

Original Research Article

The study of mannose receptors status in HIV-1 discordant couples

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ABSTRACT

Background: It is more than two decades the presence of HIV virus has created a pandemic in human community. The current study was designed to study the levels of mannose receptors on vaginal epithelial cells of female partners of seronegative couple and of seronegative wife of seropositive husband.

Methods: It is a case control observational study. In the study, 30 controls: seronegative couples- husband seronegative and wife seronegative and 30 cases (study group): serodiscordant couples-husband seropositive and wife seronegative were included. The gynecological examination of the female partner was carried out, both per speculum (PS) and per vaginal (PV). Any lesions or inflammation was noted. The area was cleaned, and smear was collected. The smear was immediately fixed with 1:1 ether alcohol. The gynecological examination of the female partner was carried out, both per speculum (PS) and per vaginal (PV). Any lesions or inflammation was noted. The area was cleaned, and smear was collected. The smear was immediately fixed with 1:1 ether alcohol.

Results: Although sexual mode of transmission is highest in India, large numbers of couples are serodiscordant. In this study, the discordant couples were married for an average of 19.3 years. Their youngest child had a mean age of 8.34 years. The mother being seronegative, all children were seronegative. The Husband's qualified for ART at the time of detection of HIV status depending on their CD4 value. It means that they were harboring the virus for more than 10 years. Despite unprotected sex, the virus was not transmitted to the wife. Search for alternate pathway of HIV entry through vaginal mucosa showed: The females in control group revealed >98% epithelial cells had mannose receptors in almost all the females. As against serodiscordant females had mannose receptors in < 10% vaginal epithelial cells. There is a significant difference ($p < 0.01$) between the control group and study group. Thus, the absence of mannose receptors probably prevents the HIV transmission.

Conclusions: This observation will be helpful in developing effective microbicide and will open new frontiers for drug development which will halt sexual transmission of HIV and will also help in vaccine development.

Keywords: Discordant couples, HIV-1, Mannose receptors

INTRODUCTION

It is more than two decades the presence of HIV virus has created a pandemic in human community.¹ Though this world-wide evolution of HIV has been simultaneously accompanied by a continuous flow of knowledge about its genome, characteristics, pathogenesis, treatment, etc. many of its aspects are still a mystery.

The HIV virus spreads primarily by

- Sexual transmission: anal and vaginal route
- Contaminated needles
 - IV drug users
 - Needle stick injuries
 - Unsterile injections
- Mother to child transmission

- In Utero
- At birth
- breast milk
- Blood transfusion from infected person.²

The lower female genital tract is lined by stratified squamous epithelium (vagina and ectocervix) or a single epithelial minelayer (endocervix). It is still a gray zone how HIV-1 penetrates the intact female genital mucosa and initiates infection.

It has been demonstrated that intact normal cervical and vaginal mucosa provide significant barrier to HIV-1 infection with its innate immunity.

However, the exact ultra-structural anatomy of this defense is yet to be outlined.³

The current study was designed to study the levels of mannose receptors on vaginal epithelial cells of female partners of seronegative couple and of seronegative wife of seropositive husband.

The results of this study have the potential to stimulate further innovative ideas and subsequently help in devising novel preventive strategies for controlling this catastrophic bane.

METHODS

The aims and objectives of the study was to study the demographic profile of serodiscordant couples. To study the presence of mannose receptors in vaginal epithelial cells of seronegative wife of seronegative husband (seronegative couple).

To study the presence of mannose receptors in vaginal epithelial cells of seronegative wife of seropositive husband (serodiscordant couples). To quantitate the number of mannose receptors on vaginal epithelial cells of wives of seronegative couple and serodiscordant couple.

To study the correlation if any between the quantity of mannose receptors and serodiscordance. Study type: case control observational study. Sample size: 30 controls: seronegative couples- husband seronegative and wife seronegative.

30 cases (study group): serodiscordant couples-husband seropositive and wife seronegative.

