

Carriage of high-risk human papillomavirus infection in male subjects in Ouagadougou, Burkina Faso

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HPV, real time PCR, genotypes, Men, Burkina Faso

Abstract

Background Human papillomavirus (HPV) infection is a public health problem in Africa because of its frequency and its various consequences in both men and women. In Burkina Faso, there is little data on the carriage of HPV infection in male subjects. This study aimed to determine the carriage of HPV infection in male subjects in Ouagadougou, Burkina Faso.

Methods This was a cross-sectional study conducted from December 2015 to September 2016. During this study period, a total of 124 male subjects who gave informed consent were recruited as part of a fertility assessment. Semen samples were analyzed by Real-Time PCR using Real TM Quant High Risk Screen HPV kit to detect 14 high-risk HPV genotypes. The Chi square test was used for comparisons.

Results Of the 124 male subjects, 22 were positive for at least one high-risk HPV, with a prevalence of 17.7%. HPV 56 was the most represented genotype with a frequency of 20%, followed by HPV 39 (11%) and HPV 68 (11%), HPV 16 (8%). The genotypes HPV 18, HPV33, HPV35, HPV51 and HPV52 also had the same frequency of 6% each and the HPV 31, 45, 59 and 66 had the same frequency of 3% each. Of the 22 positive cases, 10 were multiple infections. Spermocytogram of patients showed at least one abnormality in 87.9% of cases. Necrozoospermia and oligozoospermia were the most abnormalities observed with 42% and 40% respectively followed by asthenospermia (18%); teratozoospermia (16%) and azoospermia (14%).

Conclusion This study showed that like women, men are infected by HPV with a relatively high prevalence. In addition, the predominant genotypes in the male subjects of this study are not those targeted by the HPV vaccines available in our countries. Hence the need to reinforce the strategies for controlling HPV infection and HPV prophylactic vaccination for young boys.

Background

Human papillomavirus (HPV) is the most common cause of viral infection of the reproductive tract and causes a wide range of conditions in women as men, including precancerous lesions that may progress to cancer [1]. Persistent oncogenic HPV infections have been reported in most cases of cervical cancer and HPV is increasingly associated with penile tumors in humans and in certain anal, cerebral and cervical tumors [2, 3]. More than 200 types of HPV exist and are divided into 2 groups

according to their oncogenic potential: high-risk HPV (HR-HPV) and low-risk HPV (LR-HPV) [4]. HPV 16 and 18 are the most important genotypes of HR-HPV causing cancer worldwide [3, 5] and are implicated in penile cancer and cancer cervix. Especially, HPV 16 is familiar in brain and neck cancers [2].

According to the World Health Organization (WHO), genital HPV infections among men in sub-Saharan Africa have a prevalence of any type, at least 19.1% [6]. Men are considered to be carriers, therefore reservoir and act as transmitters of the virus. Several authors detected HPV DNA in samples from human internal organs such as vasectomy vas deferens, epididymis from non-tuberculous epididymectomy, testicular biopsy of infertile subjects or from specimens from anogenital external organs often combined with urine and sperm which are readily available samples [7-9]. In addition, it is recognized that HPV DNA in urine and sperm positively reflect the presence of HPV in the " internal " reservoirs [10]. Studies have reported that HPV infection affects fertility parameters in humans [11, 12]. In Burkina Faso, previous studies have been carried out in women and reported the frequency of other high-risk HPV genotypes outside genotypes 16 and 18 [13-17]. Since HPV infection is a sexually transmitted infection, it is not surprising that some genotypes are found in both women and men. In order to obtain data on HPV infection in humans in Ouagadougou, Burkina Faso, this study proposes to search for HPV in males visiting a laboratory in Ouagadougou for sperm analysis in the context of a fertility screening.

Methods

Type and population of study

This is a cross-sectional study conducted from December 2015 to September 2016 and involved male subjects who presented themselves to the biomedical laboratory to perform a spermogram and spermocytogram in the study period and gave their free and informed consent.

Collection of samples and data

Male subjects of this study were recruited from three medical clinics: Philadelphie, Sainte Elisabeth and Sandof. Semen samples were collected in a collection tube after 3 to 6 days of abstinence according to the WHO guidelines [18] and transported to the Pietro Annigoni Biomolecular Research

Center (CERBA/LABIOGENE) for the molecular characterization of HPV.

Spermogram and spermocytogram

For each sample, assessment of sperm characteristics and sperm was done to identify the following parameters: the volume of sperm, sperm motility, sperm concentration, sperm morphology and vitality using an optical microscope.

