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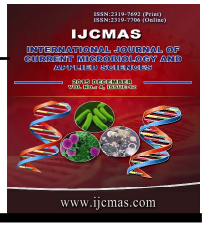

Original Research Article

Impact of Oral Contraceptives and Smoking on the Susceptibility of Reproductive Tract Infections (RTIS) in Immunosuppressed Women: A Hospital Based Study

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A B S T R A C T

The present study has been designed to investigate the prevalence and risk factors of RTIs in HIV positive/negative women in India population. As, Infection with HIV causes immune suppression of the host, which make them more susceptible for various RTIs. The study included 120 HIV seropositive and 100 seronegative women. Participants were interviewed regarding complaints and risk factors. Cervical scrapes samples were tested for Human papillomavirus(HPV), *Chlamydia trachomatis*(CT), *Trichomonas vaginalis*(TV) and *Neisseria gonorrhoeae*(NG) infection by using PCR and *Bacterial vaginosis*(BV) was detected through cervical cytology. Among the HIV-seropositive cases, positivity for the infections including HPV 19%(23/120), CT 12%(14/120), TV 6%(7/120) and BV 21%(25/120) was observed. In control subjects, the prevalence of infections was HPV 4% (4/100), CT 2%(2/100), TV 2%(2/100) and BV 4%(4/100) was observed. Multiple partners (p=0.0005), use of oral contraceptives (p=0.0011) and smoking habits (p=0.0164) were found to be positively associated as risk factors for RTIs. NG infection was not observed in any sample. The use of oral contraceptives and smoking habits may be a risk factor for RTIs in HIV infected women in India. Hence, there is a need to continuously screen, counsel, treat and monitor the trends of RTIs.

Keywords

HIV, *Chlamydia trachomatis*, *Trichomonas*, Sexually transmitted diseases HPV, CD4

Introduction

The human immunodeficiency virus (HIV), a sexually transmitted infection (STI), has an enormous impact on adults with an estimate of around 34.2 million population

infected with the virus and acquired immune deficiency syndrome (HIV/AIDS) causing 1.7 million deaths, worldwide. In India about 2.5 million people are infected with HIV and among them 38% are women (WHO Global). Moreover, HIV patients are more susceptible to other reproductive tract infections (RTIs) because of lowered resistance. The rate of RTIs among women of low economic and social conditions is higher because which creates a barrier in preventing new infections (Rao et al., 2004).

RTIs symbolize a major public health problem in developing countries (Dasgupta Aparajita and Sarkar Madhutandra, 2008). They are allied with unsympathetic health outcomes such as pelvic inflammatory disease (PID), ectopic pregnancy, miscarriage, cervical cancer and an increased risk of HIV transmission (Wasserheit, 1992;Griffin et al., 1999). RTIs include various infections such as Human Papillomavirus (HPV), *Chlamydia trachomatis* (*CT*), *Trichomonas vaginalis* (*TV*), *Neisseria gonorrhoeae* (*NG*), *Bacterial vaginosis* (*BV*) etc.

HPV is the major etiological agent in the development of cervical carcinogenesis. Many studies have shown that the high risk HPV (HR-HPV) infection is the main cause in initiating the progressive alteration that leads to cervical intraepithelial neoplasia (CIN) and to cervical cancer (Scheurer et al., 2005). *CT* and *NG* are among the most common sexually transmitted bacterial infections. According to WHO, globally 106 million cases were occurring annually with approximately two-third of these cases reported from developing countries. In women, *CT* and *NG* infections are a major cause of urethritis, cervicitis, and pelvic inflammatory disease. *TV* is another commonly prevalent STIs after HPV and *CT*, caused by a motile flagellate non-invasive parasitic protozoan *TV*. It has been associated with serious consequences such as prematurity, cervical cancer, atypical PID, low birth weight, infertility and respiratory tract infection in neonates (Miller and Nyirjesy, 2011). WHO estimates that more than 170 -190 million people are annually infected by this protozoan throughout the world. *BV* is a common vaginal infection in women aged 15-44 years. According to the Centre for Disease Control and Prevention (CDC), U.S. the most common risk factor is multiple sex partners (CDC fact sheet, 2012).

However, no comprehensive report is available analyzing the various risk factors for multiple RTI infection among HIV positive women; therefore, the objective of the present study was to investigate the risk factors including CD4 count for various RTI infections in HIV positive women and their correlation with social and behavioral factors in Indian population.

Materials and Methods

Sample Collection

A total of 220 consecutive subjects (married women aged 21- 60 years) were enrolled for the study; of these, 120 were HIV seropositive subjects attending the ART clinic and 100 were control subjects from Gynecological clinic. The age and ethnicity-matched controls having no history of any severe infection such as HIV and with normal cervical cytology were included as control in the present study. The women were then subjected to per speculum examination, cervical scrapes for the detection of RTIs by molecular method was collected from endocervical canal using cytobrush and from ectocervix using an Ayre spatula for cytology. CD_4 count of all HIV positive women were determined.

