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Detection of Human Papillomavirus from Sperm Impaired Male Patients Presented at Reproductive Health Clinic of Ahmadu Bello University Teaching Hospital, Zaria, Nigeria

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Abstract

Human papillomavirus (HPV) is the most common sexually transmitted infection worldwide, which affects both males and females. Much research on HPV has focused on women, but men are equally affected. This study aimed to determine impairment of sperm parameters and detect HPV genome among patients presented at the Reproductive Health Clinic of Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. Semen samples from 156 male patients were processed, and the study found an overall prevalence of 6.7% of HPV among male patients. The presence of the HPV genome was confirmed using the GeneXpert system. Semen quality examination revealed the occurrence of 41.7% abnormal sperm motility and 38.5% abnormal sperm morphology. A total of 41.0% of the patients had low sperm count (0-37.9 x 10^6 mL). The study on the prevalence of HPV among men with reproductive impairment serves as a valuable addition to the knowledge available to the general public. It sheds light on an underexplored aspect of HPV's impact on male reproductive health, encourages open dialogue about sexual health, and reiterates the importance of preventive measures like vaccination and also emphasises the importance of awareness of potential consequences of HPV infection, safe sexual practices, and vaccination for those planning parenthood.

Keywords: HPV, Semen, Genome, GeneXpert system

INTRODUCTION

Human Papilloma Virus (HPV) is a non-enveloped Deoxyribonucleic Acid (DNA) virus belonging to the family Papillomaviridae(Aminu *et al.*, 2014 and Palatnic *et al.*, 2024). This family includes more than 130 genotypes, many of which infect the mucosal areas of the human upper digestive tract and the anogenital region through sexual contact, leading to an increased risk of cancer development (Haruna, 2017 and Han *et al.*, 2024). Papillomavirus is a small icosahedral-shaped virus of approximately 50-60 nm in diameter. It contains a circular, double-stranded DNA genome (7000-8000 bp) that exists in a chromatinized state (Doorbar *et al.*, 2019 and Evande *et al.*, 2023). There are 16 different genera, which are divided into four major levels known as alpha, beta, gamma and delta (according to a phylogenetic analysis among the sequences of 118 Papillomavirus types) (Gayathri *et al.*, 2016 and Cosper *et al.*, 2021). Human Papilloma Virus (HPV) infection is one of the most common sexually transmitted infections worldwide (Wolf *et al.*, 2024), and can be divided into two different groups according to their ability to induce malignancy. Low-risk types are responsible for benign lesions such as genital warts and respiratory papillomatosis, whereas persistent infection with high-risk types can lead to malignant transformations in both the anogenital and aero-digestive regions (Dunne and Park, 2013 and Sung *et al.*, 2020).

Transmission of HPV usually occurs through sexual intercourse, and its prevalence in sexually active, healthy young women and men ranges from 20% to 60% and from 45% to 50%, respectively (Han *et al.*, 2017; Soheil *et al.*, 2021 and Milano *et al.*, 2023). Also, Garolla *et al.* (2024) described a high prevalence of HPV, ranging between 50% and 70%, in the male penile shaft, glans penis/coronal sulcus, and semen, as well as in scrotal, perianal, and anal regions. This suggests a possible role for males as reservoirs of HPV infection. Thus, HPV infection is thought to be one of the most common sexually transmitted infections in both genders.

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Human Papilloma Virus infection in females has been associated with adverse pregnancy outcomes in many reports (Souho *et al.*, 2015). On the other hand, the impact of HPV infection on male reproductive parameters is currently under debate (Luttmer*et al.*, 2016; Weinberg *et al.*, 2020). Recent meta-analyses have revealed that HPV is commonly present in semen samples, with an estimated prevalence of 16% in infertile men and 10% in the general population, and has negative effects on sperm concentration, motility and morphology (Weinberg *et al.*, 2020). A previous study reported an association between HPV infection of the penile epithelium and the presence of HPV in semen, suggesting that the presence of HPV in semen may result from exfoliation of HPV infected penile keratinocytes (Luttmer *et al.*, 2015). Moreover, HPV-DNA has been found not only in the penile epithelium, glans, urethra and urothelial cells (Kato *et al.*, 2021), but also in the genital tract, including the ductus deferens, epididymis and testis (Lyu *et al.*, 2017 and Capra *et al.*, 2022). Doorbar*et al.* (2019) demonstrated that HPV16 capsids adsorb to distinct sites on the surface of the head of the sperm at the equatorial segment, suggesting that these sites may mediate HPV binding (Doorbar*et al.*, 2019). However, it remains unclear whether the presence of HPV-DNA in semen indicates that the virus is located inside the sperm cell, in exfoliated urothelial epithelial cells or in the prostatic fluid (Luttmer *et al.*, 2015 and Doorbar *et et al.*, 2019).

