

PREVALENCE OF HIV-1 CCR5 TROPISM (GENOTYPIC ASSAY): JAIPUR

Shifa Yadav¹, Dinesh Mathur², Manisha Nijhawan³, Savita Agarwal⁴, Aakanksha Singh⁵, Vibhor Goel⁶, Shilpa Soni⁷, Rakesh Jangra⁸

HOW TO CITE THIS ARTICLE:

Shifa Yadav, Dinesh Mathur, Manisha Nijhawan, Savita Agarwal, Aakanksha Singh, Vibhor Goel, Shilpa Soni, Rakesh Jangra. "Prevalence of HIV-1 CCR5 Tropism (Genotypic Assay): Jaipur". Journal of Evolution of Medical and Dental Sciences 2014; Vol. 3, Issue 48, September 29; Page: 11594-11600, DOI: 10.14260/jemds/2014/3519

ABSTRACT: INTRODUCTION: HIV most commonly uses CCR5 and/or CXCR4 as a co-receptor to enter its target cells. Several chemokine receptors can function as viral co-receptors, but CCR5 is likely the most physiologically important co receptor during natural infection. C-C chemokine receptor type 5, also known as CCR5 or CD195, is a protein on the surface of white blood cells that is involved in the immune system as it acts as a receptor for chemokines. This is the process by which T cells are attracted to specific tissue and organ targets. Most forms of HIV, the virus that causes AIDS, initially use CCR5 to enter and infect host cells. We studied the prevalence of CCR5 tropism HIV1 amongst HIV positive patients attending Mahatma Gandhi hospital Jaipur. **STUDY DESIGN:** This was epidemiological, cross-sectional, and non-interventional study between March and April 2014 in HIV positive patients in Jaipur. **METHODS:** Co-receptor tropism assay was done in total nine patients who were HIV positive attending Mahatma Gandhi Hospital Dermatology & Venereology of Mahatma Gandhi Hospital OPD in one month duration time. Total nine patients were studied, seven patients were already on ART (anti-retroviral treatment) and two patients were not taking any treatment as their CD4 count were above 500 cells/UL with low viral load. Co-Receptor tropism assay (genetic assay) CCR5 (with the help of Emcure pharmaceuticals) was done in all of them. **RESULT:** Only one patient had FPR below 15 %, rest 8 patients had FPR above 15%. The study showed that the prevalence of CCR5 positivity was 88.8%, whereas CXCR4 prevalence was only 11.1%.

KEYWORDS: HIV-1; CCR5- tropism; genotypic assays.

INTRODUCTION: HIV most commonly uses CCR5 as a co-receptor to enter its target cells. Several chemokine receptors can function as viral co receptors, but CCR5 is likely the most physiologically important co receptor during natural infection. C-C chemokine receptor type 5, also known as CCR5 or CD195, is a protein on the surface of white blood cells that is involved in the immune system as it acts as a receptor for chemokines. This is the process by which T cells are attracted to specific tissue and organ targets. Most forms of HIV, the virus that causes AIDS, initially use CCR5 to enter and infect host cells. We studied the prevalence of CCR5 tropism HIV1 amongst HIV positive patients attending Mahatma Gandhi hospital Jaipur.

STUDY DESIGN: This was epidemiological, cross-sectional, and non-interventional study between March and April 2014 in HIV positive patients in Jaipur.

METHODS: Co-receptor tropism assay was done in total nine patients who were HIV positive attending Mahatma Gandhi Hospital Dermatology & Venereology of Mahatma Gandhi Hospital OPD in one month duration time.

ORIGINAL ARTICLE

RESULT: Total nine patients were studied, seven patients were already on ART(anti-retroviral treatment) and two patients were not taking any treatment as their CD4 count were above 500 cells/UL with low viral load. Co-Receptor tropism assay (genetic assay) CCR5 (with the help of Emcure pharmaceuticals) was done in all of them. HIV with a V3 loop sequence FPR<5% (False positive ratio) is considered X4 and with an FPR>=15% it is considered to be R5.

