Original Article

Prevalence and Genotype of Human Papilloma Virus (HPV) in Cervical Smear in Sokoto, North-Western Nigeria: A sentinel study to guide vaccination.

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Abstract

Background: Cancer of the cervix uteri is the most common gynaecological cancer and a leading cause of cancer mortality among women in the developing countries, including Nigeria, with high-risk HPV being the major aetiological factor. **Aims:** This study is aimed at estimating the prevalence of high-risk HPV infection in Sokoto, North-Western Nigeria. **Methodology**: This was a prospective cross-sectional study. Relevant information social and demographic information were obtained through personal interviews using structured questionnaire. Two Samples (LBC and HPV) were collected from 84 consenting participants who visited the gynaecological clinic during the study period. LBC smear was obtained, and smear results were classified using The Bethesda System of classification, 2014. Hybribio 21 HPV Geno array test kit with polymerase Chain Reaction for the HPV DNA detection was used. **Results**: Out of 84 sample tested for HR-HPV, 72 (88.9.%) cases were negative, while 12 cases were positive for HR-HPV constituting 14.3 % of the cases. The HR-HPV were seen more among the age group of 25-30yrs (33.3%) and the HPV genotype identified include 13, 16, 31, 33, 39, 45, 51, 52 and 58 respectively. Out of the 12 positive cases, three of the cases shows co-infections with other HR-HPV genotypes (16 & 52, 31 &33, and 39 & 51 respectively) and single infection by the 13,31,45,52, and 58 respectively. Three cases each were infected by HR-HPV 13 and 58 being the most common infections, followed by HR-HPV 31 and 52 infecting two cases each and the remaining (16,33,39,45,51), one case each. **Conclusion**: This study shows that HR- HPV 13 and 58 are the most common HPV subtype, follow by HPV 31 and 52 in our environment.

Keywords: Prevalence, HR- HPV genotype, cervical smear, Sokoto,

INTRODUCTION

Cervical cancer is the second most common malignancy in women worldwide and is a major cause of cancer mortality among women.[1] Worldwide, about 604,127(13.3%) new cases are diagnosed every year with approximately 341,831(7.3%) of deaths occurring in developing countries of the world.[1] In Nigeria the incidence of cervical cancer is 32.9 per 100,000 and the mortality rate is 9,659 per 100,000.[1,2]

Epidemiologic and virologic studies have clearly demonstrated that specific human papillomavirus (HPV) types play a central role in the development of cervical cancer and its intraepithelial precursor lesions.[2,3] The association between HPV, Cervical Intraepithelial Neoplasia (CIN) and cancer has been confirmed.[4,5]

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Although the number of known genital HPV types now exceeds 40, certain types (notably, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) account for nearly 90% of the HPV types detected in high-grade Squamous intraepithelial lesions (HSILs) and cancer.[3]These HPVs are often described as "cancer-associated types," with HPV types 16, 18, 45 and 31 being the most common type detected in cervical cancers in women from all geographic areas surveyed worldwide. [2,6-9]

The prevalence of HPV infection is being reported to be between 10-20% and it is dependent on the age of the patient and the presence of cytological abnormalities. In some populations, cross-sectional studies show that 20%-40% of sexually active young women have detectable HPV infection and that prevalence decreases with age.[10]

METHODOLOGY

This was a descriptive prospective cross-sectional study carried out at the gynaecological clinics of Usmanu Danfodiyo University Teaching Hospital (UDUTH), Specialist Hospital Sokoto and Maryam Abacha Women and Children Hospital, Sokoto. Relevant information (such as age, parity, history of vaginal discharges, hysterectomy, previous biopsy or cytology etc.) from consenting patient within reproductive age group and post-menopausal women were obtained, through personal interviews using structured questionnaire. Samples were collected from 84 consenting patients who visited the clinic during the study period. Two separate samples were taken from each patient (one for LBC and the other for HPV genotype).