Molecular characterization of high-risk HPV by real-time PCR

Extraction of HPV DNA was done from semen using the DNA-Sorb-A Kit (Sacace Biotechnologie, Como, Italy) according to the manufacturer's instructions. The real-time PCR amplification was carried out with " HPV Genotypes 14 Real-TM Quant" kit which allows the detection of 14 high-risk HPV genotypes of clinical interest that are: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. The amplification was done with the SaCycler-96™ thermocycler (Sacace Biotechnologies, Como, Italy) according to the following program: 95 °C for 15 minutes, for 1 cycle; 95 °C for 05 seconds, 60 °C for 20 seconds and 72 °C for 15 seconds, for 5 cycles; 95 °C for 05 seconds, 60 °C for 30 seconds, 72 °C for 15 seconds for 40 cycles.

Statistical analyzes

The data was analyzed with IBM SPSS software version 21.0. The Chi square test was used for comparisons and any value was considered statistically significant for $p < 0.05$.

Results

Characteristics of the study population

A total of 124 voluntary male subjects participated in this study. The age of the patients was ranged from 21 to 72 years with an average of 37.12 +/- 7.56 years. The male subjects of more than 30 years were the most represented (92.85%). This study did not observe an aspermal case. The mean volume of ejaculate was 2.82 +/- 1.27 mL with a minimum volume of 0.6 mL and the maximum of 7.5 mL.

The average concentration of sperm from the ejaculate was 13,697,011 sperms/ μ L with an average number of 90,140,661 sperms and a maximum to 911 840 1 000 sperms.

Sperm collection patterns in male subjects were masturbation (66.13%) and coitus interruption (33.87%). Subjects aged more than 30 years and over mostly took their samples from the laboratory

by masturbation (56.09%) and coitus interruptus was performed at home in 52.38% of cases (Table 1).

Table 1: Method of sperm collection by place and age of male subjects

Sampling mode		Sampling place			Total n (%)
			Home n (%)	Laboratory n (%)	
Coitus interruptus	Age classes	30 years	1 (2.38)	2 (4.76)	3 (7.14)
		≥ 30 years	22 (52.38)	17 (40.47)	39 (92.85)
		Total	23 (54.76)	19 (45.23)	42 (100)
Masturbation	Age classes	30 years	3 (3.65)	7 (8.53)	10 (12.19)
		≥ 30 years	26 (31.70)	46 (56.09)	72 (87.80)
		Total	29 (35.36)	53 (64.63)	82 (100)

NB: Seven (7) patients did not indicate their ages.

Sperm abnormalities of the subjects of the study

Most subjects in this study had their spermogram and spermocytogram results. They had at least one abnormality in 87.9% of cases. The most observed abnormalities were oligozoospermia and necrozoospermia with 40% and 42% respectively followed by asthenospermia (18%); teratozoospermia (16%) and azoospermia (14%).

Prevalence and distribution of high-risk HPV in male subjects

In this study, the prevalence of high-risk HPV infection was 17.7% in male subjects. Of the 14 genotypes sought, 13 were identified with frequencies ranging from 3 to 20% (Figure 1). The most common genotypes were HPV 56, HPV 31, HPV39 and HPV68; follow by of HPV 16, HPV 18, HPV 33, HPV 35, HPV 51, HPV 52, HPV 45, HPV 59 and HPV 66. HPV 16 and HPV 18 genotypes had a cumulative frequency of 14% and other HR-HPV accounted for 86%.

Carriage of HR-HPV infection according to sperm abnormality in male subjects

HR-HPV was found in 17.59% of men with sperm abnormality. Table 2 shows the frequency of sperm abnormality according to the presence or absence of infection with high-risk HPV.

Table 2: Sperm abnormality according to the presence or absence of high-risk HPV

	Sperm abnormality N (%)	Absence of anomaly N (%)	Total N (%)	p va
Positive HPV-HR	19 (17.59)	3 (18.75)	22 (17.74)	0.57
Negative HPV-HR	89 (82.41)	13 (81.25)	102 (82,26)	
Total	108 (100)	16 (100)	124 (100)	

Different types of sperm abnormalities according to HR-HPV genotypes in isolated or multiple infections are presented in Table 3. Oligospermia and necrospermia were more detected in male subjects having HPV 56.