A questionnaire was designed to assess their socio demographic, sexual and reproductive history, lifestyle, abortion, contraception and any symptoms of genital infections, followed by general and systemic examination. The study was approved by the ethics committee of the institution and prior written informed consent was taken from the patients before enrolment in the study. The study was carried out in accordance with the principles of Helsinki declaration (World Medical Association, 2000).

DNA Extraction and PCR Detection of Multiple RTIs

High molecular-weight genomic DNA was extracted from fresh cervical scrapes samples by the standard method with proteinase K digestion followed by phenol/chloroform/isopropanol treatment (Sambrook Ra, 2001). The quality and quantity of DNA were estimated by using NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Walth am, MA, USA) and extracted DNA was stored at -20^{o}C for further use. Extracted DNA was used for the detection of RTIs including HPV, *CT*, *TV* and *NG* by polymerase chain reaction (PCR) using type specific primers.

HPV diagnosis was done by using consensus primers MY09 and MY11 and further typing was done using type-specific primers of HPV-16 and HPV-18 (Singhal et al., 2013), *CT* was detected by PCR amplifying the gyrA gene of 468bp using the specific primer sequence reported by (Patel et al., 2010), *TV* was detected by amplification of pfoB gene of 333bp by using the primer sequence reported by Saluja et al., (patent number - 1098/DEL/2013 was published on Jan. 3, under issue no. 01/2014), *NG* was detected by amplifying the Orf1 gene of 250 bp, by using the primer sequence reported by Chaudhry et al., (Chaudhry and Saluja, 2002) and *BV* was diagnosed through cervical cytology.

Statistical Analysis

All statistical analyses were performed by using the software GraphPad InStat version 3.0. Chi-square test/Fisher's Exact Test was used to compare the prevalence of RTIs between HIV seropositive women/healthy controls. The odds ratio (OR) and its 95% confidence intervals (CIs), and multivariate analysis were also done as a measure of the association between demographic details and different infections and their risk. The findings were considered statistically significant at p-value < 0.05.

Results and Discussion

Analysis Based on Demographic and Clinical Profile of Studied Population

Different socio-demographic characteristics of the studied population are given in Table 1. The average age of women participating in the study was 32 years. In HIV seropositive women, 53% of women got married before the age of 20 years while for controls it was 71%. 50% of HIV patients had the history of multiple abortions while in controls it was 28%. Most of the women who attended ART clinic belonged to rural area, where as controls comprised of communities from rural as well as from urban set up. Furthermore, 23% of HIV patients had multiple sexual partners but in controls it was estimated to be 9% only. In addition, 68% of HIV positive women were used the contraceptives frequently while in controls it was 30%. However, 39% HIV patients had high intake of chewing tobacco or smoking while in control group it was only 11%. The age of marriage, number of abortions, multiple sexual partners, use of contraceptives and smoking habits were

found to be significantly associated with HIV infection ($p < 0.05$). The differences in age, parity and economic status were not found to be statistically significant in the studied population.

What is the Prevalence of Different RTIs in HIV Positive and HIV Negative Women?

We determined the prevalence of reproductive tract infections like HPV, *CT*, *TV*, *NG*, and *BV* in both HIV positive and negative women (Table 2). Scrapes samples were screened for different RTI infections by using the type-specific primer set, we found 18% (22/120) samples were positive for multiple RTI infections in HIV positive women, but in controls the prevalence of different RTIs were 4% (4/100) only.

Therefore, there is significant difference in prevalence of RTIs between cases and controls. CT infection was revealed 12% (14/120) in HIV patients but in controls it was found to be 2% (2/100) ($p < 0.0001$). The Odd Ratio of infection was found to be 6-7 fold (OR=6.682, 95% CI=1.45-30.6; $p = 0.0126$) higher than control subjects, Therefore, women with HIV infection are at a higher risk of getting *CT* infection. The frequency of *TV* was found to be 6% (7/120) in cases while 2% (2/100) in controls, but was not statistically significant. *NG* was not detected in any sample of the studied population.

The prevalence of HPV was found to be 19% (23/120) in HIV positive women while in controls group it was 4% (4/100) only which indicated a significant association of HPV infection in HIV patients, and showed 5-6 fold (OR= 5.69, 95% CI=1.89-17.07; $p = 0.0013$) higher risk of HPV infection in HIV positive women when compared to control group. On further stratification of HPV typing we observed that the prevalence of HPV 16 was 89.5% (17/19), while that of HPV 18 was 10.5% (2/19).

