death. Kaplan–Meier survival analysis using Cox proportional regression model did not reveal any significant survival benefit (p value <0.05) for any of the 3 ARGs individually or in combination either to seroconversion or disease progression. This is the first study on host genetic polymorphism amongst the Eastern, Northeastern and Gorkha regions in India. We are also the first to report the MCP1-2518 G/A polymorphism in India that is

known to increase the rate of neuropsychological impairment (NPI) in AIDS patients.

Keywords CCR2-64I · SDF1-3'A · MCP1-2518 G/A · Northeastern states (NES) · Gorkha · West Bengal · Host gene polymorphism · AIDS restriction genes (ARG's)

Introduction

The seven sister states of Northeastern India (NE states) are Assam, Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland and Tripura. The populations residing in these states are genetically diverse and culturally distinct. This region is primarily inhabited by Tibeto-Burman speakers who are genetically distinct from the Indo-European and Dravidian speakers present in the other geographical regions of India [2]. This region has numerous tribes and ethnic communities e.g. Mizo, Naga, Hynniewtre, Garos, Meiteis, Kuki-Chins, Tripuri etc.

The Gorkha population primarily hails from the Himalayan state of Nepal, Bhutan and Darjeeling and comprises exclusively of the Tibeto-Burman speakers. It is estimated that more than one million Gorkha migrants are working in India including the Armed forces.

This region has a porous border with the neighboring countries, and a conduit for entry of contraband drugs and narcotics. These factors in combination have led this region to become a hotbed for spread of HIV and AIDS [20]. In the 1980 s, Manipur, Mizoram and Nagaland, the three NE states of India bordering Myanmar a rapid spread of HIV was observed among injecting drug users (IDUs) [15].

Considering the distinct genetic makeup of the population of this region, we decided to undertake a study on the host genetic polymorphisms of the three AIDS restriction

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genes (ARG's) namely CCR2, SDF1 and MCP1 amongst a specific *high risk population* belonging to the NE states, Gorkha population and West Bengal. The study period was of 41 months (April 2004 to Aug 2007).

Chemokine receptors such as CCR5 and CXCR4 are known to be associated with entry of HIV into susceptible cells like T lymphocytes, macrophages and Langerhan's cell [13, 16, 30]. Primarily, the macrophage line viruses also called the Non syncitium inducing (NSI) strains or R5 viruses utilize the CCR5 receptors whereas the T cell line viruses also known as the Syncitium inducing (SI) strains or X4 viruses use the CXCR4 receptors for entry into the cell [6, 9–11]. Considerable research in the present decade has shown that specific mutations in the host genes expressing these chemokine receptors can significantly alter the host susceptibility to HIV infection and progression to AIDS [12, 19].

CCR2, a member of the superfamily of the seven transmembrane domain G protein occupied receptors acts as a co-receptor for HIV-1 target cell entry as well as a receptor for MCP-1 (CC chemokine). A G to A transition at position 190 leading to a valine to isoleucine substitution characterises the CCR2-64I mutation. This mutation has been identified as an important factor in delaying progression to AIDS [22, 25].

Monocyte chemoattractant protein1 (MCP1) is a β chemokine and a ligand of CCR2 and is thought to be an important mediator in recruitment of monocytes and T lymphocytes in acute inflammation and plays a role in chronic inflammation too. A 2518 G/A polymorphism in the promoter region of MCP1 is thought to be associated with rapid disease progression and HIV associated dementia (HAD) [21].

Stromal derived factor1 (SDF1) is the chemokine ligand of CXCR4, the coreceptor used by the more pathogenic X4 strains for cell entry. A G to A substitution at position 801 in the 3' untranslated region (UTR) of the SDF1 gene (SDF1-3'A) has been reported to slow disease progression [28] although in subsequent studies this claim has been refuted [7, 14].

Materials and Methods

Population Samples

A total of 568 serum samples were collected in vacutainers (Becton–Dickinson) over a 3 month period from males belonging to a young, sexually active, specific high risk professional group who typically work in uncongenial and high altitude terrain and are involved in frequent moves including overseas missions. Their family lives are severely disrupted. Being financially independent and having an

inherent risk taking ethos typical to the profession are actively sought after by FSWs and their agents while on move or during leave. All samples were obtained after detailed pre-test counseling and informed written consent. Necessary permission to carry out the study was obtained from the ethical committee of the National Institute of Cholera and Enteric Diseases, Kolkata.

