

**Human Papillomavirus in Semen at the University Teaching
Hospital, Lusaka, Zambia**

(Conference ID: CFP/489/2017)

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The International Journal of Multi-Disciplinary Research

ISSN: 3471-7102

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Abstract

Background: The Human Papillomavirus (HPV) has recently been demonstrated to have a negative influence on sperm parameters, fertilization process and abortion rate. Several studies have identified HPV in human semen, mainly from men recruited at fertility clinics and also in semen from sperm donors with a prevalence ranging from 1–82%. The prevalence of HPV in semen at the University Teaching Hospital (UTH) is not known and hence the goal of this study was to generate baseline data on the prevalence of HPV in semen with a view to promoting further research on the effects of HPV infection on male fertility and the possibility of female HPV infection through semen.

Methods: The study utilized cross section design involving 78 Semen samples from males seeking fertility medical assistance. Samples were obtained by masturbation after 3 days of sexual abstinence. DNA extraction was conducted using the ZR Viral DNA Kit. The presence of HPV infection was detected by polymerase chain reaction assay of the extracted DNA using the HPV consensus primers (MY09 and MY11).

Results: The patients' age range was from 21-56 years, with the mean age of 35 years. Of the total 78 samples that were PCR amplified, three (4%) tested positive for HPV.

Discussion and Conclusion: The study found presence of HPV in semen at the rate of 4%. The presence of HPV in the semen warrants further investigation in the potential role for transmission to females. We recommend infection prevention strategies such as vaccination against HPV in males and education on reducing the number of sexual partners to be employed more especially with the high incidence of cervical cancer in the country.

Key words: semen, human papillomavirus, Zambia, university teaching hospital

Background

Human papillomaviruses (HPV) are one of the most common and sexually transmitted viruses. They comprise a group of small, circular, non-enveloped, double stranded DNA viruses that infect both cutaneous and mucous squamous epithelia. They are a member of the papovaviridae family. The HPV infection is commonly associated with genital and non-genital warts or cervical cancer. The virus genotypes are divided into high-risk types and low-risk types depending on the correlation between its genotypes and causation of cancer (De Villiers *et al.*, 2009). More than 120 HPV genotypes are known to infect the skin and mucous membranes. This includes the pubic area, oral cavity and perianal region (Munoz *et al.*, 2003). Both males and females from different regions are generally susceptible (Palefsky, 2010 and Giuliano *et al.*, 2011). There is no global or regional statistics on HPV semen infection.

Male HPV infection has rarely been of concern in Zambia. Human Papillomavirus semen infection among Zambian males has not been reported before. Although HPV infection is highly linked to males and its characteristics of infection types and histological distributions are similar to those in females, most HPV infections among males are benign (e.g. genital warts) with a rare tendency for malignancy. Human Papillomavirus DNA may lie not only in the perianal region and external genitalia, including the penis foreskin, scrotum and glans penis but also in the urethra, ductus deferens, epididymis or testis (Weidner *et al.*, 1999).

This study was aimed at investigating the detection rate of HPV in semen from males submitting specimens for semen analysis for fertility purposes in parasitology laboratory at the University Teaching Hospital (UTH). The significance of this study lies in the demonstration of HPV in semen implicating HPV in observed sperm abnormalities that may lead to infertility and the potential role that HPV in semen may play in the transmission to females.

Methods

Study area and population

The study was conducted at the Children's Hospital of the University Teaching Hospitals, Parasitology Laboratory and Kaposi Sarcoma- Human Herpesvirus 8 (KS-HHV8) Laboratory in the Department of Paediatrics and Child Health. Collection of semen samples for detection of HPV infection from the samples submitted for semen analysis in the Parasitology Laboratory was done. Polymerase chain reaction for HPV DNA detection was done at KS-HHV8 laboratory. The study population comprised all those that met the eligibility criteria from the requesting sites and had submitted the specimen to the parasitology laboratory.