The study was divided into two phases

Phase 1: control group

- 30 age-matched seronegative couples attending the medicine OPD of a tertiary care hospital, Mumbai were selected,

- Written informed valid consent of both the partners for the HIV test as well as for the study was taken,
- iii. HIV status of both the partners was done as per NACO protocol after pre-test counseling,
- If the HIV test of both the partners was negative, the wife was called for further investigations.

Phase 2: this consisted of the study group

- Case records of the HIV positive males attending the ART OPD of a tertiary care hospital, Mumbai whose are HIV negative were selected,
- These were the couples who at the time of detection of HIV status had CD4 count below 250 which indicated that they have been harboring the virus since atleast 8 to 10years. Also, they were bearing 3 or 4 children which meant that they had unprotected sexual intercourse at the time when the husband was asymptomatic, and the wife was still seronegative. These couples were contacted and were explained about the study. the first 30 of them whom who agreed to follow with us were selected,
- Written informed valid consent of both the partners was taken,
- The date of HIV status of male, the initial CD4 count, whether ART was started and what is current CD4 count were noted,
- For the females in this group, HIV test at the beginning of the detection of the husband 's HIV status was noted (as per NOCO protocol) and a repeat test was done at the time of study,
- The duration of marriage was noted,
- Obstetric, the total number of children and age of the youngest child was documented,
- The wife of the serodiscordant couple was then called for further investigations,
- The gynecological examination of the female partner was carried out, both per speculum (PS) and per vaginal (PV). Any lesions or inflammation was noted. the area was cleaned and smearwas collected. The smear was immediately fixed with 1:1 ether alcohol,
- They were then subjected to flouoroisothiocyanate (FITC) stain to see for the presence of mannose receptors,
- Using fluorescent microscope 300 vagina cells were counted for the presence of mannose receptors stained by FITC and on fluorescent microscope they appeared green in color,

The unpaired' t' test was applied to the quantification of mannose receptors in both the study group and the cases group.

RESULTS

Age matched cases were taken in control and study group as follows.

Table 1: HIV status according to age.

	Controls (N=30)		Cases (N=30)	
Definition	Husband: HIV Negative Wife: HIV negative		Husband: HIV Wife: HIV negative	
Age (Mean+SD)	M	41.16+3.37	41.12.73+	
	F	37.52+3.11	38.46+3.08	
HIV status tested	Husband: Negative Spouse: Negative		Husband: Positive Spouse: Negative	

The demographic profile as the duration of marriage, duration of detection of HIV status, the initial CD4 count of the cases group males and their CD4 count at the end of study were taken.

Table 2: Demographic profile of male spouse in control and study group.

Profile	Study group males (N=30)
Duration of marriage (mean)	19.3 years + 3.06
Duration of known seropositive Status	9.79+2.21
Age of youngest child	8.34+2.44
Initial CD4 count	142.6+95.19
Current CD4 count after ART	405.17+167.16

Table 2 shows the demographic profile of male spouse in control and study group. The mean age in both the groups was 41.1 years is comparable. Control and study group had average duration of married life for 19 years. In the study group, the male spouse was on ART (antiretroviral therapy). The duration of known seropositivity was 3 to v10 years. the health seeking behaviour of our patients is different. These patients were detected seropositive only on the advice of doctors when they attended hospital for some health problems (symptomatic). in other words people had done voluntary testing after their risk behaviours. It indicates their ignorance, denial or apathy to get tested. Being asymptomatic and oblivious to their HIV status, the couples had regular sex. The age of the youngest child was noted on all these cases. Mean age of youngest child was 8 years. mother being seronegative, the children were negative.