The prevalence of *BV* was 21% in HIV patients and 4% in controls. We also observed that the frequency of associated *BV* infection was almost 6-7 folds (OR=6.3, CI=2.1-19.3; p=0.0006) higher in HIV positive women than HIV negative group.

How CD4 Count is Correlated with Different RTIs in HIV Positive Women?

The influence of CD4 count on different infections is shown in Figure 1(a). We noticed an increase in the prevalence of HPV (39%), *CT* (18%) and *BV* (39%) infections with the reduction of CD4 cell count (<200 cells/µl). We further, made different combinations of infections having two infections in each group, to evaluate the combinatorial impact of these two infections with CD4 cell count which is presented in Figure 1(b). Interestingly, we noticed that patients having CD4 cell count <200 cell/µl, were more prone to get infected with HPV, *BV* and *CT* individually as well as in association with each other. The chances of getting infected with *TV* were found to be associated neither with CD4 cell count nor with any other infection.

Whether Demographic Characteristics Play Some Role in the Prevalence of RTIs?

To find out the influence of demographic characteristics, we did multivariate analysis in which we adjusted the data against age at marriage, number of abortions, multiple sexual partners, contraception and smoking. After analysis, we noticed the frequency of CT, HPV and BV was found to be significantly different between HIV positive and negative women. Although these

findings were alike with univariate analysis but in this analysis we noticed that the level of significance was much higher when we nullified the effect of demographic factor which suggest some possible role of all/either of the demographic factor in the occurrence of RTIs.

Which Demographic Factors are the major Players of RTIs?

For this analysis we made two groups of HIV positive women i.e. infected and uninfected of RTIs. Women having any of the RTIs were designated as infected women while women who had none of the RTIs were designated as uninfected women. To reveal the role of demographic factors on overall RTIs we did univariate as well as multivariate analysis. In univariate analysis, multiple sexual partner and smoking were revealed as the major player with odds ratio 5.17 and 3.89 respectively. It was our interest to find out the exact role of each of the demographic factor for this we did multivariate analysis. In this analysis we checked the effect of only one demographic factor while nullify/constant the effect of all other demographic factors on the risk of RTIs. After this analysis, amusingly, multiple sexual partners were not found to be significantly associated while use of contraceptives and smoking were emerged out as an independent factor to be associated with the risk of RTIs.

These findings suggest that smoking and contraceptives is the two major players to decide whether an immunosuppressed women will have RTIs or not. After having these findings in hand we were immensely willing to know if the mode of having contraception and source of having smoking also have some impact on RTIs or not.

How the Different Modes of Having Contraception Influence the Risk of RTIs?

As there are lots of modes to have contraception but we divide our data in three categories i.e. hormonal contraceptives, intra uterine devices (IUDs) and condoms Figure 2. Interestingly, we observed that 55% of infected women were using hormonal contraception, 39% were on IUDs while only 6% were using condoms. This pattern of having contraception was almost opposite when we checked it in uninfected women. In uninfected women, the frequency of using condoms was almost 10 times higher as compare to infected women while the frequency of having IUDs and hormonal contraceptives was lesser than half. This result recommends that immunosuppressed women who are using IUDs and hormonal contraception were at higher risk of having RTIs as compared to those who were using condoms.

Whether the Source of Smoking also Affects the Prevalence of RTIs?

We also stratify our data on the basis of different source of smoking i.e. Bidi, cigarette and hookah Figure 3. We noticed that the frequency of bidi users was much higher in infected women as compared to cigarette and hookah. On the other hand uninfected women were noticed to use hookah most frequently. These observations suggest that women having bidi is also at a higher risk of having RTIs. We also tried to look if bidi and hormonal/IUDs contraception have some combined effect on RTIs but we could not find any significant differences.

Association of use of Oral Contraceptives and Smoking Habits with the Susceptibility to RTIs

Oral contraceptives used by the HIV infected women only those having the RTIs are mentioned in Table 3, contraceptives were found to be used by more than 70% by RTIs infected women while its 64% in uninfected population. Moreover, the habit of smoking varies up to more than 63% in cases while in control group it was found to be less. In present study, the data showed that the use of oral contraceptives and smoking habits were more in HIV positive women and in those women who were infected with RTIs.

We reported a high prevalence of RTIs in an immunosuppressed or in HIV positive women in India. STIs/RTIs and HIV/AIDS constitute a serious public health problem worldwide. RTIs often go undiagnosed and untreated resulting in severe morbidity and persistence (Sharma and Gupta, 2009). HIV infection causes immune suppression of the host favoring acquisition of various RTIs in HIV seropositive women (Philip et al., 2013). Immune suppression provides a background for the development of neoplasia by allowing neoplastic proliferations to escape immune surveillance and other host regulatory mechanism. In India, the prevalence of these RTIs is higher may be because of low socio-economic conditions, early marriage, use of contraceptives, multiple child birth, unhygienic conditions and lack of awareness about routine health check up programs.

