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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 13 (19): 951-955, 2010
ISSN 1028-8880

Human Papillomaviruses Prevalence and Genital Co-infections in HIV-seropositive Women in Ouagadougou (Burkina Faso)

[1,2]T. Sagna, [1,2]F. Djigma, [1,2]M. Zeba, [1,2]C. Bisseye, [1,3]S.D. Karou, [1,2]D. Ouermi,
[1]V. Pietra, [1,2]C. Gnoula, [1]K. Sanogo, [1,2]J.B. Nikiema and [1,2]J. Simpore
[1]Biomolecular Research Center Pietro Annigoni,
CERBA/LABIOGENE-Saint Camille Medical Center, 01 BP 364 Ouagadougou 01, Burkina Faso
[2]University of Ouagadougou, 07 BP 5252 Ouagadougou-Burkina Faso
[3]Ecole Supérieure des Techniques Biologiques et Alimentaires (ESTBA-UL),
Université de Lomé, BP 1515, Lomé, Togo

Abstract: The vaginal swabs among HIV-positive women in Africa often revealed opportunistic infections such as human Papillomavirus (HPV) and *Mycoplasma* that induce respectively cervix cancer and diseases such as vaginosis, abortions, infertility in through salpingitis. The purposes of this study were to: (1) seek for, the prevalence of pathogens such as HPV and *Mycoplasma*; (2) characterize the strains of HPV and estimate their prevalence; (3) identify among these women, those who were co-infected by these pathogens in order to cure them. From February 2009 to January 2010, 156 HIV-positive women attending our medical centers and aged from 19-45 years (mean age 33.65±5.75 years) had voluntarily accepted vaginal specimen's tests. PCR, ELISA and molecular hybridization were used for the identification and characterization of these pathogens. The results revealed the presence of *Mycoplasma* and HPV in 25.64 and 58.33% cases, respectively. The following HPV genotypes and the following prevalence were recorded: HPV-50'S (24.11%), HPV-18 (21.28%), HPV-30'S (18.44%) and HPV-16 (5.67%). The study also enable the identification of co-infections such as HPV-18 strains with HPV-30'S (5.67%) and HPV-30'S with HPV-50'S (3.55%). Other germs infecting the female genital tract including *Candida albicans* (20.51%), *Escherichia coli* (12.18%), *Treponema pallidum* (3.85%), *Streptococcus agalactiae* (3.21%) and *Staphylococcus aureus* (1.92%) were isolated. This preliminary research work showed the incidence of several genital pathogens, this could be a springboard for nationwide epidemiological study on HPV strains circulating in Burkina Faso.

Key words: Human papillomavirus, *Mycoplasma*, HIV, women, Burkina Faso

INTRODUCTION

The immunodeficiency caused by HIV infection, raises the problem of opportunistic infections (OIs). Indeed, women infected with HIV are mainly exposed to infections with human Papillomavirus (HPV), *Mycoplasma*, *Candida albicans* and *Escherichia coli* (Moradi and Talat, 2006). The risk of HPV infection increases along with the level of immunodeficiency and therefore the risk to develop cervix cancer (Spano *et al*., 2005). HPV cervix cancer is the second most common cancer worldwide and the first in Sub-Saharan Africa. The use of High Active Antiretroviral therapy (HAART) has reduced significantly the mortality rate among patients infected with HIV. However, in spite of better control of viral replication and immunity by HAART, the emergence of co-infection among patients infected with HIV has become a serious cause of morbidity and mortality (Spano *et al*., 2006).

HIV-positive women have in general *Mycoplasma* infection (Irwin *et al*., 2000; Bebear and Bebear, 2007) those are also isolated in the cases of bacterial vaginosis (Anukam and Reid, 2007). *Mycoplasma* pathogens that generally infect the female genital tract are mainly: *Mycoplasma hominis*, *Ureaplasma urealyticum* and *Mycoplasma genitalium* (Judlin, 2003).

The human Papillomaviruses (HPV) are viruses that infect skin and mucous. Genital HPV are sexually transmitted and they are the most common cause of sexually transmitted infections (STI) (Louie *et al*., 2008). The clinical manifestations of HPV depend on viral genotype and include skin lesions, mucosal benign

Corresponding Author: J. Simpore, Directeur du Centre de Recherche Biomoléculaire Saint Camille/CERBA/LABIOGENE
Université de Ouagadougou 01 BP 364 Ouagadougou 01, Burkina Faso
Tel: +22670230792 Fax: +22650363242

(verruca vulgaris, plantar warts, flat warts, anogenital warts, genital warts, epidermodysplasia verruciformis and laryngeal papillomas), cervical intraepithelial neoplasia and cervix cancers (Heard, 2005). Among the 500,000 new cases of cancer of the cervix diagnosed each year, 80% are women living in developing countries (Cutts *et al*., 2007). Cervix cancer, due to HPV, is the second most common cancer, after the breast cancer in women; and in terms of mortality, it is the second leading cause of cancer deaths in women worldwide (Pyeon *et al*., 2009).