Considering the incubation period of HIV to be 8 to 10 years, the male spouse was in the stage of clinical/immunological AIDS, indicates that he was harboring the virus for more than 8-10 years. The birth of the youngest child indicates that even when husband was HIV infected he had unprotected sex with wife which he continued to have till he became symptomatic and was detected HIV positive. Therefore, it can logically and practically be concluded that these women had regular sex with their husband for many years; they produced

children but still remained seronegative. Thus, it is called as serodiscordance. The negativity of the wife was not the result of condom use. the table also that the CD4 count on day 0 when he was started on antiretroviral therapy (ART). the mean CD4 count was 142+95.19 indicating advanced immunodeficient state. These cases after starting on triple ART had very good CD4. CD4 after ART was 405+167.16. The duration of ART varied from 1 to 4 years. At the initiation of ART all patients were counselled on safer sex practices. Hence at the time of enrollment for this study, Most of them were interviewed and they admitted either abstinence or regular condom use.

Presence of mannose receptors in the control group and cases group females were studied using FITC stain at NIRRH.

The findings of mannose receptors are shown in Table 3. The table shows that 29/30 women in control group had more than 98% mannose receptors. Only 1 had mannose receptor in the range of 80-90% amongst the study group women, 29 of them had mannose receptors in the range of 20-29%.

Table 3: Mannose receptors in control and cases group females.

Mannose receptors	Control group (n=30)	Study group (n=30)
>98%	29	0
70-79%	1	0
70-79%	0	0
60-69%	0	0
50-59%	0	0
40-49%	0	0
30-39%	0	0
20-29%	0	1
10-19%	0	0
<10%	0	29

Here it can be seen that in the control group females all of them are consented in around 95 to 100% range and of the cases group, majority are concentrated around < 10% range. The significance of this was calculated using standard error of proportion. This was calculated to be <0.01.

DISCUSSION

Immunity refers to the resistance exhibited by the host towards injury caused by microorganisms and their products. it is of two types: innate and acquired. The immunity that a person obtains as a part of being a human being is said to be the innate immunity and the resistance the individual acquires during life is known as the acquired immunity. Whenever an organism enters the human body, it has to face resistance from immunity at various levels.⁴

Epithelial surfaces

The intact skin and mucosa protect the body from invasion by microorganisms. Healthy skin possess bactericidal activity to which salt in drying sweat, sebaceous secretions and long chain fatty acids contribute. The mucosa has got defenses like its ciliary, local secretions like the bactericidal activity of saliva, lysozyme in tears, spermine and zinc in semen, etc.⁴

Blood and tissues

Bactericidal substances like beta lysine, lactoperoxidase in milk, leukins in blood from leucocytes and plains from platelets, lactic acid in muscle tissue, etc. cellular factors like neutrophils and macrophages, etc. form a part of the innate immunity.⁴ Similarly, for HIV virus for entering in the human body and surviving has to pass through these barriers. For heterosexual transmission, various studies have been conducted on vaginal epithelial cells, semen and the germinal cells to explain the role of various receptors for its attachment and transmission.

Studies had initially shown that leucocytes were the cells responsible for HIV-1 transmission. However, it was leucocytes were the semen that semen without leucocytes was also infectious. It was by various techniques like atomic force microscopy and polymerase chain reaction, concluded the spermatozoa were also responsible for HIV-1 transmission. But these germ cells did not express CD4, CCR5, CXCR4 molecules, the conventional receptors for HIV binding and transmission and hence the mechanism by which the infection spread was still an enigma.⁵

Studies conducted on vaginal epithelial cells by florin H et al had shown that both intraepithelial CD4 cells and Langerhans cells in the human vagina were primary targets for HIV infection.⁶ Langerhans cells or dendritic cells could play an important role in the initial spread of HIV, as the latter was identified by electron microscopy in some Langerhans cells from AIDS patients and purified Langerhans cells and dendritic cells can get infected with HIV in vitro.⁷

However, other studies failed to find HIV in Langerhans cells in HIV infected patients.⁸ Hence, the exact role of Langerhans cells in HIV transmission through heterosexual route still needs to be determined. These results further led to search for alternative pathways. Many such pathways are under scrutiny and have generated a tremendous interest amongst researchers.