There were some studies showed the higher prevalence of HPV is more in HIV positive than HIV negative women (Ezechi et al., 2014). The present study demonstrate the 5.6 fold higher risk HPV infection in HIV positive women, which is almost similar to previous studies (Palefsky et al., 1999;Sharma and Gupta, 2009). In the present study the prevalence of HPV was found to be 19% which matches a previous study from North India (Aggarwal et al., 2012). In contrast, the prevalence of HPV was reported 33%-45% (highest) in Sothern – Eastern India (Sahasrabuddhe et al., 2010;Isaakidis et al., 2013) whereas 9% (lowest) in Western India, Gujarat (Aggarwal et al., 2012). Possible explanation for these discrepancies may be attributable to geographic and climatic variations in different regions of India. The HPV is also found in healthy women which can be cleared by host immune system but in HIV infected women persisting HPV infection leads to tumor formation (Ameur et al., 2014).

The bacterial infection, *CT* was detected 12% (14/120) and 2% (2/100) in HIV positive and HIV negative women, respectively. These results were similar to previous studies from different populations worldwide including India (Patel et al., 2010). The prevalence of *CT* was found to be 6.7- fold higher in HIV positive women when compared to HIV negative (Low et al., 2014;Vandenhoudt et al., 2013).

Prevalence of RTIs caused by *TV* and *BV* was higher of in immunosuppressed women, a finding in good agreement with some previous reports throughout the world (Vandenhoudt et al., 2013). Some studies failed to establish any association between *BV* and HIV infection (Remis et al., 2013). These differences in prevalence rate may be due to low HIV concentrations in cervicovaginal fluids (Sha et al., 2005). We noticed that HIV positive women were at 3-4 fold higher risk of *TV* and *BV* respectively in comparison to HIV negative women.

In this study, we could not find any HIV women positive for *NG* infection. However the reports on *NG* –HIV association are very

disparate and unrelated to each other. Some of them documents very low frequency of *NG* in HIV positive women (Low et al., 2014), other record a very high frequency (Vandenhoudt et al., 2013). Such dissimilarity may simply reflect the prevalence of *NG* in particular regions, with the high-risk groups acquiring HIV in the aftermath of *NG*, facilitating HIV acquisition. Behavioral disinhibition while on ART may render and individual vulnerable to RTIs and the etiology in such cases may reflect the prevalent pathogen in the region.

HIV is responsible for the progressive depletion of CD4 cells which ultimately weakens the immunity against any infection (Mehandru et al., 2004). Women with HIV infection are about 10% more prone to RTI infections, especially HPV, than uninfected women and subsequent invasive cervical carcinoma. Therefore, to improve our understanding of cervical disease in HIV positive women, we carried out a prospective case vs. control study to determine the prevalence of multiple RTIs among HIV positive women and compared them with HIV negative women.

In the present study we also attempted to correlate the multiple reproductive tract infection with CD4 count. We found a significant inverse correlation between CD4 count and RTIs i.e. there was increase in the prevalence of RTIs when CD4 was decreased. The frequency of HPV, *BV* and *CT* with CD4 count < 200 cells/µl, was found to be higher. Infection rates of *TV* were found to be higher in our study, when CD4 count ranged between 200-500 cells/µl. Overall, the analysis in the present study showed that major RTIs increase when CD4+ counts are low. These results were similar to several previous studies (Massad et al., 2014;Palefsky et al., 1999).

Further, we determined the association of demographic characteristics with the risk of RTIs in HIV positive women. We observed that HIV infected women having history of smoking and multi-sex partners were significantly associated with RTIs. On analyzing the individual effect of demographic characteristics in multivariate analysis, we noticed that contraceptives and smoking was significantly associated with RTIs in HIV positive women.

Women already having the HIV infection with history of smoking were at high risk of acquiring RTIs and cervical carcinoma (Lieberman et al., 2008). HPV/*CT* infection has been linked to smoking/tobacco in several other studies (Wolf and Freedman, 2000), In our study we found more consumption of bidi may be a risk factor for RTIs in HIV infected women, that is because of reason bidi contains raw/unfiltered tobacco, it can be absorbed directly by the body, due to which biological changes occurred which affect the cervical mucosa, and creates interruption in the production of inflammatory cytokines. Increased number of cyto-toxic T-lymphocytes, which can suppress the activity of T- lymphocytes, decreased the number of natural killer lymphocytes (Johnson et al., 1990). These effects extensively in decreased number of Langerhans cells in the cervix of smokers (Poppe et al., 1996). One of the main reason of more consumption of bidi, it is not expensive as compared to cigarette in India. (Narayan et al., 1996). Most of women in present study belongs to rural areas that may be the reason, they consumpt more bidis because it is inexpensive and more affordable.