HPV genotypes are classified according to their nucleotide sequence homology. The ranking is based on comparisons of specific genomic regions (E6, E7 and L1) (Spano *et al*., 2005). Currently, more than 100 genotypes, including those with anogenital tropism (approximately 40), have been identified. They are classified according to their oncogenic potential in high-risk genotype (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) or low risk (6, 11, 26, 34, 40, 42-44, 53-55, 62, 66) (Santin *et al*., 1999). The prevalence of genotypes involved in cervical cancer varies according to geographic regions (Louie *et al*., 2008). However, the human Papillomavirus type 16 (HPV-16) are the most common genotype in the world. Porting these germs could possibly complicate immunocompromised patient state.

Thus this study aimed to: (1) seek for the prevalence of pathogens such as HPV, *Mycoplasma hominis*, *Candida albicans*, *Escherichia coli*, *Streptococcus agalactiae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Trichomonas vaginalis*, *Treponema pallidum*, (2) determine HPV genotypes in Burkina Faso and their prevalence (3) identify HIV-positive women, co-infected by these pathogens for appropriated medical care.

MATERIALS AND METHODS

Patients and the study: This study was conducted at the Saint Camille Medical Center (CMSC) and the Center for Biomolecular Research Pietro Annigoni (CERBA/LABIOGENE), University of Ouagadougou, Burkina Faso. From February 2009 to January 2010, 156 HIV-positive women attending the CMSC and CERBA and aged from 19-45 years old (average age of 33.65±5.75 years) freely accepted to participate to the study. Indeed, each woman was asked to sign a consent form certifying her agreement with the form which was edited to explain the design and the importance of the study. Pregnant women and virgins were not included.

Sample collection: From each woman, three endocervical swabs were prepared. The first swab was used for bacterial cultivation and identification. The second swab was used for microscopic observation of fresh and stained sample. The third swab was used for molecular detection of HPV by PCR / hybridization.

Mycoplasma and other bacteria detection

Mycoplasma identification: Mycoplasmas were identified using the kit Mycoplasma system plus of Diagnostic Liofilhem ®). The vaginal swab was plunged into 7 mL of saline solution provide by the manufacturer. The obtained suspension was used to inoculate 24 well microplates (0.2 mL well) and plates were incubated at 37°C for 24 to 48 h prior to *Mycoplasma* identification. The turn colour (from yellow to red) of the wells during the alkalinization of medium after 24 or 48 h of cultivation at 37°C enables the identification and enumeration of Mycoplasma denominated in units of colour change per milliliter (CCU mL^{-1}) and from 0 to > 105 CCU mL^{-1}.

Other germs identification: The above suspension was used to inoculate muller hinton, Sabouraud and chocolate agar + polyvitex agar plates. These media were incubated at 37°C for 24 h. Positive cultures were characterized by determining their biochemical characteristics according to conventional methods used in the center.

PCR/Hybridization for HPV identification

DNA extraction: Viral DNA was extract from each sample using a kit Instant Virus DNA of bio® analytkjena solutions by following the protocol provided by the manufacturer.

PCR/hybridization: The DNA obtained was used for the detection of HPV genotypes using a kit "HPV Star blotting Diatech® using nitrocellulose strips. This kit can detect HPV genotype following: 16, 18, 30'S (31, 33, 35, 39), 45, 50'S (51, 52, 53, 56, 58, 59), 6, 11 and other HPV genotype high-risk and low risk.

Ethical committee: The Committee of Ethics of the CMSC and CERBA made sure that each person provided an informed consent before sample was taken for this study.