Study design and Sampling method

This was a cross-sectional study involving 78 semen samples that had been submitted to the Parasitology Laboratory at UTH. All the eligible samples collected during the study period were included in the study. To be included in the study, consecutive samples were required to have the query for infertility.

Inclusion Criteria

All semen samples submitted for analysis when infertility was suspected were eligible for inclusion in the study.

Exclusion Criteria

Semen with low volume that could not allow extra analysis from the normal routine parameters was excluded from the study. Additionally, semen samples submitted for reasons other than infertility, such as haemospermia and vasectomy were excluded.

DNA Extraction

This was done using the ZR Viral DNA Kit™ (Zymo Research, Irvin, CA, USA). Prior to use, 24ml of anhydrous (100%) ethanol was added to each 6ml of DNA Wash Buffer concentrate to obtain the final wash buffer solution. As per recommendation by the manufacturer of the extraction kit, 250µl of beta-mercaptoethanol was added to each 50 ml of ZR Viral DNA Buffer to make the final dilution of 0.5% (V/V). All centrifugation steps were performed at 12000x g.

A volume of 200µl of semen sample was put in a 1.5 ml microcentrifuge tube and 800µl of ZR Viral DNA Buffer were added. The mixture was then vortexed and incubated at room temperature for 1 hour. After incubation, the sample /ZR Viral DNA Buffer mixture was centrifuged, to remove particulate matter that could clog the column, and transferred to the Zymo-spin™ IC Column in a collection tube. The column in the collection tube was then centrifuged for 1 minute and the flow through was discarded from the collection tube.

Next, 300µl of DNA Wash Buffer was added to the column and centrifuged for 1 minute. The flow through was then discarded from the collection tube. This step was repeated twice to increase the purity of the extracted DNA.

Lastly the Zymo-Spin™ IC Column was placed into a new microcentrifuge tube and 30µl of DNA Elution Buffer was added. The mixture was incubated at room temperature for 5 minutes and later centrifuged for 1 minute to elute the DNA.

DNA Estimation

The concentration of DNA was estimated by ultraviolet spectroscopy at 260nm using a Nano Drop Spectrophotometer 2000 (Thermo Scientific, USA). A DNA sample with an optical density (OD) of 1 at 260nm corresponded to a DNA concentration of 50µg/ml of double-stranded DNA. The purity of the DNA was determined by a DNA/protein absorbance ratio of 260nm/280nm and DNA was considered pure at >1.8. The DNA was stored at -20°C until required for use.

HPV DNA Detection

The presence of HPV DNA was detected by Polymerase Chain Reaction (PCR). The consensus primers, MY09 and MY11, were used. These primers amplify a fragment of 450 base pairs of the HPV L1 region. Amplifications were carried out in the Applied Biosystems 2720 Thermal Cycler version 2.09. The β -Actin primers were used as internal quality control. Briefly the PCR thermal profile reaction conditions were set as follows; 94°C for 1 minute (94°C for 30 seconds, 55°C for 1 minute, 72°C for 90 seconds and 72°C for 10 minutes) for 30 cycles and then hold at 4°C. The amplified products were applied to a 2% agarose gel which was stained with 10 μ l ethidium bromide and then gel electrophoresis was performed at 60 volts for 60 minutes using the Fisher Scientific Electrophoresis. The bands were visualized under UV illumination at 302 nm by photography using the UV Transilluminator (BioDoc-It™ Imaging System. Upland, CA, USA).

Data Analysis

Descriptive statistics was used to analyse the data and the results were presented as a ratio and percentage.

Ethical Approval

This study was approved by the University of Zambia Biomedical Research Ethics Committee (UNZABREC), Ref. No.009-11-15. Permission to conduct the study at the University Teaching Hospital (UTH) was granted by the hospital management. The main ethical issue surrounding this project was confidentiality and to guarantee privacy. Therefore, each semen specimen was given a new identification code and number.