A few studies had shown the absence of CD4 receptors on vaginal epithelial cells. This was further confirmed in studies conducted by Bandivedkar et al.⁹ This study had shown the presence of mannose receptors on both the sperms and vaginal epithelial cells as a potential target binding and transmission of HIV.

Considering the possibility of mannose receptor as a factor for HIV binding and transmission, its role in HIV negative females with HIV positive husband and peripheral immune mechanisms. A study conducted by Jennes W. et al suggested that many cellular factors play a role in protection of HIV transmission through this route. The study was conducted on HIV exposed seronegative female sex workers (FSWs). It shows that these females had significantly decreased expression levels of C-X-C chemokine receptor 4, but not of c-c chemokine receptor 5, on both memory ($p < 0.001$) and naïve ($p = 0.041$) CD4 T cells as compared with the blood donors. Also, CD38 expression on CD8 T cells was significantly increased among these FSWs, compared with that among blood donors ($p = 0.017$). In this study, there were no significant differences in HLA-DR and CD62L expression between blood donors and these FSWs. And these FSWs.¹⁰

Apart from chemokine receptors, the scientists also studied the role of humoral immunity. A study conducted by Sandra M. et al shows that HIV- specific IgA but not IgG was present in urine and vaginal wash samples from HIV-exposed seronegative individuals, whereas both IgA and IgG were observed in their seropositive partners suggestive that humoral immunity may also play a role in protecting from infection. This study also shows that Env peptide stimulated peripheral blood mononuclear cells produced more IL-2 and less IL-10 compared with those of HIV infected individuals. This study demonstrated no differences in chemokine production or CCR5 expression.¹¹

Another study conducted by Schenal M. et al in 2005 had shown that gag-specific T lymphocytes are present in seronegative individuals exposed to HIV. These gag-specific T cells secrete IL-2 whereas those in HIV positive individuals secrete interferon gamma. In addition the CD4/CD8 and the memory/naïve ratios terminally differentiated in seronegative HIV exposed individuals.¹² Similar results were obtained by Lo Caputo S. et al when they studied the cellular and humoral immune parameters in peripheral lymphocytes, seminal fluid and urethral swabs of HIV exposed seronegative individual and compared with seropositive individuals and healthy controls. They have shown that Env- and Gag-specific IFN gamma- producing CD4 and CD8 lymphocytes were present in these HIV exposed seronegative individuals only on seminal leucocytes but not on peripheral lymphocytes. But they were present on both seminal leucocytes and peripheral leucocytes in HIV positive individuals. They have also shown high urethral concentrations of HIV-1 specific IgA in these HIV exposed seronegative individuals.¹³ Kaul R. et al have shown that HIV-specific CD8 T cell frequencies to be higher in blood in HIV exposed seronegative individuals than the HIV positive individuals. HIV-specific CD8+ T cell responses in the absence of detectable HIV individuals may be playing an important role in

protective immunity against heterosexual HIV-1 transmission.¹⁴

A novel study to assess the role of mannose receptors in both the control group and study group females Presence of mannose receptors was studied with FITC staining. It was seen that majority of the control group females had presence of mannose receptors in 98% of vaginal epithelial cells. This gives us a baseline of the presence of mannose receptors in the general population. All of the cases group females had presence of mannose receptors in less than 10% of epithelial cells. On the application of unpaired 't' test the difference in the presence of mannose receptors in both the group shows a p value, 0.05. This shows that the seronegative female partner in the discordant couple had significantly low number of mannose receptors.

The breakthrough in the mystery of serodiscordant couples will have far reaching implications. It will certainly assist and direct the specific microbicide development which will prevent HIV transmission. It will empower women who have no power to refuse undesired sex. The mannose receptor inhibitors or antibodies against these receptors may add to preventive efforts. Similarly, it will help in HIV vaccine development.