Statistical analysis: Demographic and clinical profiles were recorded on computer files and analyzed by standard software SPSS-10 and EpiInfo-6. Statistical significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Clinical records Analysis allowed us to show that 95/156 (60.90%) women in the study did not exceed the

Table 1: Level of study, occupation and condoms use by diagnosed women

	Study level			Occupation		
Condoms use	Without level	Primary	College and higher school	Housewives	Traders	Employee
No[1] 38	34.04% (16/47)	35.42% (17/48)	27.87% (17/61)	32.48% (38/117)	41.18% (7/17)	22.73% (5/22)
Yes[2] 82	65.96% (31/47)	64.58% (31/48)	72.13% (44/61)	67.52% (79/117)	58.82% (10/17)	77.27% (17/22)
Total (156)	47/156 (30.13%)	48/156 (30.77%)	61/156 (39.10%)	117/156 (75.00%)	17/156 (10.90%)	22/156 (14.10%)
P: 1→2	=0.002	= 0.004	<0.001	<0.001	=0.303(NS)	<0.001

Table 2: Carriage of Mycoplasma and HPV according to the age groups

	Mycoplasma carriage			HPV carriage		
Age	Negative	Positive	p-value	Negative	Positive	p-value
X<30	33.62% (39)	27.5% (11)	0.001	32.31% (21)	31.87% (29)	<0.01
X>30	66.38% (77)	72.5% (29)		67.69% (44)	68.13% (62)	
Total N = 156	(116)	40/156		(65)	91/156	
		25.64%			58.33%	

Table 3: Other genital infectious microorganisms isolated from diagnosed Women

Germ	Number	Percentage
Candida albicans	32/67	47.76
Escherichia coli	19/67	28.36
Treponema pallidum	6/67	08.96
Streptococcus agalactiae	5/67	7.46
Staphylococcus aureus	3/67	4.48
Enterobacter sp.	1/67	1.49
Klebsiella pneumoniae	1/67	1.49
Klebsiella oxytoca	0/67	0.00
Trichomonas vaginalis	0/67	0.00
Staphylococcus epidermidis	0/67	0.00

Table 4: HPV genotypes among the most experienced the women of the study

HPV genotypes	Number	Percentage
50'S	34	24.11
18	30	21.28
30'S	26	18.44
16	8	5.67
6	6	4.25
18+30'S	8	5.67
18+50'S	6	4.25
30'S+50'S	5	3.55
45	4	2.84
16+18	3	2.13
HR (High risk)	8	5.67
LR (Low risk)	3	2.14
Total	141	100.00

level of primary school and rarely used condoms 33/156 (21.15%). Table 1 shows that 39.10% of women had a higher level of study. 77.27% of employed women were using prophylactic against 67.52% among housewives and 58.82% among traders.

We detected the presence of Mycoplasma into 25.64% [19.15 to 33.36] women of the study. There was statistically significant difference between the presence of Mycoplasma and the age groups of women ($p<0.001$). According to Table 2 women below 30 years old had a low proportion in our sample (50/156 = 32.05%).

The rate of HPV infection in this group compared to the infected population was 31.87% (29/91). The HPV test showed that 58.33% (91/156) [50.17 to 66.08] of women were infected.

Despite *Mycoplasma* and HPV, women were also diagnosed for the presence of genital infectious microorganisms such as *Candida albicans*, *Escherichia coli*, *Treponema pallidum*, *Streptococcus agalactiae*, *Staphylococcus aureus* (Table 3). According to our results there was neither *Trichomonas vaginalis*, *Staphylococcus epidermidis* nor *Klebsiella oxytoca*.

The present study revealed that the following HPV strains HPV-50'S (26.77%); HPV-18 (23.62%); HPV-30'S (20.47%) and HPV-16 (6.30%) were the most common in the diagnosed women. The study also pointed out co-infections such as HPV-30 strains with HPV-50'S (3.55%); HPV-18 with HPV-30'S (5.67%) (Table 4).

Among HPV infected-women, 3.30% were co-infected with Mycoplasma and *Candida*; while, this co infection rate raised to 6.15% among HPV negative women (Table 5).

This research revealed that HIV-positive women suffered from many gynecological infections. In our study, among the 156 HIV-positive, 117 (75.00%) were infected with at least one of the following germs: HPV, *Mycoplasma* and *C. albicans*. While, Minkoff *et al.* (1999) showed in their investigation that 46.9% of HIV-infected women had at least one gynecological problem that required medical care.

