http://jmscr.igmpublication.org/home/
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v9i4.21

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Serologic Evidence of High-Risk Human Papillomavirus 16 and 18 Infections and Risk of Prostate Cancer in Northwestern Nigeria

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Abstract
Prostate cancer is the leading cause of cancer death among men in Nigeria and second most common cancers of men worldwide with sexual history as a consistent risk factor. Since human papillomavirus (HPV) infection was first identified as a risk factor for cervical cancer, several sero-epidemiologic and tissue-based studies have investigated HPV in relation to prostate cancer, another common genitourinary malignancy with mixed results. To further inform this potential association, we conducted a prospective and case-control investigation of HPV types 16 and 18 in relation to risk of prostate cancer. Serum samples from 300 men were tested for IgG antibodies against HPV-16 and -18 virus-like particles (VLPs) using enzyme-linked immune sorbent assays (ELISA) specific for each type, and also screened for the level of prostate specific antigen (PSA) as well as performed digital rectal examination (DRE). However, of the 300 men, only 167 consented to undergo DRE procedure. Men with PSA level ≥ 4ng/ml and or abnormal DRE were referred for prostate biopsy. The data demonstrated that 24.0% of those screened had abnormal DRE finding and PSA level of ≥ 4ng/ml which is suggestive of prostate cancer and 28.7% had HPV infection. Of the total number infected with HPV of any type, 29.1% and 23.3% of them were infected with HPV 16 and HPV 18 respectively. The data further revealed that both HPV-16 and -18 infections occurred more ($P<0.05$) among the cases (those suspected of prostate cancer) than in the controls (those without prostate cancer). Analysis of the data also revealed that the most common risk factors for prostate cancer are age, family history of prostate cancer, occupation, nature of diet and cigarette smoking, while infection with high risk human papillomaviruses appeared to be an etiologic risk factor. This study represents for the first time the prevalence of HPV infection in prostate cancer in Nigerian population and strengthens the hypothesis that HPV infection could be one of the co factors associated with risk of prostate cancer.
Keywords: *Prevalence, Prostate cancer, HPV 16 and 18, PSA, DRE, Risk factors, Northwestern Nigeria.*

Introduction

Prostate cancer rates in African-American and Afro-Caribbean black men have been reported to be high, suggesting genetic predisposition. However, only 10% of prostate cancer is due to the familial or genetic type, while 90% is considered sporadic, due to the combination or interaction of environmental and genetic factors (Flora *et al*., 2003). The growing epidemiological studies have suggested that prostate tissue is prone

to sexually transmitted infection with several viruses having oncogenic potential such as polyomavirus (SV40), human papillomavirus (HPVs), and members of the herpes virus family. It has also been implicated that cellular transformation in prostate cancer is carried out by viral oncogenesis of polyomavirus, or the E6 and E7 proteins of HPVs. Human papillomavirus is a small, non-enveloped DNA virus with a circular, double stranded DNA genome of approximately 8kb genome size. They are widely distributed throughout the animal kingdom. Approximately 30-50% of the general population is positive for HPV DNA. Harald Zur Harsens laboratory was the first to demonstrate that genital warts contain HPV genomes. It is estimated that 15% of all cancers are etiologically linked to viral infection. Infection with high-risk (oncogenic) types of HPV (HPV 16, -18, -13, -33, -35, -39, -45, -52, -56, -58, and -68) is a well documented/ established risk factors for the development of cervical carcinoma. Human papillomaviruses are the etiological agents of cervical and other anogenital malignancies and low-risks HPVs induce only benign genital warts.

The best evidence supporting the role of HPV in the development of urological malignancy is for squamous cell carcinoma of the penis. Evidence supporting the oncogenic role of HPV in other urological malignancies is conflicting, but HPV is unlikely to be a major factor.

The oncogenic potential of HPV is determined by E6 and E7 oncogenes that interact with and inhibit the activities of critical components of cell-cycle regulatory systems, in particular E6 with p53 and E7 with Rb. In previous studies, there were reports that oncogenic subtypes of HPV, with the most common types 16 and 18, have a strong association cervical cancer. It has been speculated that cervical and prostate cancers may represent, in some aspects, homologous cancers in females and males, respectively. Both of cancer types are influenced by similar factors like sexual activities and infection status and are most commonly occurring cancer in the developing countries like Nigeria, with a roughly equal lifetime risk. There is a growing evidence of the role of sexually transmitted disease in prostate cancer; therefore, it is possible that there would be an association between oncogenic HPV and prostate cancer. Although as stated earlier, studies have reported contrasting roles of HPV infection in the pathogenesis of prostate cancer, molecular studies have reported that E6 and E7 oncoproteins of HPV types 16 at 18 can immortalize prostate epithelial cells.