The concern about male HPV infection stems from both the disease burden and the potential risk of its transmission from males to females (Orya *et al.*, 2024). To date, the prevalence and incidence of HPV infection in males is much less established compared to females (Lewis *et al.*, 2021). Because male sexual behaviour affects rates of HPV infection and disease in female partners, an improved understanding of the infection in men is an essential component of HPV-related disease prevention (Zou *et al.*, 2022). Substantial progress has been made in the understanding of the efficacy of the interventions for prevention, such as vaccination to reduce HPV-related diseases in women (Milano *et al.*, 2023). However, little is known about HPV infection in men, with only a few small prospective studies undertaken in Europe (Milano *et al.*, 2023), Latin America (Vaccarella et al., 2017), and the USA (Milano, 2023). Human Papilloma Virus infection among Nigeria males has been neglected, and the focus is only on their female counterparts. (Abdulkadir *et al.*, 2017), (Yakubu, 2016) and (Dimie *et al.*, 2013) carried out studies on HPV in Kano, Kaduna, and Zaria, Nigeria, respectively. Therefore, the optimum strategies for the prevention of HPV infection in men are not known.

MATERIALS AND METHODS

Study area

The Samples were collected at the Ahmadu Bello University Teaching Hospital, Zaria, Nigeria, located at latitude 11°04'N. N and longitude 7° 42' E. The hospital is attended by communities such as Shika, Samaru, Sabon Gari, and Funtua, to mention a few.

Study Population/ Inclusion Criteria

All consenting male patients presenting at the reproductive health Clinic of the Ahmadu Bello University Teaching Hospital, Zaria, Nigeria.

Ethical Approval

The Ethical approval was obtained from the Ethical Committee Board of the Ahmadu Bello University Teaching Hospital, Zaria, Nigeria prior to the study.

Data Collection

A well-structured questionnaire was administered to consenting patients to determine the factors that predispose men to HPV infection. The questionnaire carried the following information: alcohol drinking habit, smoking habit, health-related status like hypertension, diabetes, sexual habit (Number of sexual partners), knowledge and current status of STI.

Sample Size

A previous prevalence of 11.5, as reported in a study carried out in Zaria, Kaduna, North Central Nigeria (Dimie et al., 2013), was used to determine the sample size using Fisher's equation. Thus, for the purpose of this research, a total of 156 male patients were enrolled on the study.

Collection and Processing of Samples

Semen samples were obtained from male patients after 3 days of sexual abstinence. After liquefaction at room temperature, semen volume, pH, sperm count, concentration, motility and normal morphology were determined according to the World Health Organisation guidelines for semen analysis

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(WHO, 2021). Half of each sample was placed in a tube containing 2.5 mL of a preservative solution for liquid-based cytology (TACAS Amber; MBL Medical & Biological Laboratories Co., Ltd., Tokyo, Japan) and stored at 4°C in cryopreservation bottles until HPV-DNA measurement. All semen samples with impaired parameters were enlisted for GeneXpert analysis.

GeneXpert HPV Assay Procedure

The samples were obtained from Ahmadu Bello University Teaching Hospital, Shika, Zaria, and transported to the National Tuberculosis and Leprosy Training Centre, Saye, Zaria. All Sample manipulations were done by a Scientist using the Biological Safety Cabinet II.

Samples were allowed to thaw at room temperature. Two millilitres of the sample were pipetted and dispensed into the cartridges. Using the Modules 4 and 16 GeneXpert systems, each cartridge was scanned using a barcode scanner, and then it was loaded on the machine. The bar code scanner validates the cartridge serial number, lot number and expiry dates. It also helps in the selection of the assay type based on the system configuration.

The system then automated the process, including nucleic acid extraction, amplification, and detection. Each cartridge loaded on the GeneXpert machine took 57 minutes to complete the assay, and the data was obtained from the real-time detection and the generated results were displayed on a computer monitor in Microsoft Excel format for further analysis.

Once the assay is completed, the GeneXpert system generates and displays the results. The interpretation of the results, including the identification of HPV genotypes, was done based on the system's software or guidelines provided by the manufacturer.

