Original Article

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Microbiology Section

Correlation of CD4 T Cell Count and Plasma Viral Load with Reproductive Tract Infections/Sexually Transmitted Infections in HIV Infected Females

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ABSTRACT

Background: Sexually transmitted infections (STIs) plays a major role in the spread of Human immunodeficiency virus (HIV) due to common route of transmission. These infections display an epidemiological synergy with HIV.

Aim: The aim of this study was to analyse the correlation of CD4 T lymphocyte cell count, HIV-1 plasma viral load with Reproductive tract infections/Sexually transmitted infections (RTIs/STIs) in HIV infected females.

Materials and Methods: The study included 60 HIV infected females. An informed consent was taken from all the study subjects. Relevant specimens (genital specimen and blood) were collected for laboratory diagnosis of various RTIs/STIs, CD4 cell count and plasma viral load estimation.

Results: Mean CD4 count of females with bacterial vaginosis, vaginal candidiasis, trichomoniasis, syphilis and herpes simplex infection were lower as compared to other HIV infected cases and mean plasma viral load of bacterial vaginosis, vaginal candidiasis, trichomoniasis and syphilis were higher as compared to other HIV infected cases but this difference was not statistically significant.

Conclusion: This study highlights the importance of routine screening for STIs/RTIs of all the HIV infected females for RTIs/STIs irrespective of CD4 cell count and plasma viral load.

Keywords: Bacterial vaginosis, Herpes simplex infection, Syphilis, Trichomoniasis, Vaginal candidiasis

INTRODUCTION

The Human immunodeficiency virus (HIV) infection is a global pandemic. HIV continues to be a burden globally and presents serious public health problems in the developing countries, especially in India. Currently almost half of all new HIV infections are being reported in women. Of these less than 50% are aware of their infection and 84% are in reproductive age group i.e. 15-45 y [1]. RTI/STI most commonly affect this age group. Both ulcerative (syphilis, herpes) and nonulcerative infections (trichomoniasis, *Chlamydia and gonorrhea*) and other sexually transmitted RTIs that involve inflammation of the reproductive tract especially in women have been implicated in the spread of HIV [2]. The presence of STI facilitates shedding of HIV and increases the HIV disease progression, possibly by increasing the plasma vireamia. STI and HIV facilitate the sexual transmission of one another and this interrelationship is commonly referred to as epidemiologic synergy regimens [3-5].

The standard methods used to monitor HIV infection at present are clinical assessment, flow cytometry based CD4+ T lymphocyte count (CD4 counts) measurement, and molecular assays to quantify plasma viral load (PVL).

There are very few studies done in India which has correlated the CD4 T lymphocyte cell count, HIV-1 plasma viral load with RTI/STI in HIV infected females. We have carried out this study to analyse the correlation of these immunological and virological marker with RTI/STI in HIV infected females.

MATERIALS AND METHODS

This study was conducted prospectively between March 2010 to January 2011 at Integrated Counseling and Testing Centre, Department of Microbiology, Maulana Azad Medical College, New Delhi, India. Study subjects included 60 HIV infected females. All study subjects were recruited in the study after taking informed consent and proper counseling. Socio-demographic data and clinical signs and symptoms of all subjects were recorded in a proforma and their clinical, serological and immunological evaluations were performed.

At recruitment, 5ml of blood was collected in plain vacutainer for syphilis serology and detection of HSV antibody. Serological tests for syphilis were done using Veneral Disease Research Laboratory test (VDRL) using VDRL antigen of Laboratories of Serologist, Government of India, Calcutta and for specimens reactive on VDRL were confirmed by Treponema Pallidum Haemagglutination Assay (TPHA) (Plasmatec Laboratory Products Ltd). Presence of IgM antibodies to HSV (1+2) was determined by Enzyme Linked Immunosorbent Assay (DIALAB). Four ml blood was collected in a K3 EDTA vial, for absolute CD4 T lymphocyte count and plasma viral load estimation. Absolute CD4 T lymphocyte count was done by the FACSCount system (Becton, Dickinson and Company, San Jose, CA, USA). and plasma was separated and stored at -70°C for plasma viral load estimation. PVL was estimated using an Amplicor HIV-1 Monitor Test, version 1.5 (Roche Diagnostics, Branchburg, NJ, USA) by the standard procedure. (Detection limit 47 - 10,000,000 copies/mL)