Family history, age, testosterone, ethnic origin, environment and genetic factors are the only firmly established risk factors for prostate cancer. In addition to these, a history of sexually transmitted infection has emerged as one of the stronger and more consistently reported risk factors. Recently, based on observations using the polymerase chain reaction (PCR) amplification assay, HPV types 16 and 18 specific DNA sequences have been detected in prostate cancer specimen obtained by transurethral resection.

The inconsistent result across many of these epidemiological studies could possibly be related to the variation in ethnicity and population of one geographic region to another, or different sexual behavior and lifestyle patterns as well as method of detection. Based on the above findings, this current pioneering study is designed to evaluate the HPV infection status in prostate cancer cases in comparison with healthy controls and find its correlation if any with the various risk factors like mean age, PSA level, DRE status, family history and sexual pattern in northern Nigerian population.

Materials and Methods

This study was conducted in six major health care centers across the three senatorial zones of Kaduna state in northeastern Nigeria. Majority of the people of the state are Gbari and Hausa with majority being Muslims.

This study was approved by the ethical committee of Ahmadu Bello University Teaching Hospital and the ethical committee of Kaduna State Ministry of Health. Questionnaires which

comprised of questions regarding socio demographic details, clinical, dietary, occupational and family history of prostate cancer, history of tobacco and alcohol consumption on one hand, and information regarding sexual behavior and clinical manifestations of human papillomavirus infections on the other hand, was completed by each participant or assisted by members of the research team after initial brief health talk. Venous blood was aseptically collected with the help of vacutainer needle into specimen tubes and labeled. The participants then underwent a digital rectal examination (DRE) procedure conducted by trained health professionals. Serum was extracted appropriately and stored until use. It was then analyzed for the presence of HPV and examined for the level of prostate specific antigen (PSA). Invitation for transrectal prostate biopsy was based on the results of serum PSA and DRE status. Men with serum PSA ≤ 4.0ng/ml with abnormal DRE, men with serum PSA between 4.0-10.0ng/ml with abnormal DRE, and all men with serum PSA >10.0ng/ml irrespective of DRE status were referred for transrectal prostate biopsy.

The data obtained was analysed using SPSS 21.0 for windows statistical software. Fischer's exact test and chi square were used whenever applicable.

Results

A total of 300 men were included in the study and screened for Human papillomavirus DNA and level of prostate specific antigen as well as digital rectal examination. However, data for DRE was unavailable for 133 (44.3%) men who refused the procedure. Therefore, only 167 (55.7%) men were included in the final analysis. A total of 40 men with prostate cancer served as cases whereas 127 men without prostate cancer served as control subjects. Table 1 summarizes the soci0-demographic profile of population that participated in the research study. Distribution by age showed that 82 (27.3%), 88 (29.3%), 69 (23.0%), 46 (15.3%) and 15 (5.0%) belonged to age group 30-40years, 41-50years, 51-60years, 61-70years and above 70years respectively. The highest number of men was found in the age group of 41-50 years. Distribution according to literacy level showed that 18 (6.0%) had no formal education whereas 172 (57.3%) had tertiary education as shown table 1

Table 2 shows the occurrence of human papillomavirus among those with prostate cancer (Cases) and those without (Controls). Analysis of the relationship between human papillomavirus infection and prostate cancer risk showed that there is a significant association between HPV infection and early prostate cancer risk (P <0.05). Further stratification of Human Papillomavirus showed that high risk HPV -16 and -18 occurred more in people with prostate cancer (cases) than in those without prostate cancer who served as control subjects. Of the 40 people with prostate cancer (cases), 20 (50.0%) had HPV infection while 30.7% (39/127) had HPV infection in the control group. This difference is however statistically significant (P=0.026). Also 25.0% of the cases had HPV 16 infection as against 8.7% in the control group. This association between HPV -16 infection and the risk of prostate cancer is highly significant ($\chi 2$=7.386, df=1, P=0.007). Finally there was also a positive association between the differences in the infection of HPV 18 between the case and the control groups (P=0.010) showing a prevalence of 20.0% and 6.3% respectively. This showed that infection with HPV especially HRHPV -16 and -18 may be an important co factor in the risk of prostate cancer.