Results

All the 78 samples were adequate and contained sufficient beta-Globin as demonstrated by the beta- Actin PCR amplification. The patients' age range was from 21-56 years, with the mean age of 35 years. The presence of human papillomavirus (HPV) DNA in semen samples was demonstrated by the approximately 450bp amplicon in the pool of extracted genomic DNA (Figure 1). Out of the total 78 samples, 3 samples tested positive for HPV after amplification with the MY09/MY11 primers, giving a 4% rate of HPV detection. The three positive samples belonged to the age groups 29, 32 and 35 year old patients.

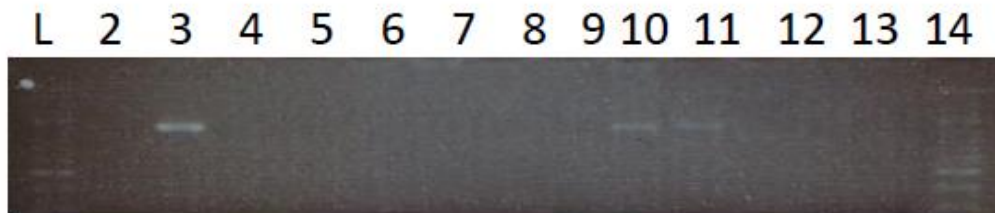


Figure 1; Representative Image of Gel Electrophoresis: Image of a 2% agarose gel with bands of the ladder (L), positive control (3), two positive samples (10 and 11) and negative control (2).

Discussion

Human Papillomavirus is prevalent in all sexually active populations and is frequently presented clinically as anogenital warts in both males and females. High-risk, oncogenic, HPV types are associated with 99.7% of all cervical cancers, whereas low-risk HPV types are responsible for almost all cases of genital warts (Ault, 2006). Human Papillomavirus infection rates in men range from 1.0% to 88.9% (Caliskan *et al.*, 2010

and Giovannelli *et al.*, 2007). This wide range may be due to the variation in the clinical material analyzed such as penile surface, glans, scrotum, urethra, semen, and urine (Caliskan *et al.*, 2010). Another reason for the wide range is the status of the source of the clinical material such as from symptomatic i.e. (genital warts patients) or asymptomatic patients for HPV.

Human Papillomavirus prevalence in our study was found to be 4% (3/78). A study conducted in Italy in 2010 by Foresta *et al.*, reported HPV semen infection in 6.1% of the 98 cryopreserved samples from a cohort of patients who had banked sperm as a result of testicular cancer (Foresta *et al.*, 2011). In the same study, 3.3% prevalence was reported in 60 health young males who were used as controls (Foresta *et al.*, 2011). In another study conducted in Turkey by Caliskan in 2010, the HPV prevalence of asymptomatic fertile and infertile men was reported to be 1.1% (2/175) (Caliskan *et al.*, 2010). The above results are in accordance with, and comparable to our results in this study.

Sperm infection with HPV has been demonstrated to reduce sperm motility, sperm–ovum interaction, the pregnancy rate, and increase the abortion rate (Perino *et al.*, 2011, Spandorfer *et al.*, 2006 and Weyn *et al.*, 2006). The presence of HPV DNA in semen samples of our study population may have similar clinical implications.

In 1997, Lai *et al.* reported the presence of HPV DNA and RNA in 24 sperm samples of subfertile men. They reported a 75% incidence of asthenozoospermia among patients infected with HPV and 8% in those without HPV in their sperm cells. Curvilinear velocity, straight-line velocity, and mean amplitude of lateral head displacement was performed and reported significantly lower in HPV infected specimens. The differences of linearity, beat cross frequency, and straightness were not statistically significant. With these findings, they concluded that, 1) certain HPV specific genes are actively transcribed; 2) that the presence of HPV in sperm cells may affect sperm motility parameters; 3) and that asthenozoospermia may be associated with sperm HPV infection (Lai *et al.*, 1997). In 2010, Foresta *et al.* confirmed these findings (Foresta *et al.*, 2010a).