Table.1 Socio- Demographic Characteristics of Studied Population

Characteristics		Cases (HIV+) N=120	Controls (HIV-) N=100	P-Value	OR (95% CI)
Age	<30	57(48)	54(54)	Reference	
	>30	63 (52)	46 (46)	0.4794	1.27 [.73-2.217]
Age at Marriage	<21	64 (53)	71 (71)	Reference	
	>21	56 (47)	29 (29)	**0.0111**	2.142 [1.22-3.75]
Parity	<2	45 (37)	30 (30)	Reference	
	>2	75 (63)	70 (70)	0.3050	0.7143 [0.4058-1.257]
Abortion	<2	60 (50)	28 (28)	Reference	
	>2	60 (50)	72 (72)	**0.0015**	0.3889 [0.2211-0.6839]
Socio-economic Status	Low	61 (51)	46 (46)	Reference	
	High	59 (49)	54 (54)	0.5628	0.8239 [0.4841-1.42]
No. of partner	=1	93 (77)	91 (91)	Reference	
	>1	27 (23)	9 (9)	**0.0120**	2.935 [1.308-6.58]
Contraceptives	Yes	82 (68)	30 (30)	Reference	
	No	38 (32)	70 (70)	**<0.0001**	5.096 [2.9-9.05]
Smoking	Yes	39 (33)	11 (11)	Reference	
	No	81 (67)	89 (89)	**0.0003**	3.895 [1.87-8.11]

OR, odds ratio; CI, 95% confidence interval; p-value, probability from the $\chi 2$ test comparing the distribution of characteristics of studied population for controls and cases. Significant p-values are in bold

Table.2 Prevalence of Different Reproductive Tract Infections in Immunosupressed and Healthy Women

Reproductive tract infections		Cases (HIV+) N=120	Controls (HIV-) N=100	Univariate Analysis		Multivariate Analysis	
				P-Value	OR (95% CI)	Adjusted OR (95% CI)	P-value
Chlamydia Trachomatis	Negative	106 (88)	98 (98)	Reference			
	Positive	14 (12)	02 (02)	**0.0126**	6.682 [1.454-30.696]	2.994[0.74-1.3]	**<0.0001**
Trichomonas vaginalis	Negative	113 (94)	98 (98)	Reference		0.265[0.2810-0.2140]	0.7911
	Positive	07 (06)	02 (98)	0.2768	3.035 [0.6159-14.959]		
Neisseria Gonorrhea	Negative	120 (100)	100 (100)	Reference		NA	
	Positive	0	0	NA			
Human Papillomavirus	Negative	97 (81)	96 (96)	Reference		1.042[0.58-1.5]	**<0.0001**
	Positive	23 (19)	04 (04)	**0.0013**	5.69 [1.89-17.077]		
Bacterial vaginosis	Negative	95 (79)	96 (96)	Reference		4.192 [0.1673-0.4610]	**<0.0001**
	Positive	25 (21)	4 (4)	**0.0006**	6.380 (2.1-19.362)		

OR, odds ratio; CI, 95% confidence interval; p-value, probability from the $\chi 2$ test comparing the distribution of characteristics of studied population for controls and cases. Significant p-values are in bold

Table.3 Analysis of Different Demographical Characteristics in Association of RTIs

Demographic characteristics		Any of the infections (HPV/CT/TV/BV/ MT) (N=40)	No infection (N=80)	Univariate Analysis		Multivariate Analysis	
				P-value	OR (95%CI)	Adjusted OR (95% CI)	P-value
Age at Marriage	<21	24 (60)	40 (50)	0.4003	1.5[0.69-3.23]	1.59 [1.1-1.438]	0.1146
	>21	16 (40)	40 (50)				
Abortion	<2	23 (58)	37 (46)	0.3329	1.57 [0.73-3.4]	1.64 [1.04-1.34]	0.1030
	>2	17 (42)	43 (54)				
No. of partner	= 1	23 (58)	70 (88)	**0.0005**	5.174 [2.1-0.48]	3.2 [0.9752-1.32]	0.45
	>1	17 (42)	10 (12)				
Contraceptives	Yes	31 (77)	51 (64)	0.1874	0.5106 [0.21-1.22]	0.75 [0.7792-1.128]	**0.0003**
	No	09 (23)	29 (36)				
Smoking	Yes	25 (63)	14(18)	**0.0001**	7.857 [3.31-18.5]	2.31[1.511-2.03]	**0.0227**
	No	15 (37)	66 (82)				