Results were as follows:

Sl. No.	Age	Sex	FPR value	CD4 count (cell/mm ³)	Viral load (Copies/ml)
1.	55 yrs	M	90.9%	640	14090
2.	43 yrs	M	0%	250	1700
3.	35 yrs	M	31%	430	2500
4.	29 yrs	F	90.9%	390	45894
5.	35 yrs	M	89.1%	46	1946
6.	50 yrs	F	90.7%	427	27796
7.	30 yrs	F	87.8%	584	49358
8.	40 yrs	M	87.4%	450	34000
9.	35 yrs	M	31%	284	30500

Only one patient had FPR below 15 %, rest 8 patients had FPR above 15%.The study showed that the prevalence of CCR5 positivity was 88.8%, whereas CXCR4 prevalence was only 11.1%.

Eight patients showed CD4 count between 200-700 cell/mm³ among them one patient was CCR5 negative and showed CD4 count 250 with viral load of 1700. One patient who was CCR5 positive showed 46 CD4 count with viral load of 1946 as the patient developed pulmonary tuberculosis so his ART was stopped, which might be the reason for his low CD4 count.

DISCUSSION: HIV enters cells by a complex process that involves sequential attachment to the CD4 receptor followed by binding to either the CCR5 or CXCR4 molecules and fusion of the viral and cellular membranes.¹ CCR5 co-receptor antagonists prevents HIV entry into target cells by binding to the CCR5 receptors². Phenotypic and, to a lesser degree, genotypic assays have been developed that can determine or predict the co-receptor tropism (i.e., CCR5,CXCR4, or both) of the patients dominant virus population. HIV tropism can be assessed by either genotypic or phenotypic methods. Phenotype is most commonly used in current clinical practice and in clinical trials.

Table 1: Shows the comparison of genotype and phenotype tropism.

Cell Based (Phenotype)	Genotype Based
Advantages: <ul style="list-style-type: none"> • Biologically relevant. • Assesses entire envelope product. • Validated in clinical trials of CCR5 antagonists. • Sensitivity of 0.3% 	Advantages: <ul style="list-style-type: none"> • Low cost (roughly \$1000). • Less technically difficult. • Sensitivity of 1%. • Short turn-around time: 3-10 days.
Disadvantages: <ul style="list-style-type: none"> • Cost (roughly \$2000). • Long turn-around time: 2-3 weeks. • High rate of non-reportable results. • Requires growth on cell lines which may differ from in vivo growth conditions. 	Disadvantages: <ul style="list-style-type: none"> • Lower sensitivity may miss X4-using strains. • Sequence analysis limited to V3 loop. • Lack of clinical trial data. • Poor hybridization to R5 probe scored as X4; may be highly divergent R5. • Sequencing of bulk DNA can miss minority species (<25%).

Table 1: Comparison of Tropism Test Methods

Tropism predictions based on genotype rely on sequencing the envelope gp120, which can be technically challenging due to a high degree of sequence heterogeneity. Limiting analysis to the V3 loop of gp120 is less difficult but may yield less accurate results since regions outside V3 are also involved in viral tropism.

Viruses in many untreated patients eventually exhibit a shift in co-receptor tropism from CCR5 usage to either CXCR4 or both CCR5 and CXCR4 tropism (i. e, dual-or mixed –tropic, D/M-tropic). This shift is temporally associated with a more rapid decline in CD4 T-cell counts.^{3,4} but whether this tropism shift is a cause or a consequence of progressive immunodeficiency remains undetermined¹. Antiretroviral treated patients with extensive drug resistance are more likely to harbor X4 or D/M-tropic variants than untreated patients with comparable CD4 counts.⁵

The prevalence of X4-or D/M- tropic variants increases to more than 50% in treated patients who have CD4 counts<100 cells/mm.^{3,5,6} Phenotypic assay characterize the co-receptor usage of plasma –derived virus. In vitro the phenotypic assay can detect CXCR4- utilizing clones with 100% sensitivity when those clones represent 0.3% or more virus population.⁷ This assay takes about two weeks to perform and requires a plasma HIV RNA level >1,000 copies/ml. In patients with plasma HIV-1 RNA below the limit of detection, co-receptor usage can be determined from pro viral DNA obtained from peripheral blood mononuclear cells, however, the clinical utility of this assay remains to be determined.⁸

Genotypic determination of HIV-1 co-receptor usage is based on sequencing of the V3 –coding region of HIV1 envelop, the principal determinant of co-receptor usage. In our study we have used the genotypic assay method. When compared to the phenotypic assay, genotypic methods show high specificity (90%) but only modest sensitivity (50%-70%) for the presence of a CXCR4 –utilizing virus.