Of the 568 samples, 347 persons hailed from the 7 NE states of India (Assam, Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland and Tripura), 96 from West Bengal, 101 belonged to the Gorkha population (Nepal and Darjeeling), and 24 persons belonged to the various other states of India. We included 181 HIV positive cases of which 92 cases belonged to the specified high risk group admitted in a tertiary care hospital at Kolkata for medical management; 61 cases were male intravenous drug users (IDUs) from the state of Manipur and 28 cases were female sex workers (FSW's) from Kolkata. The blood samples of these 89 cases from Manipur (IDUs) and Kolkata (FSWs) were processed strictly on "Unlinked and Anonymous" basis.

All 568 samples were serologically tested for Anti-HIV antibodies 1&2; HBsAg; Anti-HCV antibodies by Enzyme linked immune sorbent assay (ELISA) and Treponema pallidum hemagglutination test (TPHA). Post-test counseling was done after laboratory reports were available.

A detailed demographic study was undertaken and parameters like age, duration of service in the present high risk profession, marital status, family size, specific history of contact with FSW and its frequency, casual sex with amateurs, blood or blood product transfusion, tattooing, intravenous drug usage, AIDS defining illnesses etc. were sought and recorded. Each person was specifically questioned about HIV and AIDS; Routes of disease transmission; Safe sex; Condom usage and prognosis.

Follow up of Patients

All the HIV seronegative persons were followed up for a period of 41 months as on Aug 2007 through half yearly medical examination and appropriate investigations. All HIV positive cases that were transferred in from the various peripheral medicare centres located in the Eastern and NE states, India were admitted, extensively investigated and HIV status confirmed by 'Western blot' examination at National Institute of Cholera and Enteric Diseases (NICED), Kolkata. Meanwhile, other tests e.g. CD4/CD8 counts, Chest X-ray, detailed hematological and biochemical parameters and other special tests like USG and CT scans were conducted to assess the progress of the disease. Monthly follow up by the local medical officer who documented the progress of the disease with special reference to AIDS defining criteria and prompt management of any infections was done in addition to reinforcement of advice

pertaining to safe sex, condom usage and contact sports. All fresh HIV positive persons were advised for serological testing of their spouse and children against anti HIV antibodies. The duration of HIV infection was calculated retrospectively through interactions with HIV counselors, family members and scrutiny of medical documents.

Genotyping

A total of 568 seronegative samples and 181 seropositive samples were genotyped. Total DNA was extracted from PBMC using a Q1 Amp Blood Kit (QIAGEN, CA) and genotyping was carried out by PCR/RFLP assays as described earlier by Winkler et al. [28]. Standard PCR conditions and primers namely for CCR-64I Fwd: 5'-CTCGG ATCTTGTGGG-CAACATGATGG-3', Rev: 5'-CTGTGAATA A-TTTGCACATTGC-3'; SDF1-3'A Fwd: 5'-GACCAGTCAACCTGGGCAAA-3', Rev: 5'-CACATGATGATGGATGAGACAGAGAA-3' and; MCP-1-2518-G/A Fwd: 5'-TCA CGCCAGCACTGAC-3', Rev: 5'-ACTTCAGGAAGGAGTTGT-3' were used in a PTC 200 system and PCR amplified products were digested with restriction endonuclease (*Bsa*BI, *Msp*I and *Pvu*II respectively) for 3 h and then separated in 2.0 % agarose-gel electrophoresis [1, 28].

Data Analysis

Descriptive analysis of the demographic data was carried out. Grubb's test was conducted to detect the outliers and the unpaired 't' test values were calculated. The genotype frequency, allelic frequency, standard error and Hardy-Weinberg equilibrium values were calculated. Kaplan-Meier survival curves were plotted and Relative hazard (RH) values were calculated for each genotype and seroconversion rates were compared for Homozygotes for the normal allele (GG Homozygote or wild type); Homozygote for the susceptible allele (AA Homozygote or mutant type) and the Heterozygote (AG type) for the seronegative cohort using online calculators at <http://statpages.org>. Survival analysis was done for wild genotypes and compared with the mutant genotypes in case of the 92 HIV positive cases that could be followed up in our hospital. The other 89 cases from Manipur (IDU) and Kolkata (FSW) were lost to follow up. The AIDS definitions of 1993 and death were only considered since all HIV positive cases were under routine follow up [4, 5, 9].