OR, odds ratio; CI, 95% confidence interval; p-value, probability from the $\chi 2$ test comparing the distribution of characteristics of studied population for controls and cases. Significant p-values are in bold

Figure.1 (a) Bar Diagram Showing the Correlation of Different Reproductive Tract Infections with CD4 Count in Immunosuppressed Women; (b) Bar Diagram Showing the Impact of CD4 Count on the Prevalence of Multiple RTIs in Different Combinations

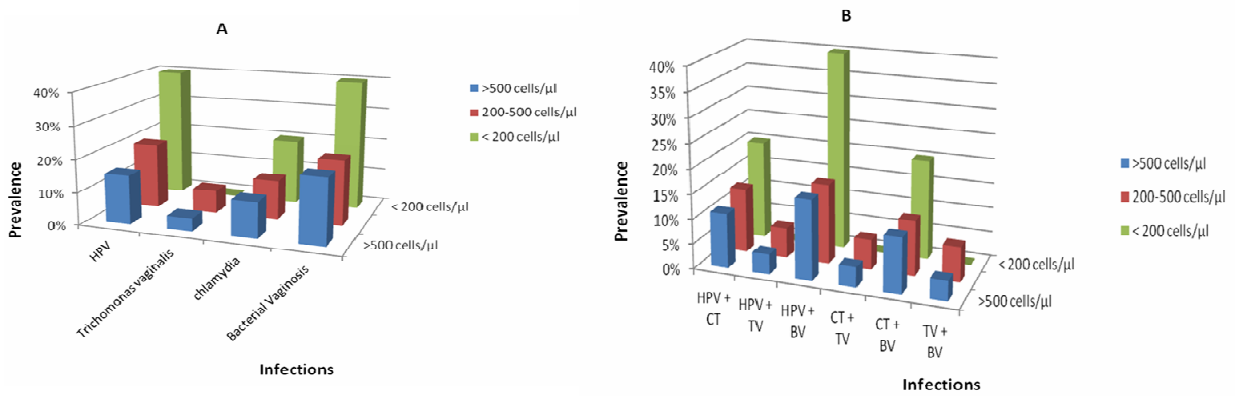


Figure.2 Distribution of use of Hormonal/oral Contraceptives in RTIs Uninfected and Infected Women in HIV Positive Women

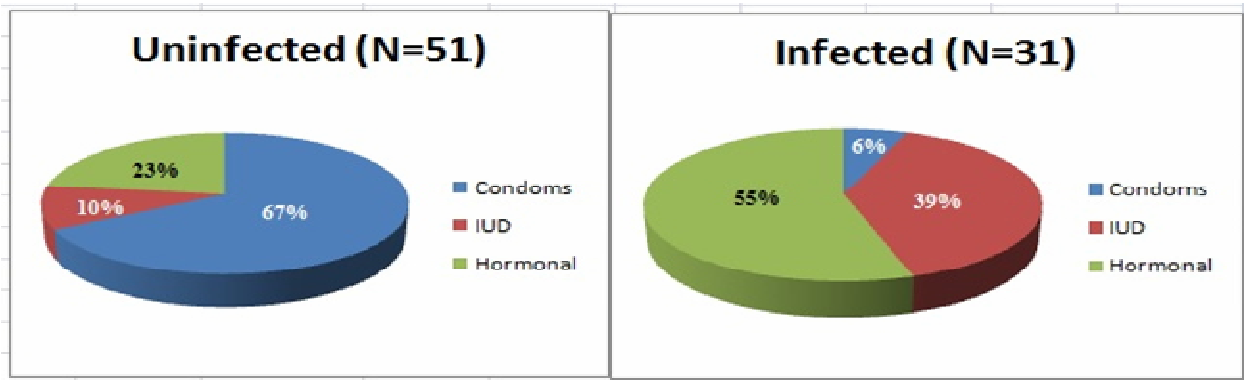
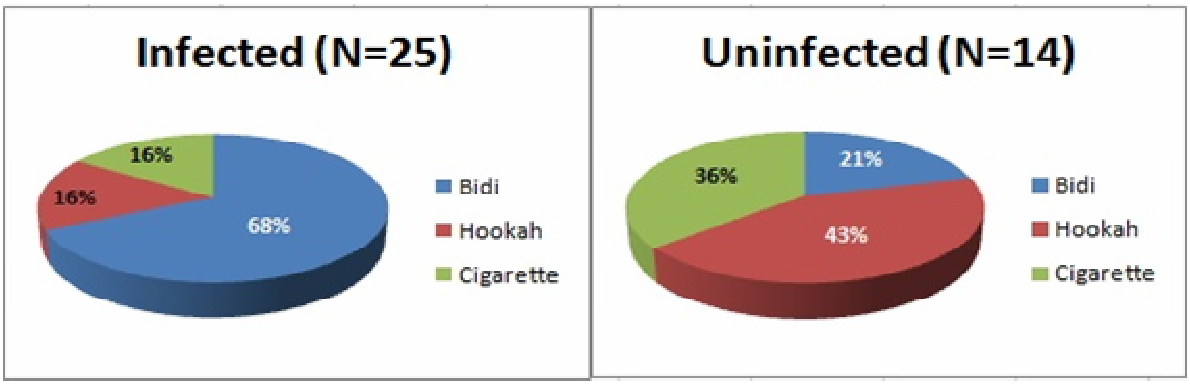