Statistical Analysis

Data on socio-demographic, risk factors and symptoms of impairment of sperm parameters and HPV infection among 156 male patients presenting at the Reproductive health clinic of Ahmadu bello University Teaching Hospital, Zaria together with laboratory findings were subjected to statistical analyses by Chi Square (x^2) and odd ratio using IBM SPSS version 23 at 95% confidence interval (CI). Final results were simplified using charts and tables.

RESULTS

Out of 156 male patients with a mean age of 37 years presenting at the Reproductive Health Clinics of Ahmadu Bello University Teaching Hospital, Zaria, their semen quality examination revealed an occurrence of 41.7% abnormal sperm motility and 38.5% abnormal sperm morphology. The frequency of men with low sperm count (0-37.9 x 10^6) cells was 41.0% (Table 1). The overall prevalence of HPV among the male patients presenting at the Reproductive Health Clinics of Ahmadu Bello University Teaching Hospital, Zaria was found to be 4(6.7%), while 56 (93.3%) were not infected (Figure 1).

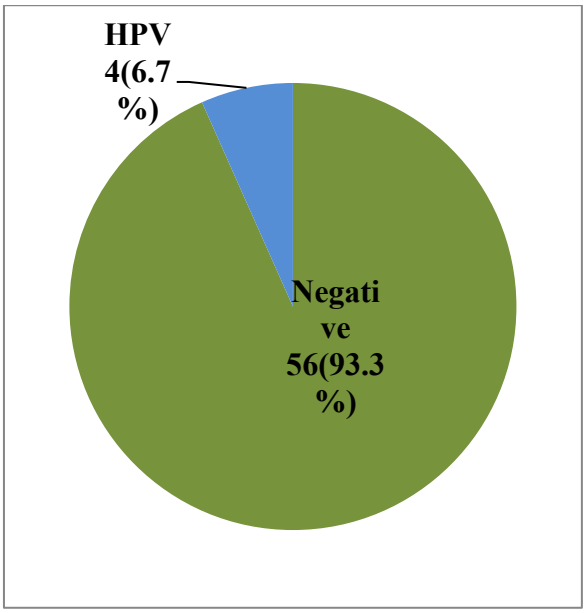

Figure 1: Overall prevalence of HPV among male patients presenting at the Reproductive Health Clinics of Ahmadu Bello University Teaching Hospital, Zaria.

Table 1: Semen quality among male patients presenting at the Reproductive Health Clinics of Ahmadu Bello University Teaching Hospital, Zaria

Sperm parameters n=156	Number positive	Occurrence (%)
Abnormal sperm motility	65	41.7
Abnormal sperm morphology	60	38.5
Low sperm count	64	41.0

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Table 2: Clinical Signs and symptoms of poor semen quality and HPV among male patients presenting at the Reproductive Health Clinics of Ahmadu Bello University Teaching Hospital, Zaria

Sign/symptom	Number examined	Abnormal sperm motility; Number positive (%)	Abnormal sperm morphology; Number positive (%)	Low sperm count; Number positive (%)	n	HPV; Number positive (%)
Difficulty to urinate						
[a]No	142	54(38.0)	50(35.2)	57(40.1)	50	2(4.0)
[b]Yes	14	11(78.6)	10(71.4)	7(50.0)	10	2(20.0)
		x^2=8.618, df =1, P=0.003, [a]OR =0.167; [b]OR =5.975	x^2=7.062, df =1, P=0.008, [a]OR =0.217; [b]OR =4.600	x^2=0.512, df =1, P=0.474, [a]OR =0.671; [b]OR =1.491		x^2=3.429, df =1, P=0.064, [a]OR =6.000; [b]OR =0.167
Difficulty controlling urine flow						
[a]No	145	56(38.6)	52(35.9)	57(39.3)	52	3(5.8)
[b]Yes	11	9(81.8)	8(72.7)	7(63.6)	8	1(12.5)
		x^2=7.850, df =1, P=0.005, [a]OR =0.140; [b]OR =7.152	x^2=5.870, df =1, P=0.015, [a]OR =0.210; [b]OR =4.770	x^2=2.501, df =1, P=0.114, [a]OR =0.370; [b]OR =2.702		x^2=0.505, df =1, P=0.477, [a]OR =2.333; [b]OR =0.429
Painful urination						
[a]No	148	59(39.9)	53(35.8)	59(39.9)	53	3(5.7)
[b]Yes	8	6(75.0)	7(87.5)	5(62.5)	7	1(14.3)
		x^2=3.855, df =1, P=0.050, [a]OR =0.221; [b]OR =4.525	x^2=8.567, df =1, P=0.003, [a]OR =0.080; [b]OR =12.547	x^2=1.607, df =1, P=0.205, [a]OR =0.398; [b]OR =2.514		x^2=0.739, df =1, P=0.390, [a]OR =2.778; [b]OR =0.360
Haematuria						
[a]No	153	63(41.2)	58(37.9)	62(40.5)	58	4(6.9)
[b]Yes	3	2(66.7)	2(66.7)	2(66.7)	2	0(0.0)
		x^2=0.787, df =1, P=0.375, [a]OR =0.350; [b]OR =2.857	x^2=1.028, df =1, P=0.311, [a]OR =0.305; [b]OR =3.276	x^2=0.831, df =1, P=0.362, [a]OR =0.341; [b]OR =2.935		x^2=0.148, df =1, P=0.701, [a]OR =0.931; [b]OR =n.a
Genital warts						
[a]No	153	64(41.8)	58(37.9)	64(41.8)	58	3(5.2)
[b]Yes	3	1(33.3)	2(66.7)	0(0.0)	2	1(50.0)
		x^2=0.087, df =1, P=0.768, [a]OR =1.438; [b]OR =0.695	x^2=1.028, df =1, P=0.311, [a]OR =0.305; [b]OR =3.276	x^2=2.128, df =1, P=0.145, [a]OR =0.582; [b]OR =n.a		x^2=6.244, df =1, P=0.012, [a]OR =18.333; [b]OR =0.055