Results

The mean age of the HIV negative population was 29.4 ± 6.89 years and 65 % belonged to an age group between 20 and 30 years and the rest 35 % belonged to an

age group of 30–40 years. The Bengal Cohort was comparatively older with a mean age of 33.3 ± 5.83 years while the Gorkha and the NE states population had a mean age of only 28.5 ± 6.73 years, the difference being significant. Similarly, the duration of service in these two groups also showed significant difference (14.9 ± 6.8 years and 9.6 ± 6.61 years). 70 % of the HIV positive population belonged to a relatively older age group with mean age being 35.5 ± 6.74 years. The difference in the mean age between the seronegative and the seropositive cohorts was found to be significant ($p < 0.0001$). Compared to the 73 % of the HIV negative men, 90 % of the HIV positive men were married. The mean duration of service in the present profession was 10.6 ± 6.9 years for seronegative and 16.3 ± 6.6 years for seropositive individuals. The difference was again found to be significant ($p < 0.0001$). The demographic profile of the study population is depicted in Table 1.

During the pre and post test counseling sessions, the HIV negative volunteers were found to be aware of HIV and AIDS, STDs and the dangers of unprotected casual sex and safe sex including condom usage. Two cases volunteered a positive history of IDU activity before joining the present service. Thirty-three people volunteered a positive history of tattooing and 04 people amongst them were found to be HBV positive (5.1 %). Eighty-seven persons (15 %) had exposure to FSWs and casual amateur sex (CAS) especially during leave and temporary moves and 3 were found positive for HBV. No person volunteered any history of MSM activity (Table 1).

Of the initial 568 samples collected, Three (03) cases were found to be HIV positive and 12 were positive for Hepatitis B (HBsAg). Seroprevalence rate for HIV and HBV in the study group was 0.52 and 2.1 % respectively. No cases of HCV or Syphilis were detected. There were no concurrent infection of HIV and HBV. Seventy-two percent of the seropositive hospital patients had repeated contact with FSWs and CAS. Fourteen percent of the cases were detected during routine screening examination for foreign travel and other illnesses. Twenty-one percent reported with combination of fever, weight loss and other features of infections. Eight percent reported with Herpes zoster. The mean duration of seropositivity was 5.3 ± 2.71 years. Twenty-one percent repeatedly denied any history of high-risk behaviour (Table 1).

Over a follow up period of 41 months 04 persons two each from Manipur and Nagaland seroconverted for HIV1 infection. Of the 92 HIV positive cases at our hospital 25 cases progressed to AIDS (27 %) as per the AIDS definitions of 1993, 53 persons (57.6 %) were exhibited HAART and 5 cases expired during this period (5.4 %) (Table 1). Unfortunately, the rest 89 HIV seropositive cases were lost to follow up.

Table 1 Demographic and clinical profile of the HIV seronegative and seropositive populations

Description	Seronegative	Seropositive
Number	568	92
Age (mean + SD)	29.4 ± 6.9 years	35.5 ± 6.7 years
Number married	417 (73 %)	83 (90 %)
Duration of service (mean + SD)	10.6 ± 6.9 years	16.3 ± 6.6 years
Duration after seroconversion (mean + SD)	NA	5.3 ± 2.7 years
Comprehensive knowledge about HIV/AIDS	100 %	100 %
Denial of any high risk activity	555 (97 %)	19 (20.6 %)
Detected positive during this study	03 (0.53 %)	13 (14.1 %)
Contact with FSW ^a and CAS ^b	87 (15 %)	66 (71.7 %)
IDU ^c activity	02 (0.35 %)	02 (2.2 %)
Blood or blood product transfusion	NA	02 (2.2 %)
MSM ^d activity	Nil	01 (1.1 %)
Tattooing	33 (5.8 %)	03 (3.3 %)
Consented for testing of spouse and children	NA	44 (48 %)
Spouse and children positive for HIV	NA	08 (8.7 %)
Seroconverted within study period	04 (0.7 %)	NA
Expired within study period	Nil	05 (5.4 %)
Incidence of Hepatitis B	12 (2.1 %)	03 (3.3 %)
Incidence of Malignant diseases	Nil	01 (1.1 %)
Incidence of Herpes zoster	Nil	07 (7.6 %)
Incidence of Mycobacterial infections	Nil	11 (11.9 %)
Progressed to AIDS (AIDS definition, 1993)	NA	25 (27.1 %)
Patients exhibited HAART	NA	53 (57.6 %)

^a Female sex worker; ^b casual amateur sex; ^c intravenous drug user;