In HPV infected semen samples, it has been shown that HPV can be localized at different levels; in spermatozoa, in exfoliated cells or in both sites. This suggests that spermatozoa or indeed semen can act as the vector for HPV infection to sexual partners. In 2006, Weyn *et al* reported HPV in the placenta. They suggested that this infection may occur not only through ascending infection from the cervix but also via infected sperm at fertilization (Weyn *et al.*, 2006). The results of HPV in semen in this study also suggest that some of the cervical cancer cases may be caused by cervical HPV infection via infected semen.

Circumcision services in Zambia and indeed many other sub-Saharan countries represent an opportunity to reduce HPV infection as well as HIV in men. This is done with a view that women would benefit indirectly from a lower prevalence of HPV in their male partners. The effects of circumcision which are, reduced acquisition rate of penile HPV infections, and increased clearance rate of pre-existing HPV from the penis are well understood. However, more studies are needed to understand if these effects due to circumcision could be observed in semen.

Finally, the presence of HPV in semen samples suggest that HPV may be transmitted through semen and that circumcision alone could not effectively reduce the burden of HPV infection in males and later on in females. To this end, there is need to employ other strategies such as vaccination of both males and females against specific strains of HPV and education of people on the importance of reducing the number of sexual partners with regard to HPV prevention.

Conclusion

The prevalence of HPV in semen of men suspected of being infertile was found to be 4%. The presence of HPV DNA in semen suggests that HPV by inducing an alteration of sperm motility may play a major role in infertility. The presence of HPV in semen also suggests that male circumcision alone may not effectively prevent the transmission of HPV to females. Therefore, females may still be at risk of suffering from cervical cancer

and other diseases that are caused by the exposure to HPV through semen. Since the presence of HPV in semen may threaten the fertility of affected men and the health of their partners, we propose that, appropriate measures should be taken in males such as vaccination against HPV in addition to circumcision.

Recommendations

The presence of HPV DNA in semen and confirmation of its genomic activity by other studies suggests that HPV-infected semen could be a health risk to male fertility and female health. For this reason, we recommend other strategies such as vaccination against specific strains of HPV in males and education on the importance of reduced number of sexual partners to be employed.

Limitation

The limitation of this study is the lack of genotyping of the isolated HPV DNA as that could have given more insight on the specific HPV genotypes isolated from these samples. This could have given more guidance on whether the current available vaccines, gardasil and cervarix, may work in this population if administered at the right time.

List of Abbreviations

ART; Assisted Reproduction Technology, DNA; Deoxyribonucleic Acid, dsDNA; Double stranded Deoxyribonucleic Acid, HPV; Human Papillomavirus, IVF; In-Vitro Fertilization, LAMU; Lusaka Apex Medical University, OD; Optical Density, bp; Base Pair, PCR; Polymerase Chain Reaction, RNA; Ribonucleic Acid, SS; Semen Specimen, STDs; Sexually Transmitted Diseases, UNZA; University Of Zambia; UNZABREC; University of Zambia Biomedical Research Ethics Committee, UTH; University Teaching Hospital, WHO; World Health Organization, KS-HHV8; Kaposi Sarcoma-Human Herpesvirus 8.

Declarations

Consent for publication

Not applicable

Availability of data and materials

All data generated and analyzed in this study are available on request from the corresponding author.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was funded by Lusaka Apex Medical University and the Moses Sinkala Award 2015.

Authors' contributions

Christopher Newton Phiri, Sody Mweetwa Munsaka and Trevor Kaile conceived the study; they also performed data analysis and interpretation. Christopher Newton Phiri, Fred Fredrick Bangara and Chrispin Chisanga performed data collection. All authors reviewed and approved the final manuscript.

Acknowledgments

The authors wish to acknowledge the Lusaka Apex Medical University (LAMU) and the 2015 Moses Sinkala Award, awarding committee for the financial support rendered towards this work.

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The International Journal of Multi-Disciplinary Research

ISSN: 3471-7102

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