Liquid Based Cytology sample was obtained using cytobrush and was immediately inserted in the LBC preservative container. The sample collected was transported to the laboratory for analysis, the laboratory technician centrifuged each labelled sample separately and then a smear was made on a glass slide and fixed immediately in 95% ethanol. The slides were stained after fixation using Papanicoloau staining procedure. Smear results were classified using Bethesda classification, 2014.

The samples for the HPV genotyping were collected in the hybribio HPV DNA collection kit provided for HPV DNA test. The samples were stored in freezers and transported to 'The Specialist Laboratory (TSL)' in Lagos for the HR-HPV genotyping via air transport system to main cold chain. In the laboratory, the cell suspensions from the hybribio HPV DNA collection kit were analysed using the hybribio 21 HPV Geno array test kit which uses PCR amplification and flow through hybridization to characterize the HPV genotypes. The results were interpreted by direct visualization of the membrane for colour change of specific genotype. Women who have not consented or who are pregnant or had undergone hysterectomy or conization were excluded from the study.

An ethical clearance and permission to conduct the research was obtained from the Research Ethics Committee (REC) of Usmanu Danfodiyo Teaching Hospital. Informed consent was obtained from each participant before questionnaire was administered and samples taken.

RESULTS

A total of eighty-four (84) samples were tested for HR-HPV in this study. Seventy-two cases accounting for 85.7% were negative while the remaining 12 cases (14.3%) were positive for HR-HPV (Table 1).

Table 1. Age Distribution for HR-HPV Genotype

Age Group (Years)	HR-HPV (Negative)	HR-HPV (Positive)	TOTAL (%)
20-24	10	3	13
25-30	13	4	17
31-34	10	1	11
35-40	14	1	15
41-44	8	1	9
45-50	6	1	7
51-54	8	1	9
55-60	3	0	3
TOTAL	72 (85.71%)	12 (14.28%)	84 (100%)

Table 2. Age Distribution for Cytological Diagnosis

	Cytological Diagnosis				
Age Group	Inadequate	Inflammatory	NILM	LSIL	Total
20-24	1	2	8	2	13
25-30	1	4	9	3	17
31-34	0	3	6	2	11
35-40	1	2	8	4	15
41-44	0	2	6	1	9
45-50	0	1	5	1	7
51-54	0	3	5	1	9
55-60	0	1	2	0	3
TOTAL	3	18	49	14	84

The HR-HPV infection were seen more among the age group of 25-30yrs representing (33.3%) of the positive cases while negative cases occurred more among 35-40 years age group (Table 1). The genotypes identified were 13, 16, 31, 33, 39, 45, 51, 52 and 58 respectively. Out of the 12 positive cases three of them showed co-infections involving HR-HPV types (16 & 52, 31 & 33, and 39 & 51 respectively) and single infection by types 13, 31 ,45, 52, and 58 respectively. Three cases each were infected by HR-HPV 13 and 58 making them the most common

genotypes identified in this study followed by HR-HPV 31 and 52 infecting two cases each and the remaining (16, 33, 39, 45, 51), one case each.

Fourteen cases (16.7%) were diagnosed as having low grade squamous intraepithelial lesion (LSIL) on cytology, while 49 (58.35%) were Negative for intraepithelial lesion or malignancy (NILM). The remaining cases includes 18 (21.4%) inflammatory lesions and 3 (3.6%) samples that were inadequate for cytological diagnosis according to The Bethesda System adequacy criteria.