High vaginal swabs were collected during per speculum examination for diagnosis of bacteria vaginosis, trichomoniasis and candidiasis. Bacterial vaginosis was diagnosed by microscopic examination of gram stained vaginal smear using Nugent's method [6]. Trichomoniasis by saline wet mount and candidiasis by Gram stained smear. The presence of pseudohyphae or budding yeast cells was considered diagnostic of candidal infection. Two endocervical swabs were collected for detection of *Neisseria gonorrhoea* and *Chlamydia trachomatis* infection. One endocervical swab was collected according to the instructions provided in the specimen collection and transport kit (Amplicor STD swab collection and transport set) for detection of *Chlamydia trachomatis* by real-time PCR (COBAS® TaqMan® CT Test, v2.0) and another for *Neisseria gonorrhoea* by direct microscopy and culture.

		Number of Study subjects	Percentage(%)
Age			
18-25		8	13.33
26-35		39	65
36-45		12	20
46-49		1	1.66
Marital status			
Married		48	80
Widowed		10	16.67
Divorced		2	3.33
HIV status of spouse			
HIV Positive		57	95
HIV Negative		3	5
Unknown		0	0
Mode of HIV transmission			
Heterosexual contact		55	91.67
Blood transfusion		4	6.66
Not known		1	1.67
Number of sexual partners			
One		56	93.33
Two		3	5
Multiple		1	1.67
Condom use (male partner)			
Yes	Regular	6	10
	Infrequently	16	26.67
No		38	63.33

[Table/Fig-1]: Demographic profile and self reported risk factors of study subjects

STATISTICAL ANALYSIS

Difference of occurrence STIs/RTIs and its correlation with CD4 cell count and plasma viral load among HIV infected subjects were compared using chi square test and accepted statistically significant when p-value is < 0.05.

RESULTS

[Table/Fig-1] shows demographic and self reported risk factors of study subjects. Of the 60 females enrolled in the study, majority of study subjects were married and 26-35 year of age. Ninety five percent of subjects were spouse of HIV infected males having a single sexual partner. Heterosexual mode was the commonest mode of HIV transmission and only six females reported regular use of condom by their sexual partner. IV drug abuse was not found as mode of transmission in any of the cases [Table/Fig-1]. The most common presentation among women was vaginal discharge and lower abdominal pain.

Mean CD4 count of females with bacterial vaginosis, vaginal candidiasis, trichomoniasis, syphilis and herpes simplex infection were lower as compared to other HIV infected cases and mean Plasma viral load of bacterial vaginosis, vaginal candidiasis, trichomoniasis and syphilis were higher as compared to other HIV infected cases but this difference was also not statistically significant [Table/Fig-2].

RTI/STI	Present/ absent	No. of Cases (%)	Mean CD4 Count	p-value	Mean PVL	p-value
Bacterial vaginosis	Present	9(15)	390.89	.934	855915.33	.108
	Absent	51(85)	419.94		149467.21	
Vaginal candidiasis	Present	7(11.67)	330.86	.350	333264.14	.451
	Absent	53(88.33)	426.77		252966.29	
Trichomoniasis	Present	2(3.33)	304.00	.422	332290.50	.402
	Absent	58(96.67)	419.43		260437.33	
Chlamydial trachomatis infection	Present	4(6.67)	486.50	.265	12908.00	.165
	Absent	56(93.33)	410.52		277159.87	
Syphilis	Present	8(13.33)	394.38	.948	588579.25	.558
	Absent	52(86.67)	418.85		208740.90	
HSV(1+2)	Present	6(10)	327.33	.482	227993.50	.185
	Absent	54(90)	425.39		267204.72	