Table 3: Isolated or multiple HR-HPV infections and sperm abnormalities

HPV-HR genotypes	Total (%)	Normal (%)	Oligospermia (%)	Asthenospermia (%)	Necrospermia (%)	Teratospermia (%)	Cryptospermia (%)	Azoospermia (%)
HPV 16, HPV 31	1 (5)	-	1 (10)	-	-	-	-	-
HPV 18	2 (9)	-	2 (20)	-	2 (18)	1 (50)	-	-
HPV 31, HPV 51	1 (5)	-	-	-	-	-	1 (100)	-
HPV 31, HPV 51, HPV 58	1 (5)	-	-	-	1 (9)	1 (50)	-	-
HPV 31, HPV 59	1 (5)	1 (33)	-	-	-	-	-	-
HPV 33	1 (5)	-	-	-	-	-	-	1 (33)
HPV 33, HPV 56	1 (5)	1 (33)	-	-	-	-	-	-
HPV 35	1 (5)	-	-	-	-	-	-	1 (33)
HPV 39	3 (14)	-	1 (10)	-	2 (18)	-	-	1 (33)
HPV 39, HPV 52	1 (5)	-	-	1 (50)	1 (9)	-	-	-
HPV 45, HPV 68	1 (5)	-	-	-	1 (9)	-	-	-
HPV 52, HPV 35, HPV 68	1 (5)	-	-	-	-	-	-	-
HPV 56	5 (23)	-	5 (50)	1 (50)	3 (27)	-	-	-
HPV 66, HPV 68	1 (5)	-	1 (10)	-	1 (9)	-	-	-
HPV 68, HPV 33, HPV 56	1 (5)	1 (33)	-	-	-	-	-	-
Total (%)	22 (100)	3 (100)	10 (100)	2 (100)	11 (100)	2 (100)	1 (100)	3 (100)

Discussion

This study was the first in Burkina Faso to detect the carrying of HPV infection in male subject.

Masturbation was the most commonly used method of semen collection especially in subjects over 30 years of age. This study reported a prevalence of HR-HPV at 17.7%. It is higher than 12.48% found in a general male population in China [19] and 16% found in Denmark in sperm donors [20]. This could be explained by the poor knowledge of the means of prevention and transmission of HPV infection by the general population of Burkina Faso. Also, many people think that HPV infection only

concerns women and ignore that males can be infected. Thirteen (13) HR-HPV genotypes were detected among which HPV 56 was the most represented genotype with a prevalence of 20%, followed by HPV 39 (11%) and HPV 68 (11%), HPV 16 (8%) and HPV 18 (6%). HPV infections were isolated or multiple. The HR-HPV genotypes found in this study were the same as those reported from sperm derived from duct biopsy with high prevalence of 31% in Finland [21]. In contrast to this study where HPV 16 and 18 were less represented, these two genotypes were predominant in other studies conducted in Amsterdam and Brazil [12, 22]. Our results are consistent with those of other authors who found that HPV 16 and 18 genotypes are more common in Europe, America and Africa [14, 16, 23–25]. Male fertility depends on parameters such as total and progressive mobility, morphology, vitality, number and concentration of spermatozoa that are determined during the spermogram and from which sperm abnormalities can also be identified according to the standards of the WHO manual version 2010 [18]. The presence of sperm abnormality can lead to male infertility. In this study, HR-HPV were found in 19 cases of sperm abnormality against 3 cases in absence of sperm abnormality. This shows that HPV infection may be a factor affecting male fertility and can weaken sperm quality including progressive mobility, morphology leading to a decline in male fertility or even cause infertility [13, 26, 27]. Indeed, Foresta et al. believe that HPV infection can have the same mechanisms as the other viruses that infect sperm and are localized in the equatorial region of the head or tail of the sperm to decrease the function and capacity of acrosome, thus affecting gamete fusion [11].

This study showed that male with the HPV 56 genotype (23%) had oligospermia (50%) and necrospermia (27%) and those with the HPV 18 genotype (9%) had oligospermia and necrospermia respectively in 20% and 18% of cases. In contrast, oligospermia was found in male with only HPV 16. Nasser et al., had also shown a relationship between HPV 16, 18, 45 and 52 with oligospermia and azoospermia [27]. The teratospermia cases in this study involved HPV genotypes 18, 31, 51, and 58. Lai et al., found that sperm mobility was significantly affected in HPV 16 and 18 infections [28]. In this same study, the incidence of asthenospermia in HR-HPV infected patients was significantly elevated compared with those who did not have HPV in sperm (75% versus 8%). However, in about 50% of

infertile men, the etiology remains unknown and is called idiopathic infertility, presenting only oligospermia, asthenospermia, teratozoospermia or other sperm abnormalities [29].