Thus, candidiasis are an important cause of morbidity in immunocompromised patients such as HIV infected-patients (Machet *et al.*, 2006). Other germs such as *E. coli*, *T. pallidum*, etc., which are often found in cases of vaginosis, have been identified in our study with relatively low prevalence rates. However, we isolated neither *Trichomonas vaginalis*, *Klebsiella oxytoca* nor *Staphylococcus epidermidis*, which are often responsible for vaginal infections. Among these women *Mycoplasma* prevalence was 25.64%, similarly (26.7%) by Djigma *et al.* (2008) obtained 26.7% in Ouagadougou and Mamadou *et al.* (2006) 30.9% in Niamey among sex workers. However, the carrier rate in our sample is higher than that reported in Ivory Coast (20%) (Faye-Kette *et al.*,

Table 5: Co-infection rate of *Mycoplasma* with *C. albicans* among women HPV positive and negative

Infection	Co-infection	N	Frequency observed	Frequency expected	No. expected	p-value
HPV+	*Mycoplasma/C. albicans*	3	3/91 = 3.30%	23/91 = 0.253 17/91 = 0.187 0.253X0.187 = 4.73%	4	1.00 (NS)
HPV-	*Mycoplasma/C. albicans*	4	4/65 = 6.15%	16/65 = 0.246 15/65 = 0.231 0.246X0.231 = 5.68%	4	0.72 (NS)
Total		7			8	

2000) and in Bangui (92,0%) (Rapelanoro *et al*., 1998). The probable reason of the low prevalence of mycoplasmas in the sample is the fact that our diagnosed women are under consistent follow up; since they are HIV infected subjects, many attention is paid to prevent opportunistic infections. Indeed, although no statistically significant difference existed, the HAART and antibiotics taking seems to have an effect on Mycoplasma carriage among these HIV-positive women (p>0.050). We observed in our study a high prevalence of *C. albicans* (20.51%) (Table 3). A low prevalence (14.2%) was previously found in Burkina Faso (Meda *et al*., 1995; Djigma *et al*., 2008). *Candida albicans* is the main yeast species that occurs in 80% of the population. The infected subjects often develop no particular symptom, indeed the microorganism behaves as a commensal saprophyte. However, it may cause fungal infections (candidiasis) mainly mucosal digestive and gynecological in certain conditions.

The study on human Papillomavirus allowed us to determine the prevalence of HPV among HIV-positive women (58.33%). This rate is comparable to that reported by Ouermi (2009) (52.66%) in Ouagadougou. However, our prevalence is lower compared to other studies carried out in Burkina Faso among HIV-positive women, in Brazil, San Paolo (Goncalves *et al*., 2008), in Rwanda (Singh *et al*., 2009) and in South Africa in Johannesburg (Firnhaber *et al*., 2009). Our results indicated that 91/156 women were predisposed to develop cervix cancer. Among the HPV positive 95.51% had a high risk for developing cervix cancer. According to present results, HPV-50, 18, 30'S and 16 strains are most experienced in the sample.

An increased prevalence of infection with human Papillomavirus (HPV) and precancerous lesions and cancer was observed among women infected with HIV. (Larsen, 1995; Monsonego, 2007; Louie *et al*., 2008). Indeed, HPV clearly play an important role in the pathogenesis of many neoplastic processes, some being considered low-risk oncogenic (HPV6, HPV11) also called LR-HPV (for Low Risk HPV) and others as responsible benign tumors (warts), and others as being at high risk oncogenic (HPV16, 18, 31, 45) named HR-HPV (for High Risk HPV) implicated in various cancers (head and neck, cervix). Although HPV infection have a key role in the occurrence of a malignant process, other factors seem necessary, as immunity (Heard, 1999; Spano *et al*., 2005). Indeed, the natural history of HPV infection is impaired in individuals infected with HIV and there is an increased risk of persistent HPV infections in this population. The HIV-positive women in particular those who are severely immunodeficients are five times more likely to contract HPV than HIV-negative women (Smits *et al*., 2005; Dames *et al*., 2009).

We isolated any viral and bacterial co-infections in our sample. However, we did not found statistically significant difference between the calculated and observed frequencies neither among HPV positive women co-infected with Candida and Mycoplasma (p = 1) or those HPV negative co-infected with *Candida* and *Mycoplasma* (p = 0.72) (Table 5).

This preliminary research showed results that have impact on public health and could be a springboard for a testing campaign and for epidemiological surveillance of *Mycoplasma* and different types of HPV strains circulating in Burkina Faso. The evidence of the high prevalence of papillomaviruses infection in our country, leads us to propose the introduction of specific vaccines against HPV strains for adolescents.

ACKNOWLEDGMENTS

The Authors are grateful to the staff of Saint Camille laboratory and CERBA, Ouagadougou. In particular, the skilful and patient collaboration of Mr Oscar Zoungrana, Hermann Somda and Mrs Fatoumata Nana. They are deeply grateful to the Italian Episcopal Conference (C.E.I) and to the RADIM House, Roma, Italy and Doctor Luigi SPARANO for the financial support.

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