Table 3 shows the relative risk of developing prostate cancer among HPV sero-positive men in comparison with other risk factors. Analysis on possible confounding factors of prostate cancer showed that both HPV types -16 and -18 sero-positivity tended to be associated with increased risk of prostate cancer. The relative risk for HPV -16 and -18 are 2.317 and 2.359 respectively (table 3). The continuous test indicated that the association of HPV -16 and prostate cancer risk is highly significant (P= 0.007) and also significant

for HPV -18 (P= 0.010) respectively. Also, increased age and family history of prostate cancer tended to be associated with prostate cancer risk with both associating at a highly significant level (P= 0.000) and relative risk for family history of prostate cancer at 3.995 (95% CI= 2.277-7.008).

Table 4 shows some clinical and epidemiologic characteristics of men with early prostate cancer. The result showed that 47.5% of them are above 60 years and all of them had abnormal DRE status. The total PSA were >20 ng/mL in 12 (30.0%) men and 10.0-20.0ng/mL in 23 (57.5%). Further evaluation of the men with prostate cancer showed that 13 (32.5%) smoke cigarette while 8 (20.0%) consume alcohol. Among men with prostate cancer, 40.0% consume food high in animal fat while the remaining 60.0% consume low-fat containing food and 11 (27.5%) of them are civil servants while 3 (5.0%) are farmers. Still 1 (2.5%) person was a tire plant worker and 3 (7.5%) were painters. Again analysis on literacy level showed that 20 (50.0%) of them had tertiary education while 10 (25.0%) had secondary education. More so, 8 (20.0%) of them had primary education while 2 (5.0%) had no formal education as shown in table 4

Table 1: Characteristics of the study population

Parameters	n = 300	%
Age		
31 - 40	82	27.3
41 - 50	88	29.3
51 - 60	69	23.0
>60	61	20.3
Marital status		
Single	39	13.0
Married	246	82.0
Widower	10	3.3
Divorced	5	1.7
Type of family		
Monogamy	145	48.3
Polygamy	100	33.3
Others	55	18.3
Level of Education		
No formal education	18	6.0
Primary	31	10.3
Secondary	79	26.3
Tertiary	172	57.3
Family History of Pca		
Yes	70	23.3
No	230	76.7
Occupation		
Civil servants	77	25.7
Farmers	19	6.3
Tire plant workers	10	3.3
Painters	23	7.7
Others	171	57.0
Alcohol consumption		
Yes	82	27.3
No	218	72.7

Smoking cigarette		
Yes	117	39.0
No	183	61.0
Nature of Diet		
High in fat	136	45.3
Low in fat	161	53.7
No fat	3	1.0

n = number,
% percentage

Table 2: Human Papillomavirus infections and Risk of Prostate Cancer among Cases and Control subjects in Northwestern Nigeria

HPV type	No. examined	Cases n=40(%)	Controls n=127(%)	χ2	P value
Any HPV					
Positive	59	20 (50.0%)	39 (30.7%)	4.955	0.026*
Negative	108	20 (50.0%)	88 (69.3%)		
HPV 16					
Positive	21	10 (25.0%)	11 (8.7%)	7.386	0.007**
Negative	146	30 (75.0%)	116 (91.3%)		
HPV 18					
Positive	16	8 (20.0%)	8 (6.3%)	6.591	0.010*
Negative	151	32 (80.0%)	119 (93.7%)		

Key: χ2 =chi square, *= significant at P≤0.05, %=percentage, No. =number

Table 3: Relative risk of developing prostate cancer among HPV-Seropositive men in comparison with other possible risk factors in Northwestern Nigeria