^d men having sex with men

Analysis of genotype frequency data did not reveal any significant deviation from the Hardy–Weinberg expectation in any of the populations (HWE). **CCR2-64I**: Of the 568 HIV negative samples 471 were wild type homozygotes (82.2 %) and 102 were mutant heterozygote (17.8 %), and out of 181 HIV positive samples 155 were wild homozygotes (85.3 %) and 26 were mutant heterozygote (14.6 %). No mutant homozygotes were detected. **SDF1-3'A**: Of the 568 HIV negative samples 349 were wild type homozygotes (61.4 %) and 201 were mutant heterozygote (35.4 %) and 18 were mutant homozygotes (3.2 %). Out of 181 HIV positive samples 96 were wild homozygotes (53 %), 75 were mutant heterozygote (41.4 %) and 10 were mutant homozygotes (5.5 %). The allele frequency for mutant

SDF1-3'A ranged within 0.11 and 0.32. There was no significant variation in frequencies between HIV negative and positive cohorts. **MCPI-2518 G/A**: Of the 568 HIV negative samples 280 were wild homozygotes (48.5 %), 215 were heterozygote (37.2 %) and 82 were mutant homozygotes (14.2 %) and allele frequency was 0.33. 61.8 % of the Mizoram population exhibited MCP1 2518 G/A polymorphism. Out of the 181 seropositive samples 74 were wild homozygotes (40.6 %), 85 were heterozygote (47.1 %) and 22 were mutant homozygotes (12.2 %). Allele frequency of q (mutant) was 0.36 (Table 2).

The RH values in seropositive cases were >0.94 for all three genes for progression to AIDS and >0.96 for the final outcome of death under AIDS definition of 1993 [4, 5, 9]. For seronegative cases the Relative risk of seroconversion was >0.90 (Table 3). Kaplan–Meier survival curves as analysed by the Cox proportional hazard regression model, the difference between the wild and the mutant genotypes of SDF1 (RR-1.61, SE-1.61, *p*-0.63); CCR2 (RR-1.28, SE-1.48, *p*-0.83); and MCP1 (RR-0.93, SE-0.93, *p*-0.95) were statistically insignificant (Fig. 1).

We calculated the allele distribution in case of the three genes. Amongst the seronegative group 24.1 % had no mutations; 45.2 % had single mutation; 27.3 % had double mutation and 3.4 % had triple mutations. The values for seropositive group were similar.

Discussion

We collected 568 samples from healthy but a high risk group from the 7 NE states of India, West Bengal and the Gorkha population and 03 were found to be seropositive indicating a prevalence rate of 0.53 %. The revised estimates of HIV prevalence in India is 0.36 % [24] that are marginally lower than our figures. Our hospital data for antenatal cases (ANC) indicates a prevalence rate of 0.11 %. The prevalence rate of HIV amongst ANC in the NE states varies from 0.46 % in Arunachal Pradesh to 1.63 % in Nagaland [17]. 12 cases were found HBV positive indicating a prevalence rate of 1.89 % that is significantly lower than the reported prevalence rates of HBV in the Eastern India (2–4 %) [8]. There was a significant difference in the mean age of seropositive (35.5 ± 6.7 years) and seronegative groups (29.36 ± 6.9 years). Interestingly, there was also a significant difference in the duration of service in the present high-risk profession. Primarily, the combined seronegative group from NE States and the Gorkha cohort (*n* = 448) was a much younger cohort with a mean age of 28.5 years in addition to low mean service tenure of 9.6 years. It is apparent from our study that this particular high-risk group contracted the disease at an older age and longer service tenure. These differences were

