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Major Risk Factors In HPV DNA Detection in Unisex Immunosuppressed Cohorts

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ABSTRACT: Risk factors in HPV/HIV co-infectivity were considered in unisex cohorts aged 16 years and above. It was a completely randomized sample collection research design; with informed consent research questionnaires administered to the subjects whose HPV and HIV status had been previously established at the earlier parts of the work. The study was conducted in FCT Abuja in Nigeria. Multivariate odd ratios (**ORs**) and 95% confidence intervals (CI) for each endpoint were obtained for risk factors after adjustment for confounding variables in their personal data which showed that the **ORs** - of medical history of Herpes simplex infection in the female and male cohorts were respectively 2.22 (95% CI=1.44-3.64) and 1.4 (95% CI =0.61-3.42): Oral contraceptives usage in the female cohorts was 3.0 (95% CI = 1.10-7.22) : Multiple sexual partners in the female and male cohorts were respectively 2.1 (95% CI = 0.41-9.42) and 2.8 (95% CI = 0.91-8.58). Cigarette smoking , following the same trend were respectively 2.10 (95% CI =0.60-6.81) and 8.0(95% CI =1.36-47.7) ; while Nulliparity in the female cohorts had 3.01 (95% CI =1.59-5.55) . Most sexually active age range of >25 -34< years in the female cohorts was 4.40 (95% CI =1.45-12.0) and in the male cohorts was 5.52(95% CI = 1.7-12.0) .Education in the female and male cohorts had ORs of 2.0(95% CI= 1.0-7.80) and 1.99(0.86-4.5) respectively. These risk factors significantly($P<0.05$) enhance HPV/HIV co-infectivity in unisex cohorts aged >16 years, awareness campaigns by various health organs of government must be stepped up for effective combat /control.

Keywords: Risk factors, Human Papilloma Virus (HPV), Herpes Simplex Virus, HIV.

Introduction

As in other cancer types, particularly cervical cancer, the onset of the disease can be promoted by specific risk factors which may be genetical or environmental.Several risk factors have been identified. Lifestyle habits influence the risk for cervical cancer, as do social circumstances (high risk in developing countries), toxic agents and drugs. (Moscicki, 2004). Some women are more at risk than others. Following factors increase the chances of cervical cancer in women:

Human Papilloma Virus (HPV): HPV is a widely spread sexually transmitted agent. The infection has been identified as the most import risk factor for cervical cancer. Among more than 100 "benign" types of HPV there are aggressive strains (most important: HPV 16 and 18) which can lead to malignant transformation of cervical cells. (Moscicki, 2000).

Multiple Sex partners: Women who have more than one sex partner are at higher risk by increasing the chance of a HPV infection.

Early Sexual activity: Women who have had had early sexual activity, before 18 years of age are more at risk as the cervical cells are very fragile at this young age.

Other STD infections: Women who have had some other Sexually Transmitted Disease (AIDS, Gonorrhoea) are more prone to Cervical Cancer.(Karl son, *e t al* , 1995).

Family history of cervical cancer: Some families show a higher incidence of cervical cancer. Some scientists believe they might carry a genetic condition making them more sensible for the negative effects of HPV infections. (Verson ,1999.) . Age seems to play a definite role as this cancer is more common in 40 plus women and quite rare in women less than 15 years of age. Cervical cancer seems to be more pronounced as age progresses which might be due to the simple reason that after women reach the menopause many of them think that there is no more need for a Pap Smear Test .(Schwartz,2006).

Contraceptive Pills: Women who are regularly on the pills may get Cervical Cancer faster as they do not use condoms which are more suited to prevent STD's. (Bleeker , 2003).

Cigarette Smoking: Traces of chemicals found in smoke and cigarettes have been isolated in the cervical tissue of women who smoke. This indicates a strong correlation between the two.

Income/socioeconomic status: Since the earning levels are directly related to the living standards ,lower income women are almost five times more at risk than higher income groups (Wang, 2003).

Race: African Asian women are at higher risk of having cervical cancer and are more likely to have an advanced stage at the time of detection than Caucasian Women. Hispanic women are also more prone to cervical cancer. (Walboomers *et al*,1999)

Unhealthy diets: Improper diet is also a reason that can put women at risk. Malnutrition is also recognized as a cause.