Figure.3 Distribution of Different Source of Smoking in RTIs Uninfected and Infected Women in HIV Positive Women



The use of oral contraceptives showed to be a risk factor for RT1s as it is because of hormonal changes. The cervical infections clearance depends on cellular immunity, and hormones controls the cytokine response. Hormones could play a role in the instigation of CIN3 in women with persistent HPV and cervical infections. Long term use of hormonal contraceptives might be affect the probability that expose to HPV results in cervical infections, it may not clear or persistence of infections which can increase the regression of pro-neoplastic and neoplastic lesions (Smith et al., 2003).

So, the present study demonstrated the higher prevalence of HPV and *Bacterial vaginosis* in HIV positive women and correlation of these RTIs with demographic factors and CD4 count. This study is a pointer for immunosuppressed women who are at risk to catch any infection easily mainly HPV and *Bacterial vaginosis*. The finding of this study, can conclude that use of oral/hormonal contraceptives and smoking habit of bidi may be a risk factor for RTIs in immunosuppressed women. This study will help the high risk women to make them aware by proper counseling; about their life style this might be help them at some instant. Future studies may focus on evaluating the role of host genetic factors in the development of RTIs and related complications in HIV infected and uninfected women.

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Reference

Aggarwal R, Sachdeva RK, Naru J, Suri V, Sharma A, and Nijhawan R. 2012. HPV genotyping in north Indian women infected with HIV. Int J Gynecol Pathol 31:475-481.

Ameur A, Meiring TL, Bunikis I, Haggqvist S, Lindau C, Lindberg JH, Gustavsson I, Mbulawa ZZ, Williamson AL, and Gyllensten U. 2014. Comprehensive profiling of the vaginal microbiome in HIV positive women using massive parallel semiconductor sequencing. Sci Rep 4:4398.

Chaudhry U and Saluja D. 2002. Detection of Neisseria gonorrhoeae by PCR using

orf1 gene as target. Sex Transm Infect 78:72.

Dasgupta A and Sarkar M 2008. A study on reproductive tract infections among married women in the reproductive age group (15-45 years) in a slum of Kolkata. 58[6], 518-522.

Ezechi OC, Ostergren PO, Nwaokorie FO, Ujah IA, and Odberg PK. 2014. The burden, distribution and risk factors for cervical oncogenic human papilloma virus infection in HIV positive Nigerian women. Virol J 11:5.

Griffin RG, Wilkinson TH, and Hoff GL. 1999. HIV surveillance: a dynamic, not static, process to assure accurate local data. Sex Transm Dis 26:291-295.

Isaakidis P, Pimple S, Varghese B, Khan S, Mansoor H, Ladomirska J, Sharma N, Silva ED, Metcalf C, Caluwaerts S, Alders P, Ntzani EE, and Reid T. 2013. HPV infection, cervical abnormalities, and cancer in HIV-infected women in Mumbai, India: 12-month follow-up. Int J Womens Health 5:487-494.

Johnson JD, Houchens DP, Kluwe WM, Craig DK, and Fisher GL. 1990. Effects of mainstream and environmental tobacco smoke on the immune system in animals and humans: a review. Crit Rev Toxicol 20:369-395.

Lieberman JA, Moscicki AB, Sumerel JL, Ma Y, and Scott ME. 2008. Determination of cytokine protein levels in cervical mucus samples from young women by a multiplex immunoassay method and assessment of correlates. Clin Vaccine Immunol 15:49-54.

Low AJ, Konate I, Nagot N, Weiss HA, Mabey D, Segondy M, Vickerman P, Meda N, van de PP, and Mayaud P. 2014. Neisseria gonorrhoeae and Chlamydia trachomatis infection in HIV-1-infected women taking antiretroviral therapy: a prospective cohort study from Burkina Faso. Sex Transm Infect 90:100-103.

Massad LS, Xie X, Minkoff H, Darragh TM, D'Souza G, Sanchez-Keeland L, Watts DH, Colie C, and Strickler HD. 2014. Abnormal pap tests and human papillomavirus infections among HIV-infected and uninfected women who have sex with women. J Low Genit Tract Dis 18:50-56.