Table 2 Genotype percentage of SDF1-3'A, CCR2-64I and MCP1-2518G/A

State	N	SDF1-3'A			CCR2-64I		MCP1-2518G/A		
		AG ^a	AA ^b	q (Mut) ^c	AG	q (Mut)	AG	AA	q (Mut)
<i>Seronegative samples</i>									
Assam	121	39.67	1.65	0.22	18.18	0.09	30.33	18.85	0.34
Arunachal Pr	27	48.15	0.00	0.24	24.14	0.12	36.67	16.67	0.35
Manipur	80	36.25	3.75	0.22	17.50	0.09	26.83	25.61	0.39
Meghalaya	9	22.22	0.00	0.11	9.09	0.05	27.27	18.18	0.32
Mizoram	53	39.62	1.89	0.22	18.18	0.09	30.91	30.91	0.46
Nagaland	45	35.56	0.00	0.18	17.78	0.09	40.82	16.33	0.37
Tripura	10	30.00	0.00	0.15	30.00	0.15	30.00	30.00	0.45
NES total	347	38.62	1.73	0.21	18.52	0.09	31.83	22.25	0.38
West Bengal	96	42.71	10.42	0.32	16.67	0.08	48.96	0.00	0.25
Gorkha	101	19.80	0.99	0.11	14.85	0.07	42.57	0.99	0.22
Others	24	25.00	4.17	0.17	24.00	0.12	48.00	8.00	0.32
Grand total	568	35.39	3.17	0.21	17.80	0.09	37.26	14.21	0.33
<i>Seropositive samples</i>									
TCH ^d , Kolkata	92	50.00	4.55	0.30	19.12	0.10	53.03	16.67	0.43
IDU, Manipur	61	32.79	6.56	0.23	13.11	0.07	42.62	3.28	0.25
FSW, Kolkata	28	38.62	1.73	0.27	7.14	0.04	42.86	21.43	0.43
Total	181	42.71	10.42	0.27	14.65	0.07	47.10	12.26	0.36

^a Heterozygote; ^b homozygote for the susceptible or mutant allele; ^c allele frequency; ^d tertiary care hospital. 61.8 % of the Mizoram population exhibited MCP1 2518 G/A polymorphism but no mutant homozygotes were detected for CCR2-64I. Overall there was no significant variation in genotype frequencies between HIV negative and positive populations

Table 3 Standard errors of allelic frequencies and relative hazard values for CCR2-64I, SDF1-3'A and MCP1-2518 G/A

Group	N	<i>p</i> -wild ^a	q-mutant ^b	HWE ^c	RH-1 ^d	RH-2 ^e
<i>CCR2-64I</i>						
Seronegative	568	0.91 (0.01)	0.09 (0.01)	5.47	0.973 (0.014)	NA
Seropositive	155	0.93 (0.01)	0.07 (0.01)	0.98	0.948 (0.079)	0.0962 (0.112)
<i>SDF1-3'A</i>						
Seronegative	568	0.79 (0.01)	0.21 (0.01)	2.92	0.937 (0.033)	NA
Seropositive	155	0.74 (0.03)	0.27 (0.03)	0.58	0.977 (0.067)	0.984 (0.068)
<i>MCP1-2518 G/A</i>						
Seronegative	568	0.67 (0.01)	0.33 (0.01)	13.92	0.977 (0.012)	NA
Seropositive	155	0.64 (0.03)	0.36 (0.03)	0.09	0.963 (0.042)	0.993 (0.036)

^a Allele frequency of GG or wild homozygote; ^b allele frequency of mutant type; ^c Hardy–Weinberg expectation; ^d relative hazard, AIDS-1993; ^e relative hazard-death

statistically significant with *p* values being consistently less than 0.0001. Furthermore, 90 % of the HIV positive population were married with 1–3 children whereas only 41 % of the HIV negative population from the NE states and Gorkha population (excluding the Bengal cohort) were married indicating the significant influence of long separation from the family as a overbearing reason for enticement by FSWs and their agents in these young and sexually active individuals.

Analysis of genotype frequency data did not reveal any significant deviation from the Hardy–Weinberg expectation

(HWE), both for seropositive and seronegative groups in any of the populations. Genotype percentage of SDF1-3'A in seronegative population ranged from 36 to 40 % (Allele frequency-0.18–0.24) amongst the NE states and West Bengal, highest being in Arunachal Pradesh i.e. 48 %. The frequency amongst the Gorkha population was 21 % being significantly lower compared to the NE states. Amongst the seropositive individuals the frequency was higher at 48 % though the difference was insignificant. Frequency of 48 and 24 % amongst a diverse population in Andhra Pradesh [18] and Northern India [26] respectively were recorded in

Fig. 1 Kaplan–Meier survival curves of seroconverters in HIV positive cohort from the tertiary care hospital at Kolkata showing relation of SDF1, CCR 2 and MCP1 genotypes to AIDS endpoints. *Left panels* Wild genotype (blue) is compared with the heterozygous (pink) and homozygous (red) genotypes to assess the Relative risk for seroconversion as per the AIDS definition of 1993. *Right panels* Wild genotype (blue) is compared with the heterozygous (pink) and

homozygous (red) genotypes to assess the Relative risk for death. *RR* Relative risk, *SE* standard error. *p* value based on the Cox proportional hazards model for all the three AIDS restriction genes (ARGs) indicate that there is no significant protection or survival benefit conferred by the gene polymorphisms either on seroconversion, progression to AIDS or death. (Color figure online)

various studies carried out amongst Indian populations. Our figures are significantly higher as compared to the north Indian population. Worldwide figures indicate an average allele frequency of 0.3 amongst Chinese population [27]; 0.16 amongst African population; 0.15–0.36 amongst American, Caucasians and other Asian population. Allele frequency was significantly higher amongst the Oceania populations (0.54–0.72) [9, 23].