Significant signs of abnormal semen among the male patients were difficulty urinating (P≤0.05, OR>1), low sperm count (OR>1). However, HPV infection occurred more frequently among

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patients who experienced difficulty urinating (20.0%) (Table 2). In Table 2, significant signs of abnormal semen among the male patients were difficulty urinating, painful urination and difficulty in controlling the flow of urination (P≤0.05, OR>1). Patients who experienced difficulties in urinating, control urine flow, painful urination, and haematuria were more at risk of low sperm count (OR>1). However, a significant sign of HPV infection among them was the presence of genital warts (50.0%; P=0.012) as presented in Table 2.

Occurrence of HPV infection did not significantly affect sperm count (P=0.057), but all the HPV infections were recorded among those with abnormal sperm motility (7.5%; P>0.05). Also, HPV infection occurred highest (100.0%; P=0.000) among those with zero semen leukocyte count (Table 3)

Table 3: Effect of HPV on semen quality of male patients presenting at the Reproductive Health Clinics of Ahmadu Bello University Teaching Hospital, Zaria

Semen parameter	N	HPV. Number positive (%)	x^2	Df	P-value	Odd Ratio (OR)
Sperm count						
0-37.9	57	3(5.3)	3.609	1	0.057	9.000
38-75.9	3	1(33.3)				0.111
Sperm motility						
Abnormal	53	4(7.5)	0.566	1	0.452	0.925
Normal	7	0(0.0)				n.a
Semen leukocyte count						
0	1	1(100.0)				
1-5	42	2(4.8)	14.262	2	0.001	n.a
6-9	17	1(5.9)				

DISCUSSIONS

Numerous epidemiologic studies of genital HPV infection in women have been conducted, but data on the prevalence of HPV among men, especially from Nigeria, are lacking. In concordance with findings of other studies on HPV prevalence in semen samples, both from the general population and men attending fertility clinics, this study revealed a prevalence of 6.7%. This prevalence is low compared to a study by Han *et al*. (2017), who evaluated a total of 1138 male subjects from the general population and found 142 cases of HPV infection in their semen (12.48%).

Prevalence of HPV infection in this study was higher compared to the work of Moghimi *et al*. (2019), who reported an overall prevalence of 5.7% of high-risk HPV DNA infection in semen. However, this study agreed with the findings of Han *et al*. (2017), who also found a prevalence of 6.7% among 523 fertile men. Also, in a cohort study by Luttmer *et al*. (2015) on 199 infertile couples undergoing ART cycles, semen samples of the male partners had a 9.5% HPV infection.

Furthermore, Doorbar *et al*. (2019) conducted a cohort study on 308 idiopathic infertile males and found a prevalence of 7.8% HPV in their semen. This current study also agreed with the findings of Foresta *et al*. (2015), who reported a prevalence of 2-31% HPV infection among the general male population and 10-35% among men with unexplained infertility.