DISCUSSION

Up to 99.7% of cervical cancers have been found to carry the HPV genome.[11, 12] Cancer associated HPV types have been detected in a smaller percentage of LSILs. In view of these findings, it is possible that HPV DNA testing would provide a useful triage for women with LSIL. Women with cancer-associated HPV types could be referred for immediate colposcopy, and those without could be followed with annual Pap smears.[3]

A Hospital based study conducted by Aggarwal R et al in northern Indian among women visiting gynaecological outpatient clinic with different complained, were subjected to pap smear with HPV testing, found that HPV type 16, 18 and 22 was the most common.[13] The prevalence of HR-HPV was said to be 12.34% among heathy women with good personal hygiene in the study population in a study conducted by Akhtar et al in communities of Bihar India.[14] The prevalence of HR-HPV among Iranian women was said to be 24% in heathy women and 78.8% among cancer patient in a study conducted by Farahmand et al.[15] Castellsagué X et al in a study conducted in rural Mozambique among age stratified pap smear and HPV genotype samples taken, found that HPV 35 was the most commonly identified genotype among HPV-positive women 17% and women with cervical Neoplasia 30 %[16], also in the same study the interpretations of Papanicolaou smear shows that 12% of the women had cervical intraepithelial neoplasia.[16] In the current study we found out the prevalence of squamous intra-epithelial lesion to be 16.7% among the study population, which is higher than in the Castellsagué X et al study even though we have different sample size which may influence the prevalence rate. The presence of HPV-DNA was determined in tumour biopsies of cervical cancer patients and in cervical swabs of non-cancer patients from Tanzania, East Africa by Ter Meulen J et al[17]. They found that types 16 and 18 with 13.2% and 17.5%, respectively, of all HPV infections. Therefore they concluded that HPV 18 is more prevalent in Tanzania than in any other geographical location so far reported.[17] In Dakar and Pikine, West Africa, Xi LF et al found that the prevalence of HPV infection was 18%, among women with negative cytological findings, and also that the prevalence of high risk HPV types increased with age with HPV16 (2.4%) and HPV58 (1.6%) were the most frequently detected HPV types in this population.[18] In Ibadan the prevalence of HPV was 26.3% as documented by Thomas JO et al, also in the same study the genotype of HPV types 16, 31, 35 and 58 was predominat.[19] In Ile-Ife, Fadahunsi OO et al documented HPV prevalence of 21.6% and The predominant HPV genotypes were HPV 16, 53, 18 and 52.[20]

Mark Schiffman et al in a study on HPV testing in cervical cancer demonstrated that there was now overwhelming evidence from randomized clinical trials that carcinogenic HPV DNA screening is more sensitive than cytological screening for detecting histological CIN3. Even more important, a negative HPV test provides long-term risk stratification: 5–10 years of reassurance (i.e., a high negative predictive value) of not developing CIN3 and even stronger reassurance of not developing invasive cancer among HPV DNA–negative women. High negative predictive value permits safe and cost-effective lengthening of the cervical screening interval when HPV testing is used.[21]

Currently, there are neither effective means of preventing HPV transmission nor cures for the clinical manifestations; infection can only be prevented via complete sexual abstinence, while treatment for clinical sequelae such as genital warts and cytological abnormalities consists of removing the problematic cells and watching for recurrence. This method consumes significant health care resources and is costly.[22] New prophylactic HPV vaccines promise to dramatically reduce the incidence of HPV infection, genital warts, and cytological abnormalities.[22] The proven effectiveness of HPV vaccine in clinical trials to date suggests that HPV vaccination may represent a viable preventive strategy in the fight against cervical cancer.[23] Because geographical variation in HPV types distribution exist, knowledge about the distribution of HPV types in cervical cancers and HPV types circulating in the communities in different regions of Nigeria would be useful in devising the optimum strategy for vaccination in Nigeria.[24]

CONCLUSION

This study shows that HR- HPV 13 and 58 are commoner than HPV 31 and 52 in our environment.

Recommendation

High prevalence of HR-HPV may be a distinctive feature of our population therefore, further studies need to be done to expand the population size in other to objectively relates the HR-HPV genotypes in our population to

commercially available vaccine types for appropriate vaccination programs implementation in Sokoto.

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Conflict of Interest**:** The authors have declared that no competing interest exist.

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