The HPV genotypes found in the male subjects in this study are the same as those reported in other studies in women in Burkina Faso [15–18]. HPV is therefore one of the most common sexually transmitted viruses in men and women [29]. Indeed, previous studies have shown that more than 50% of sexually active men or women will be infected with HPV in their lifetime, and that the risk of infection could be increased due to multiple sexual partners, unprotected sexual intercourse and smoking [30–32]. The population of Burkina Faso being very young, it is likely to be confronted with these problems. Therefore, HPV infection in men and women could increase if, however, the activities of awareness and prevention are not strengthened. It should be noted that although HPV infection is found in men and that the characteristics of infection types and histological distributions are similar to those of women, most HPV infections in men are benign [18]. HPV vaccination remains the important strategy to prevent infection but must take in count the most represented genotypes particularly in Africa context.

Conclusion

This study determined for the first time, the prevalence of HR-HPV and the distribution of HPV genotypes in male subjects in Burkina Faso. HPV-HR infection was higher in subjects with sperm abnormalities and could be responsible for lower fertility or male infertility. The identified HR-HPV genotypes are the same as those found in women, thus showing the chain of transmission between male and female sex partners.

List Of Abbreviations

DNA, Deoxyribonucleic; HPV, Human Papillomavirus; PCR, Polymerase Chain Reaction; WHO, World health Organization.

Declarations

Ethical considerations

The study was approved by the Institutional Research Ethics Committee of CERBA/LABIOGENE and the Ethical Committee of Saint Camille Hospital in Ouagadougou, Burkina Faso and by the clinical authorities where the sampling has been done. Written and informed consent was obtained

from all the participants enrolled in the study.

Competing interests

The authors declare they have no competing interests

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Authors' contributions

SFBT collected data, performed the experiments and drafted the manuscript; TMZ, STS, IMAT performed the analysis and interpreted the data and revised the manuscript; AKO, DO, RAO, FWD revised the manuscript; CO and JS designed the study. All authors gave their final approval of the manuscript.

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Figures

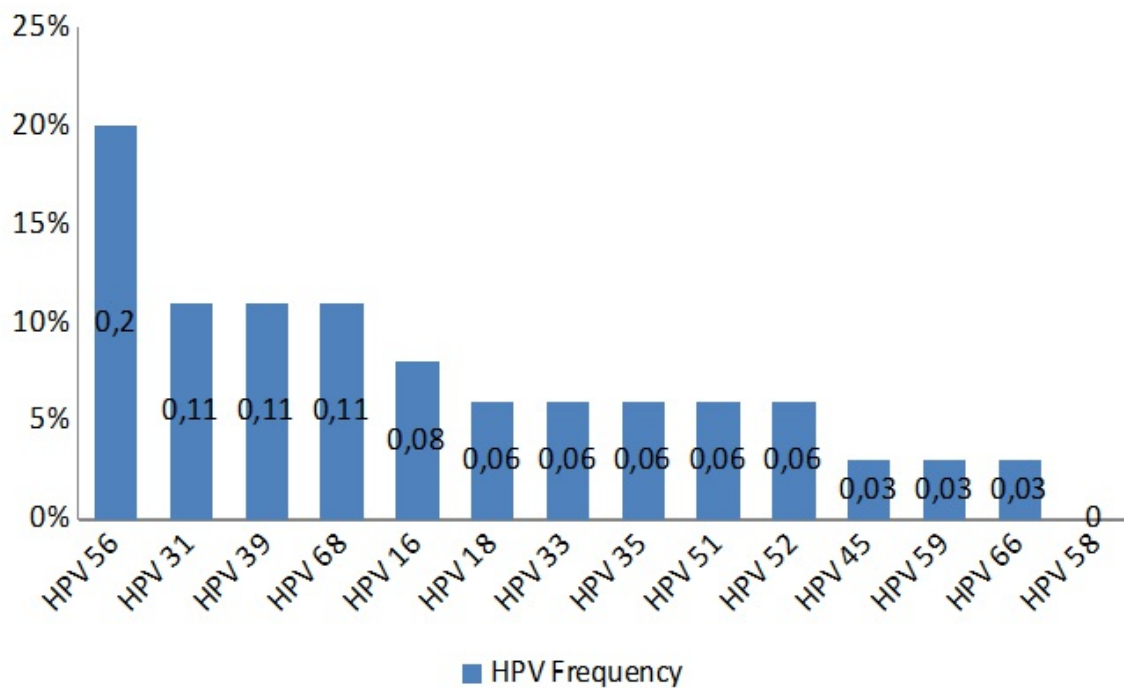


Figure 1

Frequency of high-risk HPV genotypes in male subjects