Risk factor	Cases (n=40)	Controls (n=127)	Relative risk	95% CI	P value
Age (yrs)					0.000**
30-40	0(0.0)	38(29.9)			
41-50	9(22.5)	34(26.8)			
51-60	12(30.0)	34(26.8)			
>60	19(47.5)	21(16.5)			
Age at sexual debut (years)					0.989
16-20	16(40.0)	50(39.4)			
21-25	19(47.5)	60(47.2)			
26-30	5(12.5)	17(13.4)			
No of sexual partners					0.698
0	0(0.0)	1(0.79)			
1	10(25.0)	35(27.6)			
2	13(32.5)	34(26.8)			
3	10(25.0)	24(18.9)			
>3	7(17.5)	33(26.0)			
Family history of Pca					0.000**
Yes	16(65.0)	27(21.3)	3.995	2.277-7.008	
No	14(35.0)	100(78.7)	0.581		
Alcohol consumption					0.502
Yes	8(20.0)	32(25.2)	0.794	0.399-1.580	
No	32(80.0)	95(74.8)	1.069		
Cigarette smoking					0.434
Yes	13(32.5)	50(39.4)	0.795	0.444-1.424	
No	27(67.5)	77(60.6)	1.072		
HPV 16					0.007**
Positive	10(25.0)	11(8.7)	2.317	1.337-4.018	
Negative	30(75.0)	116(91.3)	0.659		
HPV 18					0.010*
Positive	8(20.0)	8(6.3)	2.359	1.323-4.208	
Negative	32(80.0)	119(93.7)	0.634		

n= number, Pca = prostate cancer, CI = 95% confidence interval, * = significant level

Table 4: Characteristics of men with prostate cancer in Northwestern Nigeria

Parameters	(n = 40)	%
Age		
31 - 40	0	0.0
41 - 50	9	22.5
51 - 60	12	30.0
>60	19	47.5
DRE status		
Positive	40	100.0
Negative	0	0.0
Serum PSA(ng/mL)		
0.0 - 3.9	0	0.0
4.0 - 9.9	5	12.5
10.0 - 20.0	23	57.5
>20.0 0	12	30.0
HPV 16		
Positive	10	25.0
Negative	30	75.0
HPV 18		
Positive	8	20.0
Negative	32	80.0
Smoking cigarette		
Yes	13	32.5
No	27	67.5
Alcohol consumption		
Yes	8	20.0
No	32	80.0
Diet		
High in fat	16	40.0
Low in fat	24	60.0
No fat	0	0.0
Occupation		
Civil servants	11	27.5
Farmers	2	5.0
Tire plant workers	1	2.5
Painters	3	7.5
Others	23	57.5
Level of Education		
No of formal education	2	5.0
Primary	8	20.0
Secondary	10	25.0
Tertiary	20	50.0

n = number and % = percentage

Discussion

The oncogenic potential of high risk HPVs and their role as vital etiologic factors in the development of prostate cancer is well documented. Though various studies have reported the presence of HPV in prostate cancer worldwide, none of the studies till date have been reported from Nigeria. Therefore, this study was designed to establish the roles of HPV in prostate cancer from northeastern Nigeria. Previous findings have given sufficient evidences that oncogenic subtypes of HPV, with the most

common types 16 and 18, have a strong relationship with anogenital and other epithelial cancers. However, the role of HPV in prostate carcinogenesis is still debatable and need to be elucidated. This study demonstrated that a significant number of prostate cancer cases were infected with HPV accounting for up to 50.0% of cases and 30.7% in the control. This is in concordance with previous studies and recent epidemiological findings carried out world over that showed presence of HPV in as high as 65% of prostate tumors and established associations of specific HPV types as a risk factor for prostate cancer progression, as well as prevalence of 51.2% and 52.6% reported independently by Carrie *et al*., 2007 and Angelo *et al*., 2014 among men in Florida. However, this result is also higher than other reported studies such as 10.7% prevalence reported in Ghana by Domteh *et al*., 2008, and the global HPV prevalence of 11.7%. This may be due in part to the higher sensitivity of the detection method (ELISA test kits) used and partly due to the lifestyle of the population studied which is characterized by early sexual initiation, multiple partnering and low condom usage among others. Human papillomavirus infection occurred more among those that had sexual debut at an early age (16-20) and less among late sexual debutants (26-30). This is similar to previous reports by Beatriz *et al*., 2008, Oliver *et al*., 2014 and Thomas *et al*., 2004 in Ibadan, Nigeria. This is particularly so because those that have had early sexual debut are much more likely to get exposed to HPV infections, than those who have been initiated into sex at an advanced age. Therefore, HPV infection is associated with the period of sexual initiation ($P<0.05$).