CONCLUSION

Although sexual mode of transmission is highest in India, large numbers of couples are serodiscordant. In this study, the discordant couples were married for an average of 19.3 years. Their youngest child had a mean age of 8.34 years. The mother being seronegative, all children were seronegative. The Husband's qualified for ART at the time of detection of HIV status depending on their CD4 value. It means that they were harboring the virus for more than 10 years. Despite unprotected sex, the virus was not transmitted to the wife. Search for alternate pathway of HIV entry through vaginal mucosa showed. The females in control group revealed >98% epithelial cells had mannose receptors in almost all the females. As against serodiscordant females had mannose receptors in < 10% vaginal epithelial cells.

There is a significant difference ($p < 0.01$) between the control group and study group. Thus, the absence of mannose receptors probably prevents the HIV transmission. This observation will be helpful in developing effective microbicide and will open new frontiers for drug development which will halt sexual transmission of HIV and will also help in vaccine development.

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REFERENCES

1. Adler MW. ABC of AIDS, Michael Adler 5th edition. London: ELST; 2001 p 2.
2. Fauci, Braunwald, Kasper, Hauser, Longo, Jameson editors. Harrison's principles of Internal Medicine. 17th edition. Mc Graw Hill Medical; 2008;1:1137-1203.
3. Hu Q, Frank L, Williams V, Santose JJ, Watts. Blockade of Attachment and Fusion Receptors Inhibits HIV-1 Infection of Human Cervical Tissue. J Exp Med. 2004;199:1065-75.
4. Anathnarayan R, Paniker CKJ. Textbook of Microbiology. 6th edition India: Orient longman private limited. 2001;6774.
5. Cardona-maya W, The Role of mannose Receptor on HIV-1 Entry into Human Spermatozoa. Amer J Repro Immun. 2006;33:241-45.
6. Hladik F. Initial Events in Establishing Vaginal Entry and Infection by Human Immunodeficiency Virus Type-1. Immunity. 2007;26(2):257-70.
7. Macedonia SE, Lau R, Patterson S, Pinching AJ, Knight SC. Dendritic cell infection Depletion and dysfunction in HIV-infected individuals. Immunology. 1990;71(1): 38-45.
8. Kalter DC, Gendelman HE, Meltzer MS. Infection of human epidermal Langerhans's cells by HIV. AIDS. 1990;4:266-7.
9. Fanibunda S, Velhal E. CD4 independent binding of HIV gp120 to Mannose Receptor on Human Spermatozoa. J Acqurd Immun Def Syn.2008;48:389-97.
10. Jennes W, Sawadogo S, Koblavi-Deme S, Vuylsteke B, Maurice C, Cellular Human immunodeficiency virus (HIV)- protective factors: a comparison of HIV- exposed seronegative female sex wrkers and female blood donors in Abidjan, Cote d' Ivoire. J Infect Dis. 2003;187(2):206-14.
11. Sandra M. HIV-1 specific mucosal and cellular immunity in HIV-seronegative partners of HIV-seropositive individuals. Nature Medic. 1997;3: 1250-7.
12. Schenal M, Lo Caputo s, Fasano F, Saresella M, Pierotti P, Villa ML, et al. Distinct patterns of HIV-specific memory T lymphocytes in HIV-1 specific HIV-1 specific immunity in HIV-exposed uninfected individuals and in HIV- infected patients AIDS. 2005;19(7):653-61.
13. Lo Caputo S, Trabattoni D, Vichi F, Piconi S, Lopalco L, Villa ML, et al. Mucosal and systemic HIV-1 specific immunity in HIV-1 exposed but uninfected heterosexual men. AIDS. 2003;17(4): 531-9.
14. Kaul R, Plummer FA, Kimani J, Dong T, Kiama P, Rostron T, et al. HIV-1 specific CD8+ lymphocyte responses in the cervix of HIV-1 prostitutes in Nairobi. J Immunol. 2000;164(3):1602-11.

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