High fasting Glucose levels: Incidences of cervical cancer are more in the women who have 140mg/DL levels of Glucose Sugar. Beta –carotene and vitamin C were common features in women with minor cervical abnormalities (Mackerras,1999)**.**

Presence of abnormal cells: Cells like Dyskaryosis increase the risk levels of cancer.

Multiple pregnancies: Multiple child birth may also increase the risk of cervical cancer in the women.

DES: Daughters of women who had used the drug DES (Diethylstilbestrol) in early 1970's are said to be on higher risk of Cervical Cancer. (Mackerras,1999)

Materials and Methods

Study Design

Completely randomized endocervical swab samples from female cohorts aged 15-60 years were collected. Seminal fluids (15ml) from male cohorts of the same age brackets as their female counterparts were also randomly collected . Informed consent research questionnaires were administered to the unisex cohorts drawn from four hospitals in Abuja Metropolis in Nigeria. Koilocytes (HPV indicators) were assayed using the Papanicolaou and the Hematoxylin /Eosin staining techniques (Baker, 2002). Positive samples were reconfirmed with the Roche PCR Assay adopted (using the GP5+/ GP6+ PCR Procedure) for pooled high risk types 16 or 18 HPV. In a laminar flow hood, 10ul of crude cell lysates(prepared from female endocervical swab of female cohorts and Seminal fluids of

male cohorts) were pipetted into PCR tubes with sterile aerosol –resistant tip. In a PCR cabinet 40ul GP5+/6+ PCR mix was added to the PCR tubes containing the crude cell lysates, using a repeat pipette with a sterile tip. This was centrifuged for 15sec at maximum speed in a micro centrifuge; the PCR tubes were then transferred to the PCR thermo cycler that was located in a physically separated laboratory. 40 cycles of GP5+/bio GP6+ PCR amplification were ran. This followed 4 min denaturation step of 94^{o}C. Each cycle included a denaturation step at 94^{o}C for 20s, an annealing step at 38^{o}C for 30s, and an elongation step at 71^{o}C for 80s. The final elongation step was prolonged for a further 4 min . In each PCR run two negative sample preparation controls and two PCR negative controls were included-- for both female and male cohorts were included. likewise, positive controls, designated, SiHa-10ng, SiHa-1 ng, and SiHa-100pg positive controls. An internal control to ensure DNA integrity was performed by amplifying the *β*-globin gene using the primers PC03 and PC04 as described previously (Saiki *et al*., 1986).

Results

Table 1 shows the result of the various risk factors considered in terms of HPV infection in the male cohorts . In ages 0<16 years the mean prevalence was 9(891), 1.0%. In ages > 16 years to < 25 years the prevalence was 273(794) 5.2%; in Age > 25 years to < 34 years the prevalence was 332(771), 38.8%.In the ages>34 years to < 43 years the mean prevalence was 47(849), 30.3% while in ages ≥43 years the mean prevalence was 20(880), 2.2%. Percentage mean prevalence of HPV DNA decreases with higher level of education. Those that had no education at all, had a mean HPV DNA prevalence of 10 .6%, 96(804); in those that had only primary education , the mean papilloma virus DNA prevalence was 18.0%, 162(643); in those that had secondary , the mean HPV DNA was 10.0%, 90(810); While those that had tertiary education, viz; University, Polytechnic, Monotechnic, e.t.c., the mean HPV DNA was 5.5%, 50(850). The mean HPV DNA was 11.1%, 100(800) in those who had never smoked cigarette while those who had ever smoked cigarette , the mean prevalence was 25.8%, 232(668). Those who had multiple lifetime sexual partners had higher HPV DNA %. From the table, those with just one sexual partner had a mean HPV DNA of 10.0%, 90(810); While those with two sexual partners the mean prevalence was 13.0% ,117(783). Those with ≥ three sexual partners the mean prevalence was 16.0%. Those that had previous Herpes virus infection and other sexually transmitted diseases (HSV and other STDS) their HPV DNA prevalence was 12.1%, while those that had never suffered this had HPV DNA prevalence of 11.1%. had HPV DNA prevalence of 11.1%.