This study showed that there was early indication of prostate cancer in 24.0% of men with complete valid data. However, the calculated prevalence of early indication of prostate cancer among the overall population is 13.3% (40/300). This (24.0%) is comparable to the report in Ibadan cancer registry who reported 23.5% while the 13.3% overall prevalence is similar to 13.3% reported by Ezenwa *et al* in 2012 in Lagos, Nigeria and 15.0% reported by Gabriel *et al* in 2008. However, both are lower than the 43.0% reported by Stephen *et al*., 2013. However, both are higher than the 0.7% reported by Soderdahl *et al* in 2012

The differences observed in this study and those observed elsewhere may be due largely to differences in exposure to major risk factors, detection practices (availability of diagnostic and screening services), awareness of early signs and symptoms and availability of treatment. Also, the country of residence or better still, the differences in lifestyle and diet of the different study population may be another reason.

The result of this study has found sero-epidemiological evidence that infection with oncogenic HPV is associated with increased risk of developing prostate cancer. The association of HPV sero-positivity with prostate cancer risk is at some variance with PCR studies reporting the presence of HPV DNA in prostate cancer tissue, since in most of these a large proportion of specimens both of benign and of malignant tissue have been positive, usually for HPV 16 (Jaudah, 2007; Cuzick, 1995; Moyret-Lalle *et al*., 1995; Suzuki *et al*., 1996; Widerof *et al*., 1996). The fact that increased prostate cancer risk was nevertheless specific to the major oncogenic HPV suggests that the association is not secondary but is probably of etiological significance. However, our study could also be comparable with a "hit-and-run" model for oncogenesis, since HPV capsid antibodies are known to persist for life even after clearance of HPV DNA. This is similar to reports of Neha *et al*., 2014 who also found an association between HR- HPV infection and risk of prostate cancer among Indian population, and report of Jaudah in 2007 in Saudi Arabia who also demonstrated positive association between HR-HPV and prostate cancer. Another report by Mariarosa *et al* in 2013 also found as association between HPV infection and prostate cancer survival. A report by Joakim *et a*l., 1998 also showed a positive association between HPV 16

and prostate cancer occurrence. In 2004, Carozzi *et al* also found 14/26 of prostate cancer patients with high-risk HPV and Leiros et al., 2005 also found 17/41 HPV DNA in prostate samples. The result is however different in studies reported by Ruth *et al*., 2012; Hans-olov *et al*., 2003; Karin *et al*., 2003; Zoltan *et al*., 2005; Saad *et al*., 1999 whose findings suggested negative association between HPV infection and risk of prostate cancer. These inconsistence associations with different HPV types may in part be due to differences in the sampling site (anal or external genital sites), differences in the population (general population and high-risk men) as well as the differences in the method of detection of both prostate cancer as well as Human papillomavirus. Another difference may be the age of the sample population; this is because while prostate cancer is more likely identifiable among those over 60 years of age, HPV is more prevalent among younger adults who are more sexually active.

An association of HPV with prostate cancer, if substantiated, would be unexpectedly good news for cancer-prevention prospects, since prophylactic papillomavirus vaccination has been spectacularly effective.

Conclusion

In this study, infection with high risk human papillomavirus type -16 and -18 were found to be associated with the risk of developing prostate cancer among men in Kaduna state, Nigeria. However, infection with any other type of HPV was also found to be an important etiologic cause of prostate cancer. Our study represents for the first time the prevalence of high risk human papillomavirus infection in prostate cancer in Nigerian population and strengthens the hypothesis that HPV infection especially high-risk types -16 and -18 could be one of the co factors associated with progression of prostate cancer among men over 35 years of age.

The findings of this study further showed that, the frequently observed clinical manifestations of prostate cancer are weak flow of urine, having blood in urine and/or semen, painful urination, frequent urination especially at night as well as difficulty in having erection.

In the final analysis therefore, this study strengthens the hypothesis that the prostate gland in males may represent a complex niche where multiple infections with oncogenic DNA viruses like HPV occur and implicates the potential role of these viruses in progression of prostate cancer. To our knowledge, this is the pioneering study evaluating the serologic evidence of HR-HPV infection in prostate cancer in Nigeria. It has thus guarantees the clinical significance of HPV infection in prostate carcinogenesis that has been underestimated till date in Nigerian population.

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