We recorded only heterozygous mutants for CCR2-64I alleles. Genotype percentage for mutant allele in seronegative as well as seropositive population ranged from 15 to

18 %, highest being 30 % amongst the Tripuri ($n = 10$) and low of 14 % amongst the Gorkha ($n = 101$) populations. Frequency of 17 and 9 % in Andhra Pradesh and North India respectively were recorded. Again our findings were significantly higher as compared to the North Indian states. Allele frequency recorded in our study revealed a figure of 0.6–0.1. Worldwide figures indicate an average allelic frequency of 0.2 amongst Chinese population; 0.14 amongst African population; 0.20 amongst American, Caucasians and other Asian population. Allele frequency was significantly lower in our study and we did not detect

any CCR2-64I/64I amongst our study population. Only sporadic cases of such homozygotes were reported by Ramana et al. [18] in Andhra Pradesh.

MCPI-2518 G/A polymorphism were studied for the first time in India. We recorded a very high genotype frequency amongst seronegative and seropositive populations. Frequencies exceeding 60 % was seen in Mizoram and Manipur of which 30 % were homozygous mutants. The frequencies were comparable (43–49 %) amongst the West Bengal and Gorkha population. Worldwide figures indicate a frequency of 64 % amongst the Chinese, Japanese and Koreans; 47 % amongst the Asians and lower frequencies of 21 % amongst the Hispanics and Afro-American populations. Our figures are comparable to those of Chinese amongst the Northeastern population that could probably be attributed to their Tibeto-Burman origin [29].

The Relative risk for seroconversion to HIV infection in the seronegative cohort was analysed and it was found that the RH values for seroconversion was >0.90. The comparative difference between the wild and the mutant genotypes of SDF1 (RR-1.61, SE-1.61, p -0.63); CCR2 (RR-1.28, SE-1.48, p -0.83); and MCP1 (RR-0.93, SE-0.93, p -0.95) was statistically insignificant. The Relative risk for development of AIDS and death revealed that SDF1-3'A/3'A apparently provided a marginal delay in progression to AIDS as compared to wild and heterozygous genotypes (Fig. 1). Our findings were consistent (though not statistically significant) with the previous findings of Winkler et al. [21] but at variance with the findings of Andrea et al. [3] who reported acceleration of the disease process while Ioannidis et al. [14] reported absence of any significant influence on disease progression.

While comparing wild and mutant genotypes of CCR2 and MCP1, no significant difference was found in seroconversion rates, disease progression or death. We analysed the combined effects of 3 wild genotypes with mutant genotypes and found no significant differences either on progression to AIDS or death.

Singh et al. [21] have reported that MCP1-2518 G/A polymorphism significantly increased the rate of neuropsychological impairment (NPI) in HIV infected patients. In our study only 2 cases exhibited features of NPI and the data was too small to be statistically significant.

In our study we followed the AIDS definition of 1993 and Death [4, 5, 9] only as we could follow up the patients and apply the criterion stringently. We did not encounter any significant effects of genetic polymorphisms on seroconversion rates amongst HIV negative population. Although 4 individuals seroconverted during this period of 36 months, a larger follow up period is necessary for accurate analysis.

Our study on genetic polymorphism of ARGs was the first one amongst the Eastern and Northeastern states of

India. We also for the first time included the Gorkha population from Nepal and West Bengal. The study group comprised of young sexually active males vulnerable to enticement by FSWs and to casual sex. HIV prevalence in our study population was comparable to the national figures despite being a very high-risk group (0.46 %). This was achieved due to better awareness, constant vigilance and timely intervention. The age and service tenure of the seronegative population was significantly lower as compared to the seropositive population. The effect of the genetic polymorphism individually or in combination on seroconversion, progression to AIDS or death showed marginal delay in respect of SDF-3'A. Genotype frequency of SDF1-3'A and CCR2-64I in our study was significantly higher as compared to the figures reported amongst the North Indian population. We are the first to report MCP1 polymorphism in India and detected a high MCP1-2518 G/A polymorphism ranging between 50 and 60 % including homozygous mutant state between 15 and 25 %.

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