Table 2 shows the result of the various risk factors considered in terms of HPV infection in the female cohorts . In ages 0<16 years the mean prevalence was 16(884), 1.8%. In ages > 16 years to < 25 years the prevalence was 45(855) 5.0%; in Age > 25 years to < 34 years the prevalence was 322(578), 35.8%.In the ages>34 years to < 43 years the mean prevalence was 18(882), 2.0% while in ages ≥43 years the mean prevalence was 20(880), 2.2%. Percentage mean prevalence of HPV DNA decreases with higher level of education. Those that had no education at all , had a mean HPV DNA prevalence of 11.0%, 99(801); in those that had only primary education , the mean papilloma virus DNA prevalence was 22.0%, 198(702); in those that had secondary education , the mean HPV DNA was 14.0%, 126(774); While those that had tertiary education , viz; University, Polytechnic, Monotechnic, e.t.c., the mean HPV DNA was 6.0%, 54(846).

The mean HPV DNA was 10.9% , 98(802) in those who had never smoked cigarette , while those who had ever smoked cigarette , the mean prevalence was 8.0%, 72(828). Those who had multiple lifetime sexual partners had higher HPV DNA %. From the table, those with just one sexual partner had a mean HPV DNA of 10.1%, 91(809); While those with two sexual partners the mean prevalence was 9.0% ,81(819). Those with ≥ three sexual partners the mean prevalence was 15.0%, 135(765). Those that had previous Herpes virus infection and other sexually transmitted diseases (HSV and other STDS) their HPV DNA prevalence was 18.0%, 182(738)while those that had never suffered from this , had HPV DNA prevalence of 10.05%, 90(801). Parity as a risk factor for HPV DNA prevalence was observed in nulliparous(childlessness) female cohorts as 31.2%, 260.2(835).Those who were moderately parous i.e. 1-2,; 3-4; or >5 had HPV DNA prevalence of 9.1% ,82(810), 5.4% 49(851) and 7.2% 35(617) respectively. On the table , those female cohorts who never had abortion had %HPV DNA of 10%, 90(810). Those that had abortion once had 13.0%, 117(783) while those who had abortion >2 times , had 22.0%, 198(702). HPV DNA prevalence thus tend to increase with the frequency of abortions in the female cohorts.

Table 1: Major risk factors for HPV DNA detection using Multiple Logistic Regression Analysis of subjects personal data in male cohorts

Personal Data	Total Positive	Total Negative	Positive (Pooled) HPV (%DNA)	OR	Male Cohort
Age (yrs.)					
<16	9	891	1.0		1 (95% CI)
16-25	273	794	5.2	0.75	(0.26-1.0)
25-34	332	771	38.8	5.52	(1.7-12.0)
34-43	47	849	30.3	4.0	(1.35-10.0)
>43	20	880	2.2	0.8	(0.29-1.95)
Education					
None	96	804	10.6		1
Primary	162	643	18.0	2.0	(0.86-4.45)
Secondary	90	810	10.0	1.82	(0.61-5.11)
Tertiary	50	850	5.5	0.80	(0.29-1.10)
Smoking					
Never	100	800	11.1		1
Ever	222	668	25.8	8.0	(1.36-47.7)
No of Life Time Sexual Partners					
1	90	910	10.0		1
2	197	783	13.0	1.7	0.40-6.90
>3	136	850	16.0	2.4	0.43-9.50
HSV History					
Never	99	801	11.1		1
Ever	117	967	12.1	1.4	(0.61-3.42)

Discussion

Age

Prepubertal male and female aged <16 years had significantly low($P<0.05$) prevalence of HPV DNA. It is suggested that this age group would be better targets for candidate vaccine preventative against HPV infection. This is due to their sexual naivity and documented strong immune responses in this age category. This agrees with the work of Nathalie and Oberdan (2000) 12 who tested the efficacy of vaccine cervarix developed by GLAXO SMITH KLINE(GSK) on this category of cohort. In the same manner, the highest prevalence, unisex, was $\leq$ 25-34 years and $\leq$ 34-43 years age brackets. It is suggested that these groups be targeted for therapeutic vaccine testing. This agrees with the earlier work of Schwartz, (2006): Frazer *et al* (2006) ; and Christopher, (2007). These groups advocated the usage of curative /therapeutic vaccines for those already infected and preventative for those at risk of acquiring the HPV infection in future oncogenic HPV infection vaccine strategies. Further, this also agrees with the findings of Gissman , (1999) who conducted experiment with this regard on Mice- aimed at stimulating CD8 T cells to target cancer associated proteins E7 and E6 of high risk HPV types 16 and 18 were detected in semen and prostatic tissues. This suggests that men probably serve as potential reservoirs for the transmission of these high risk viruses of cervical cancers.