ORIGINAL ARTICLE

Given these performance characteristics, these assays may not be sufficiently robust to completely rule out the presence of an X4 or D/M variant.⁹ European guidelines currently favor genotypic testing to determine co-receptor usage.¹⁰

Our study showed the prevalence of CCR5 positivity as 88.8%, whereas CXCR4 prevalence was only 11.1%. Moreno et al showed in their study prevalence of CCR5-tropic HIV-1 among treatment-experienced individuals in Spain and found that (68.9%) patients had CCR5-tropic HIV-1 virus, and (31.1%) had dual-tropic/mixed or CXCR4 virus variants. GlaxoSmithKline (GSK), with their compound aplaviroc, Schering-Plough with vicriviroc and Pfizer with maraviroc reached clinical trials in humans but only maraviroc has been approved by the U.S. Food and Drug Administration (FDA).

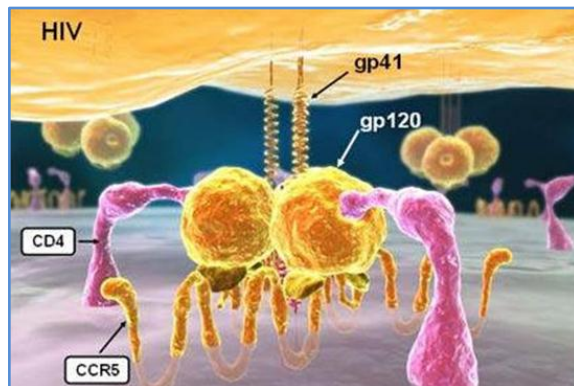
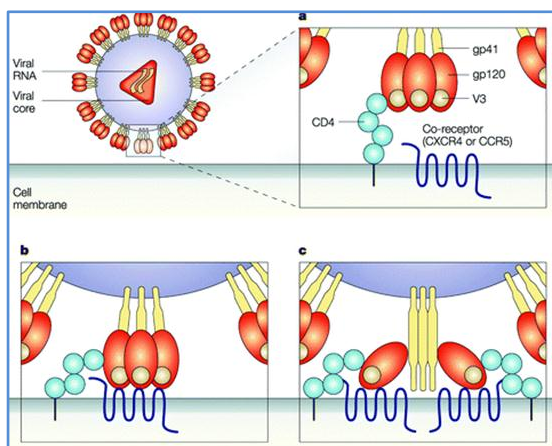
Studies in which v3 genotyping was performed on samples from patients screened for clinical trials of MVC suggest that genotyping performed as well as phenotyping in predicting the response to MVC.^{11,12} A tropism assay may potentially be used in clinical practice for prognostic purposes or to assess tropism before starting ART if future use of a CCR5 antagonist is anticipated. In our study since 7 patients were already on ART and were clinically stable so we did not shift these patients to maraviroc. Other two patients who were not on ART may be started on maraviroc in future.

REFERENCES:

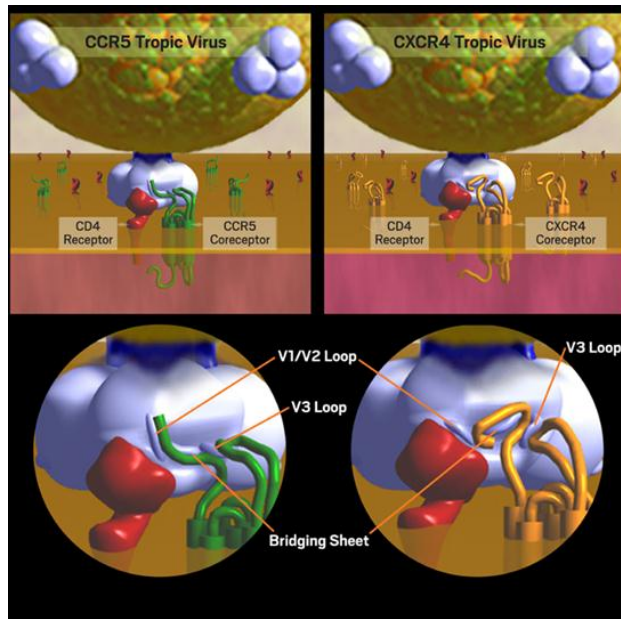
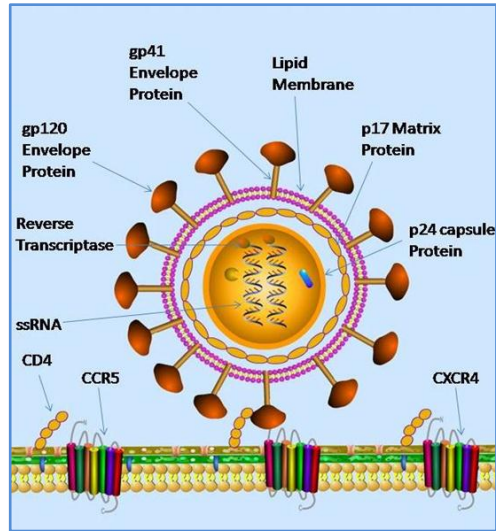
1. Moore JP, Kitchen SG, Pugach P, Zack JA. The CCR5 and CXCR4 coreceptors-central to understanding the transmission and pathogenesis of Human immunodeficiency virus type 1 infection. *AIDS Res Human Retroviruses*. 2004; 20 (1): 111-126. Available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=retrieve & db=Pub Med & dopt=citation & list_uids=15000703.
2. Fatkenheuer G, Pozniak AL, Johnson MA, et al. Efficacy of short term monotherapy with maraviroc, a new CCR5 antagonist, in patients infected with HIV-1. *Nat Med*. 2005;11(11):1170-1172. Available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=citation&list_uids=16205738.
3. Connor RI, Sheridan KE, Ceradini D, ChoeS, Landau NR. Change in coreceptor use correlates with disease progression in HIV-1 infected individuals. *J Exp Med*. 1997;185(4):621-628. Available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=retrieve&db=PubMed&dopt=citation&list_uids=9034141.
4. Koot M, Keet IP, Vos AH, et al. Prognostic value of HIV-1 syncytium-inducing phenotype for rate of CD4 + cell depletion and progression to AIDS. *Ann Intern Med*. 1993; 118 (9): 681-688. Available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=retrieve&db=PubMed&dopt=citation&list_uids=8096374.
5. Hunt PW, Haarigan PR, Huang W, et al. Prevalence of CXCR4 tropism among antiretroviral – treated HIV-1-infected patients with detectable viremia. *J Infect Dis*. 2006; 194 (7): 926-930. Available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=retrieve&db=PubMed&dopt=citation&list_uids=16960780.