The effect of HPV on sperm parameters is controversial, as the mechanism by which HPV affects sperm quality is still poorly understood. This study recorded the highest occurrence of abnormal sperm motility of 43.8% among men between 37 and 50 years old. Low sperm count was most frequent among men of 37-50 years with 43.8%, but least frequent among the youngest men 23-36 years old (39.1%). Similarly, HPV infection was 7.7% most prevalent among men between the ages of 37-50 years old, followed by 6.1% among the youngest men of 23-36 years old, but absent among the oldest men. The reason behind the high impairment of sperm parameters and a high prevalence of HPV infection among this age group may be due to the fact that men at that age (37 – 50) are at the peak of their sexual activity without recommended periods of abstinence. Sperm count can be significantly reduced by daily ejaculation, especially among male subjects diagnosed with subfertility conditions and seeking reproductive assistance. Abstinence has been shown to improve sperm count and sample

volume when compared to daily ejaculation. World Health Organisation ***suggests that men abstain from ejaculation and sex for a period of 2 - 7 days, as*** total sperm count and seminal volume per ejaculation declined and remained decreased for the duration of the daily ejaculation period (WHO, 2010).

The reasons behind spermatozoa genomic impairments could be several, from abnormalities in DNA packaging during spermatogenesis to aberrant apoptosis and exposure to oxidative stress. Environmental and pathological conditions, such as diseases, drugs, ageing, and infections, are the main triggers (Vaccarelli *et al.*, 2017). Impairment of sperm parameters was higher compared to the report of Han *et al* (2017). Most sexually active adults acquire HPV in their lifetime, and it can occur at any age (Dunne *et al.*, 2014 and Gayathri *et al.*, 2016).

In the current study, HPV decreased significantly total sperm motility, progressive motility, and morphology. In contrast, previous studies on sperm parameters in relation to HPV infection reported no significant changes in sperm parameters, which may explain the conflicting findings reported by Sun *et al.* (2025). However, Han *et al.* (2024) demonstrated that HPV infection in asymptomatic men was associated with low sperm quality and changes in progressive motility. Consistent with the findings of this study, the majority of previous studies observed reduced sperm motility in men infected with HPV, as demonstrated by Garolla *et al.* (2024). These data suggested a possible role for HPV in the impairment of sperm parameters, and the difference in prevalence might be due to the use of different diagnostic tests to detect HPV.

Based on the Number of sex partners in this study, men who had three sex partners were found with the highest occurrences of 50.0% each of abnormalities of sperm motility and morphology and low sperm count, than those with lower numbers of sex partners. This was, however, lower as compared with the findings of Sweatt *et al.* (2001), who reported 87% in men who had 5 sex partners, and consistent with the findings of Dunne and Pack (2013). In individual-based studies, the Number of sexual partners is consistently the strongest risk factor for infection in both women and men.

Based on the level of education of the study population, the highest occurrences of sperm abnormalities in motility and morphology were 83.3% each among men with informal education. Also, there was an 83.3% occurrence of low sperm count among the men with informal education. Poor semen quality was significantly associated with the informal education of the men. The highest occurrence of HPV infection was 18.2% among men with a primary level of education, followed by 5.9% among those who had undergone tertiary education.

Thus, from this study, the level of education was seen to affect the rates of sperm abnormalities in motility and morphology. Lower education level was the variable that most interfered with knowledge. The finding from this study is consistent with the findings of Grandahl *et al.* (2017) and Abreu *et al.* (2012). With respect to HPV infection, people with a primary level of education had the highest exposure. Socioeconomic factors, individual beliefs behavioural aspects are also important factors. However, HPV awareness is important as people with lower education level (informal) were associated with higher rates of HPV-related sperm abnormalities than those with higher levels (primary, secondary and tertiary).

CONCLUSION

This research has provided compelling evidence that HPV infection in men has negative effects on their sperm parameters. Semen quality examination of the patients presenting at the reproductive health clinics of Ahmadu Bello University Teaching Hospital, Zaria, revealed an occurrence of 41.7% abnormal sperm motility and 38.5% abnormal sperm morphology. The overall prevalence of HPV infection among the male patients was found to be 4(6.7%), while 56(93.3%) were not infected.

Specifically, HPV was found to be associated with impaired sperm motility, morphology and a decrease in the total sperm count among the men infected with HPV. Since HPV infection causes a significant reduction in total sperm count, reduced motility and impaired morphology, these effects can also impact male fertility. Therefore, it is crucial for individuals, especially those planning for parenthood, to be aware of the potential consequences of HPV infection and undertake appropriate preventive measures, such as practising safe sexual behaviour and getting vaccinated against HPV.

RECOMMENDATIONS

Future studies should explore the effectiveness of HPV vaccination campaigns, barriers to safe

sexual practices, optimised screening protocols, treatment adherence, partner communication strategies, and long-term outcomes of follow-up care in male populations to enhance HPV prevention and management.

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