Table 2: Major risk factors for HPV DNA detection using Multiple Logistic Regression Analysis of subjects personal data in female cohorts

Personal Data	Total Positive	Total Negative	Positive (Pooled) HPV (% DNA)	OR	Male Cohort
Age (yrs.)					
<16	16	884	1.8		1
16-25	45	855	5.0	0.38	(0.19-1.01)
25-34	322	578	35.8	4.40	(1.45-12.0)
34-43	261	639	29.0	3.18	(1.36-7.35)
>43	18	882	2.0	0.40	(0.39-7.35)
Education					
None	99	901	11.0		1
Primary	198	702	22.0	2.0	(1.0-7.80)
Secondary	126	774	14.0	1.19	(0.20-8.46)
Tertiary	54	846	6.0	0.74	(0.26-1.10)
Smoking					
Never	98	802	10.9		1
Ever	72	828	8.0	2.10	(0.60-6.81)
Parity					
None	260.52	835	31.2	3.01	(1.59-5.55)
1-2	82	818	9.1		1
3-4	49	851	5.4	0.79	(0.28-1.09)
>5	35	617	7.2	1.78	(1.40-5.0)
History of Abortion					
Never	90	810	10		1
1	117	783	13.0	1.7	(0.40-6.90)
>2	198	702	22.0	2.7	(0.80-9.10)
No of Life Time Sexual Partners					
1	91	809	10.1		1
2	81	819	9.0	1.0	(0.71-2.61)
>3	135	765	15.0	2.1	(0.41-9.42)
HSV History					
Never	90	810	10.05		1
Ever	162	738	18.0	2.22	(1.44-3.64)
Oral Contraceptive					
Never	99	801	11.0		1
In Part Only	117	783	13.0	1.54	(0.70-3.22)
Current Use	299	601	33.3	3.00	(1.10-7.22)

Archival tissue examination showed that the overall mean prevalence in Male cohort archival Prostatic/Anal tissue was 24(100), 24% . The standard deviation here was only 1.06. At 95% confidence limits , there was no significant difference(P>0.05) in % HPV DNA prevalence in male and female cohort archival tissue samples . Suggesting that tissue samples provide better results for HPV DNA diagnosis that body scrapings and fluids. From the hypothesis proposed, we retained the null hypothesis. This agreed with the report of Nicolson, (2004) who reported similar route of HPV transmission from infected men to their women sexual partners--- with semen serving as the vehicle of infection. In artificial insemination, donated semen samples should be properly screened for HPV infection in the light of the above reason , irrespective of the fact that these semen samples are usually pre-washed. This agreed with the findings of Araneta *et al* (1995) and Olatunbosun *et al* (1990) who reported that prescreening of semen for the presence of high risk HPV types would go a long way in preventing men directly transmitting the virus to women. Direct assay for high risk HPV types including HPV type 16 and HPV type 18 would also go a long way in stamping out misdiagnosis either as a result of subjectivity or error in interpreting low grade squamous intraepithelial lesions for high grade. Further the fact that this study detected HPV DNA types in prostatic and anal tissues as well as seminal fluids agrees with the finding of Olatunbosun *et al* ; (1990). This observation is contrary to the popular impression among some medical doctors. This could be attributed to the fact that in Nigeria and other parts of Africa , there is paucity of information regarding the prevalence of HPV in seminal fluid and prostate tissue. Since there are possibilities of latency and persistency (Olatunbosun *et al*, 1990) the role of HPV in benign prostate hyperplasia / and also in prostate cancer may not be ruled out. In fact , it could be an important factor in geriatric benign prostate hyperplasia-which could be due to prolonged persistence of high risk HPV types 16 and 18. This agreed with Olatunbosun *et al* (1990) who made similar observation.