Mehandru S, Poles MA, Tenner-Racz K, Horowitz A, Hurley A, Hogan C, Boden D, Racz P, and Markowitz M. 2004. Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract. J Exp Med 200:761-770.

Miller MR and Nyirjesy P. 2011. Refractory Trichomoniasis in HIV-positive and HIV-negative Subjects. Curr Infect Dis Rep 13:595-603.

Narayan KM, Chadha SL, Hanson RL, Tandon R, Shekhawat S, Fernandes RJ, and Gopinath N. 1996. Prevalence and patterns of smoking in Delhi: cross sectional study. BMJ 312:1576-1579.

Palefsky JM, Minkoff H, Kalish LA, Levine A, Sacks HS, Garcia P, Young M, Melnick S, Miotti P, and Burk R. 1999. Cervicovaginal human papillomavirus infection in human immunodeficiency virus-1 (HIV)-positive and high-risk HIV-negative women. J Natl Cancer Inst 91:226-236.

Patel AL, Sachdev D, Nagpal P, Chaudhry U, Sonkar SC, Mendiratta SL, and Saluja D. 2010. Prevalence of Chlamydia infection among women visiting a gynaecology outpatient department: evaluation of an in-house PCR assay for detection of Chlamydia trachomatis. Ann Clin Microbiol Antimicrob 9:24.

Philip PS, Benjamin AI, and Sengupta P.

2013. Prevalence of symptoms suggestive of reproductive tract infections/sexually transmitted infections in women in an urban area of Ludhiana. Indian J Sex Transm Dis 34:83-88.

Poppe WA, Drijkoningen M, Ide PS, Lauweryns JM, and Van Assche FA. 1996. Langerhans' cells and L1 antigen expression in normal and abnormal squamous epithelium of the cervical transformation zone. Gynecol Obstet Invest 41:207-213.

Rao JV, Ganguly NK, Mehendale SM, and Bollinger RC. 2004. India's response to the HIV epidemic. Lancet 364:1296-1297.

Remis RS, Liu J, Loutfy M, Tharao W, Rebbapragada A, Perusini SJ, Chieza L, Saunders M, Green-Walker L, and Kaul R. 2013. The epidemiology of sexually transmitted co-infections in HIV-positive and HIV-negative African-Caribbean women in Toronto. BMC Infect Dis 13:550.

Sahasrabuddhe VV, Bhosale RA, Joshi SN, Kavatkar AN, Nagwanshi CA, Kelkar RS, Jenkins CA, Shepherd BE, Sahay S, Risbud AR, Vermund SH, and Mehendale SM. 2010. Prevalence and predictors of colposcopic-histopathologically confirmed cervical intraepithelial neoplasia in HIV-infected women in India. PLoS One 5:e8634.

Sambrook Ra. 2001. Molecular Cloninig: A laboratory manual. Cold Spring Harbor Laboratory Press 3.

Scheurer ME, Tortolero-Luna G, and dler-Storthz K. 2005. Human papillomavirus infection: biology, epidemiology, and prevention. Int J Gynecol Cancer 15:727-746.

Sha BE, Zariffard MR, Wang QJ, Chen HY, Bremer J, Cohen MH, and Spear GT. 2005. Female genital-tract HIV load correlates inversely with Lactobacillus species but positively with bacterial vaginosis and Mycoplasma hominis. J Infect Dis 191:25-32.

Sharma S and Gupta B. 2009. The prevalence of reproductive tract infections and sexually transmitted diseases among married women in the reproductive age group in a rural area. Indian J Community Med 34:62-64.

Singhal P, Hussain S, Thakur N, Batra S, Salhan S, Bhambani S, and Bharadwaj M. 2013. Association of MDM2 and p53 polymorphisms with the advancement of cervical carcinoma. DNA Cell Biol 32:19-27.

Smith JS, Green J, Berrington de GA, Appleby P, Peto J, Plummer M, Franceschi S, and Beral V. 2003. Cervical cancer and use of hormonal contraceptives: a systematic review. Lancet 361:1159-1167.

Vandenhoudt HM, Langat L, Menten J, Odongo F, Oswago S, Luttah G, Zeh C, Crucitti T, Laserson K, Vulule J, and Buve A. 2013. Prevalence of HIV and other sexually transmitted infections among female sex workers in Kisumu, Western Kenya, 1997 and 2008. PLoS One 8:e54953.

Wasserheit JN. 1992. Epidemiological synergy. Interrelationships between human immunodeficiency virus infection and other sexually transmitted diseases. Sex Transm Dis 19:61-77.

WHO Global. http://www.who.int/reproductivehealth/topics/rtis/en/.

Wolf R and Freedman D. 2000. Cigarette smoking, sexually transmitted diseases, and HIV/AIDS. Int J Dermatol 39:1-9.