[Table/Fig-2]: Correlation of mean CD4 count and mean PVL with RTIs/STIs in study subjects
PVL: Plasma viral load

DISCUSSION

Among various factors associated with the sexual transmission of HIV, sexually transmitted Infections (STIs) seem to contribute significantly. Hence, STI control measures contribute significantly for prevention and control of HIV. In developing countries, both prevalence and incidence of STIs are very high since routine screening of STIs/RTIs is not carried out in our country due to lack of laboratory diagnostic facilities, limited resources and some fundamental social problems like poverty, illiteracy and social inequity. STIs impact the health of women adversely for a variety of reasons such as higher susceptibility compared to men, asymptomatic nature of infection, etc. Enormous evidence is available indicating that both ulcerative and inflammatory STIs increase the risk of HIV infection. STIs promote HIV transmission by facilitating HIV shedding in the genital tract, causing disruption of normal epithelial barrier and by deploying and activating HIV susceptible cells at the site [7].

In the present study, mean age of HIV infected cases was found to be 30.92 ±5.720. This compares well with the finding of another studies conducted by Sharma et al., and Vandana et al., [8,9]. The predominant age group in the HIV positive participants in our study was 26-35 y. This section of the population is more affected because they are sexually more active. This association of STIs with younger age is consistent with other studies [9,10]. This finding supports the fact that young sexually active adults should constitute a priority target group in the STI Control Program. The present study population consisted mostly married women, which is concordance with the study conducted in south India [11]. Heterosexual contact was commonest mode of transmission in HIV infected females which correlate well with earlier studies [8,12].

This study attempted to assess the extent of relationship between absolute CD4 T lymphocyte cell count and HIV-1 plasma viral load with RTI/STI in HIV infected female. It was observed that there is no significant difference in the prevalence of RTIs/STIs in females by CD4 count. This is in concordance with study conducted by Goel V et al., where they found no correlation between bacterial vaginosis or vaginal candidiasis and CD4 cell count [9]. This is also concordance with the study conducted in Brazil where no association of candidiasis, trichomoniasis and bacterial vaginosis was observed [13]. Similarly another study conducted by Magnus M et al., showed trichomoniasis is not associated with immune status [14]. This is also in agreement with the study done in Cuba

[15] where no association of *Chlamydia trachomatis* was found with CD4 cell count while on the other hand some studies have reported that candidiasis is correlated with the immunological status of the host [16,17].

Mean plasma viral load among women with bacterial vaginosis, vaginal candidiasis, trichomoniasis and syphilis were higher as compared to other HIV infected cases however this difference was not statistically significant and also there is no significant difference on the basis of symptoms of RTI/STI. However in a study conducted in Belgium presence of gonococcal infection were significantly associated with plasma viral load; vaginal trichomoniasis, syphilis, *Chlamydia* were not significantly associated with higher PVL [18]. In an earlier study conducted by Micheletti et al., no association with vaginal candidiasis, trichomoniasis and bacterial vaginosis was observed and in another study conducted in USA no correlation with bacterial vaginosis was observed [13,19].

CONCLUSION

In the light of the present findings and in the view of the substantial burden of HIV infection in India, it needs to be emphasized that controlling STI/RTI may have positive impact in the control of HIV transmission and spread. The observations of the present study highlights the importance of routine screening for STIs/RTIs of all the HIV infected females irrespective of CD4 cell count and plasma viral load as a necessary intervention to reduce the burden of STIs/RTIs and to reduce the risk of HIV and its spread.