Smoking

The analysis of the results of this research showed that male and the female cohorts who smoke cigarettes had an OR of 8.0 and 2.10 respectively for HPV DNA detection in both male and female cohorts , when standardized against those who never smoked cigarette . This suggests some level of significance (P<0.05). In other words cigarette smoking is positively associated with HPV DNA detection in both male and female cohorts This finding is in agreement with previous studies by Wang *et al*,(2003); Bauer *et al*. (1993); Olatunbosun *et al* (1990); McNicol and Dodd, (1990) - they positively associated cigarette smoking with HPV DNA infection.

Education

From the data on tables 24-25, the analysis of the questionnaire' result earlier conducted , the mean prevalence of HPV DNA was observed to decrease with the level of education of the cohorts, unisex. As seen earlier, this was22.0 % and 18.0% for those that had primary education in the females and male cohorts respectively.14.0% and 10.0% for those that had secondary education in the female and male cohorts respectively; while these were 6.0% and 5.0% for those that had secondary education in the female and male cohorts respectively. It is suggested that better literacy enhances the individuals knowledge of the HPV infection which could support strategy to prevent infection against HPV, provide useful information on how to access medical attention if such individual is already infected with HPV, and guides the individual against risky factors earlier enumerated for HPV infection.

Parity

Nulliparity, which was 31.2% in the female cohorts ,seemed to be one of the major risk factors associated with HPV DNA detection. When Standardized against the female cohorts that have parity in the normal range of one or two children per birth rate. It is suggested that their inability to have children could be directly responsible for increased rate of sexual activity, complications of various sorts , and exposure to increased risk of opportunistic infections such as Human papilloma virus ,HPV infection, herpes simplex virus, HSV, infection , Human immunodeficiency virus, HIV infection, etc This agrees with the work of Jacobs *et al*;(2000).

Abortion

It was observed that female cohorts who never had abortion , had % HPV DNA of 10% while those who had ≥ 1-2 instances of abortion had % HPV DNA of 13% and 22% respectively. In the analysis of the result done earlier on this, it was observed that frequent instances of abortion tend to significantly (P<0.05) affect the mean prevalence

of HPV DNA in the female cohorts. For females that are desperately in search of children, frequent abortion promotes more sexual activity which could lead to higher probability of acquiring HPV infection and other sexually transmitted diseases such as HIV and HSV. Other hospital acquired infections (nosochomia infections) such as Herpes virus infection, cytomegalovirus infection e.tc. are also very likely- as the woman is prone to be always in and out of the hospital. All these infections could become opportunistic –which probably may support the acquisition of HPV infection.

Number of lifetime Sexual Partners

This study showed that the most consistent risk factor for HPV infection and HPV DNA positivity was increased number of sexual partners. From the results , female cohorts , who had ≥ 3.0 sexual partners had 15% positive cases of HPV DNA prevalence., while in the male cohorts , 16% of them had HPV DNA prevalence. When these values were standardized with those who had just one sexual partner, the OR values were 2.05 and 2.8 respectively-these values were quite significant ($P<0.05$); Suggesting some level of associations between lifetime number of sex partners and genital HPV acquisition. This agrees with the works of Tarkowski *et al* , (2004) ; Wheeler *et al* (1993) ; Franceeschi *et al*,(2002) Svare *et al* ,(2002); As they reported that higher rates of sexual activity tend to enhance greater chances of human papilloma virus infection .

Conclusion

Sexually active age (26-34 years), Poor educational background with its accompanied wretched attributes of retrogressive – self development , management , and impediment to vital information and understanding ; Ever cigarette Smoking habit , ;Multiple sexual partners , all significantly ($P<0.05$) enhance HPV DNA detection in the unisex cohorts . In addition , Nulliparity, current oral contraceptive usage were significant risk factors ($P<0.05$) in HPV DNA detection in the female cohorts. Awareness campaigns of the dangers associated with these risk factors in HPV infectivity should be stepped up by the various health organs of government in various localities for effective combat /control.

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