ORIGINAL ARTICLE

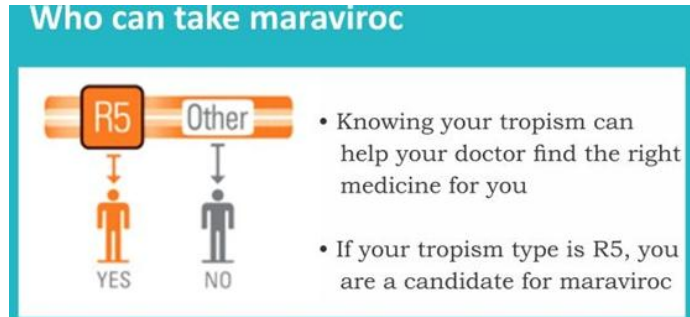
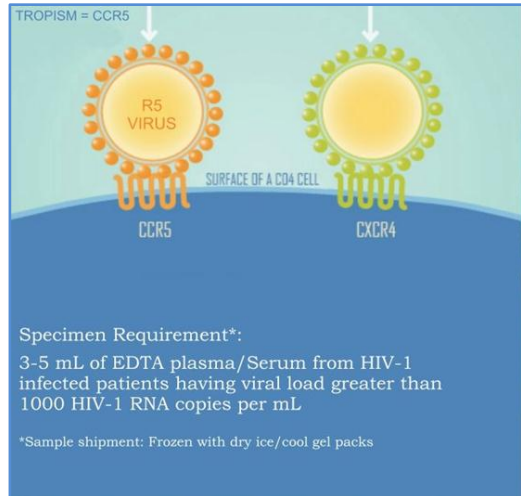
6. Wilkin TJ, Su Z, Kuritzkes DR, et al. HIV type 1 chemokine coreceptor use among antiretroviral – experienced patients screened for a clinical trial of a CCR5 inhibitor: AIDS Clinical Trial Group A5211. *Clin Infect Dis.* 2007; 44 (4): 591-595. Available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=retrieve&db=PubMed&dopt=citation&list_uids=17243065.
7. Trinh L, Han D, Huang W, et al. Technical validation of an enhanced sensitivity Trofile HIV coreceptor tropism assay for selecting patients for therapy with entry inhibitors targeting CCR5. *ANTIVIR ther.* 2008; 13 (Suppl 3): A 128.
8. Toma J, Frantzell A, Cook J, et al. Phenotypic determination of HIV-1 coreceptor tropism using cell-associated DNA derived from blood samples. Paper presented at: 17th Conference on Retroviruses and Opportunistic Infections; 2010; San Francisco, CA.
9. Lin NH, Kurtzkes DR. Tropism testing in the clinical management of HIV-1 infection. *Curropin HIV AIDS.* 2009; 4(6):481-487. Available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=retrieve&db=PubMed&dopt=citation &list_uids=20048714
10. Vandekerckhove LP, Wensing AM, Kaiser R, et al. European guidelines on the clinical management of HIV-1 tropism testing. *Lancet infect dis.* 2011; 11 (5): 394-407. Available at <http://www.ncbi.nlm.nih.gov/pubmed/21429803>.
11. Mc Govern RA, Thielen A, Mo T, et al. Population-based V3 genotypic tropism assay: a retrospective analysis using screening samples from the A4001029 and MOTIVATE studies. *AIDS.* 2010; 24 (16): 2517-2525. Available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=retrieve&db=PubMed&dopt=citation &list_uids=20736814
12. Archer J, Weber J, Henry K, et al. Use of four next-generation sequencing platforms to determine HIV-1 coreceptortropism. *PLoS One.* 2012; 7 (11): e49602. Available at <http://www.ncbi.nlm.nih.gov/pubmed/21429803>.



ORIGINAL ARTICLE



Tropism Designations:
R5 = CCR5 usage
X4 = CXCR4 usage
DM = Dual/Mixed CCR5 and CXCR4 usage
 • Viral population may include:
Dual clones
R5 clones + **Dual** clones
X4 clones + **Dual** clones
R5 clones + **X4** clones
R5 clones + **X4** clones + **Dual** clones

**AUTHORS:**

1. Shifa Yadav
2. Dinesh Mathur
3. Manisha Nijhawan
4. Savita Agarwal
5. Aakanksha Singh
6. Vibhor Goel
7. Shilpa Soni
8. Rakesh Jangra

PARTICULARS OF CONTRIBUTORS:

1. 2nd Year Resident, Department of Dermatology, Venereology and Leprosy, Mahatma Gandhi Hospital, Sitapura, Jaipur, Rajasthan.
2. HOD, Department of Dermatology, Venereology and Leprosy, Mahatma Gandhi Hospital, Sitapura, Jaipur, Rajasthan.
3. Professor, Department of Dermatology, Venereology and Leprosy, Mahatma Gandhi Hospital, Sitapura, Jaipur, Rajasthan.
4. Senior Resident, Department of Dermatology, Venereology and Leprosy, Mahatma Gandhi Hospital, Sitapura, Jaipur, Rajasthan.
5. 2nd Year Resident, Department of Dermatology, Venereology and Leprosy, Mahatma Gandhi Hospital, Sitapura, Jaipur, Rajasthan.

6. 1st Year Resident, Department of Dermatology, Venereology and Leprosy, Mahatma Gandhi Hospital, Sitapura, Jaipur, Rajasthan.
7. 1st Year Resident, Department of Dermatology, Venereology and Leprosy, Mahatma Gandhi Hospital, Sitapura, Jaipur, Rajasthan.
8. 1st Year Resident, Department of Dermatology, Venereology and Leprosy, Mahatma Gandhi Hospital, Sitapura, Jaipur, Rajasthan.

NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Shifa Yadav,
Room No. 12,
Type IV-A,
Building Old P.G. Hostel,
Mahatma Gandhi Hospital,
Sitapura, Jaipur.
Email: shifayadav@gmail.com

Date of Submission: 08/09/2014.
Date of Peer Review: 09/09/2014.
Date of Acceptance: 10/09/2014.
Date of Publishing: 27/09/2014.