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REFERENCES

[1] Women and HIV infection including mother to child transmission specialists training and reference module. *NACO*;2000.p.172-84.
[2] Wasserheit JN, Sterling JC, Kurtz JB. Viral infections. Rook/Wilkinson/Ebling Textbook of dermatology. 6th *ed. Oxford: Blackwell science.*
[3] Ministry of Health and Family Welfare Government of India. National Guidelines on Prevention, Management and Control of Reproductive Tract Infections including Sexually Transmitted Infections. *NACO*, 2007.
[4] World Bank. World Development Report, 1993: Investing in health. New York: *Oxford University Press*; 1993.
[5] Fleming DT, Wasserheit JN. From epidemiological synergy to public health policy and practice: the contribution of other sexually transmitted diseases to sexual transmission of HIV infection. *Sex Transm Infect.* 1999;75:3-17.
[6] Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *J Clin Microbiol*. 1991;29:297-301.
[7] Risbhud A. Human immunodeficiency virus & sexually transmitted diseases. *Indian J Med Res*. 2005;121:369-76.
[8] Sharma A, Marfatia YS, Modi M. Reproductive tract infections in HIV positive women: a case control study. *Indian J Sex Transm Dis*. 2009;30:16-18.
[9] Goel V, Bhalla P, Sharma A, Mala YM. Lower genital tract infections in HIV seropositive women in India. *Indian J Sex Transm Dis.* 2011;32:103-07.
[10] Osinde MO, Kakaire O, Kaye DK. Sexually transmitted infections in HIV- infected patients in Kabala Hospital, Uganda. *J Infect Dev Ctries.* 2012;6(3):276-82.
[11] Chennaveerappa PK, Halesha BR,Vittal BG, Jayashree N. A study on sociodemographic profile of the attendees at the integrated counseling and testing centre of a medical college in Southern India. *J Clin Diagn Res.* 2011;5:430-33.
[12] Natividah-Villanueva GU, Santiago E, Manalastas RM Jr, Brown HW, Ingersoll J, Caliendo AM, et al. Human immunodeficiency virus in plasma an cervicovaginal secretions in Filipino women. *Int J STD & AIDS*. 2003;14:826-29.
[13] Micheletti AM, Dutra Vde F, Murta EF , Paschoini MC, Silva-Vergara ML, Barbosa e Silva G, et al. Cervicovaginal cytological abnormalities in patients with human immunodeficiency virus infection, in relation to disease stage, CD4 cell count and viral load. *Diagn Cytopathol*. 2009;37(3):164-69.
[14] Magnus M, Clark R, Myers L, Farley T, Kissinger PJ. Trichomonas vaginalis among HIV-Infected women: Is immune status or protease inhibitor use associated with subsequent T. *vaginalis positivity? Sex Transm Dis.* 2003;30(11):839-43.
[15] Kouri V, Cartaya J, Rodriguez ME, Mune M, Soto Y, Resik S, et al. Prevalence of Chlamydia trachomatis in human immunodeficiency virus-infected women in Cuba. *Mem Inst Oswaldo Cruz, Rio de Janeiro*. 2002;97:1073-77.
[16] Rhoads JL, Wright DC, Redfield RR, Burke DS. Chronic vaginal candidiasis in women with human immunodeficiency virus infection. *JAMA.* 1987;257:3105–07.
[17] Spinillo A, Michelone G, Cavanna C, Colonna L, Capuzzo E, Nicola S. Clinical and microbiological characteristics of symptomatic vulvovaginal candidiasis in HIV-seropositive women. *Genitourin Med.* 1994;70:268-72.
[18] N kengasong JN, Kestens L, Ghys PD, Koblavi-Deme S, Bile C, Kalou M, et al. Human immunodeficiency virus type 1(HIV-1) plasma virus load and markers of immune activation among HIV-infected female sex workers with sexually transmitted diseases in Abidjan, Cote d'Ivoire. *J Infect Dis.* 2001;183:1405-08.
[19] RD Kirkcaldy, J Mika, LM Newman, J Langa, L Tian, I Jani, et al. Bacterial vaginosis, alterations in vaginal flora and HIV genital shedding among HIV-1-infected women in Mozambique.*South African Journal of HIV medicine.* 2011;12:22-24.

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