Prevalence of High Risk Oncogenic Human Papillomavirus Types in Cervical Smears of Women Attending Well Woman Clinic in Ile Ife, Nigeria

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Abstract

This study is aimed at estimating the prevalence of cervical infection with High risk Human Papillomavirus (HR-HPV) in cervical smears of women attending the Well Woman Clinic of the Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife. This is a community based clinic where women are screened for cervical, breast and other female related diseases. This is a prospective cross-sectional observational study. Information was obtained through personal interviews using structured questionnaire. Cervical samples were collected from 118 consenting women visiting the clinic during the study period. Conventional Pap smear was obtained and smear results were classified using Bethesda classification, 2001. HPV DNA was detected using the hybribio 21 HPV Geno array test kit which uses Polymerase Chain Reaction (PCR), amplification and flow through hybridization. The data obtained were analyzed using simple and inferential statistics.

The mean age of the participants was 42.9 years (SD ± 10.9). A total of nine different HR-HPV types were identified with an HPV prevalence of 21.6% overall and 22.7% among women with cervical lesions. The predominant HR-HPV types were HPV 16, 53, 18 and 52. In all, 41.7% of the infections involved more than one HPV type. Unlike in most populations studied so far, HPV prevalence was high not only among young women, but also in middle and old age. It was also observed that the prevalence of HR-HPV increases with parity.

This study shows that HPV 53 is the second most common type after HPV 16 in our environment. High prevalence of HR-HPV in all age group may be a distinctive feature of our population of women where HPV transmission continues into the middle age and cervical cancer incidence is very high.

Keywords: Cervical smears; Human papillomavirus; Cervical cancer

Background

Cervical cancer is the second most common malignancy in women worldwide and is a major cause of cancer mortality among women. Worldwide about 500,000 new cases are diagnosed every year with approximately 85% of deaths occurring in developing countries of the world [1]. In Nigeria the incidence of cervical cancer is 14,550 per 100,000 and the mortality rate is 9,659 per 100,000 [1].

The prevalence of HPV infection has been 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 [2].

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 [3]. New prophylactic HPV vaccines promise to dramatically reduce the incidence of HPV infection, genital warts, and cytological abnormalities [3]. 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 [4].

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 [5].

Material and Method

The study was conducted among women attending the Well Woman Clinic at Obafemi Awolowo University Teaching Hospital Complex (OAUTHC) Ile Ife for routine Pap smear. The Well Woman Clinic is a community based clinic where women are screened for cervical, breast and other female related diseases. All women attending the clinic usually have routine Pap smear done. Informed consent was obtained from the women and ethical clearance was obtained from the Ethical Committee of OAUTHC.

Keywords: Cervical smears; Human papillomavirus; Cervical cancer

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One hundred and eighteen (118) samples were collected between March 2010 to February 2011 (one year) from women residing in Ile Ife and its environs, attending the Well Woman Clinic for routine Pap smear during the study period.

Women who were less than 18 years of age, pregnant or had undergone hysterectomy or conization were excluded from the study.

**Sample collection and cytology**

Samples were collected using cytobrush ( cervix³) cervical cell sampler. The sampler was introduced into the cervix and the squamocolumnar junction scraped by a 360 degree rotatory movement. This was then applied to a glass slide and fixed immediately in 95% alcohol or by a fixative spray. The slides were then stained using Papanicolaou staining procedure.

The sampler was thereafter dropped in the hybribio HPV DNA collection kit provided for HPV DNA test. Cytology results were classified using Bethesda classification of 2001.

**HPV DNA typing**

The residual cell suspensions from the hybribio HPV DNA collection kit were frozen at -20°C. The frozen samples were transported to Lagos University Teaching Hospital (LUTH) in iced cold pack for DNA analysis 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 color change of specific genotype.

**Data analysis**

The data obtained was analyzed using SPSS version 15. Simple and inferential statistic was used where applicable. Level of significance was taken as P ≤ 0.05.

**Results**

Of the total 118 women recruited for this study from the Well Woman Clinic OAUTHC, 38 of them were traders, 29 were civil servants, and 29 were professionals, while artisans, undergraduates and unemployed accounted for the others. The age range was 18 to 68 years (mean age=42.9 ± 10.9 years SD). The peak age group was 35-44 years with 46 women (39%) while the lowest number of participants recorded was 5 women (4.2%) in the >65 years age group. The age group 45-54 years had 37 (31.4%) women, while age groups 25-34 years, 55-64 years and less than 25 years had 16 (13.6%), 8 (6.8%) and 6 (5.1%) women respectively (Figure 1).

One hundred and fourteen women had adequate cytological results while four had inadequate results, however the women with inadequate results did not have a repeat smear. Among the women for whom Pap smear results were available, 92 (78%) were negative for intraepithelial lesion or malignancy (NSILM), 5 (4.2%) showed Atypical Squamous Cells of Undetermined Significance (ASCUS), 17 (14.4%) had Low Grade Intraepithelial Lesion (LSIL) and none of the women had High Grade Squamous Intraepithelial (HSIL) or glandular lesion. LSIL was highest among women of the 45-54 age groups (27%) while women less than 25 years were all negative for cervical lesion. ASCUS accounted for 8.7% of lesion found in the 35-44 age group which was the highest seen while none of the women in the age group less than 25 years or greater than 65 years had ASCUS (Table 1). There is significant relationship between different age group and Cytological diagnosis (P<0.05).

**Results of HPV testing**

One hundred and eleven women had valid HPV results; among the women for which HPV test was available twenty four of them had high risk HPV accounting for 21.6% of all the women while 87 women (78.4%) were negative for HR-HPV DNA. The prevalence of HR- HPV of any type was 21.6% among all the women while the prevalence among women with cervical lesion was 22.7% and the prevalence among women with normal cytology result was 20.7%. Only nine serotypes out of the 15 HPV serotypes tested for were found in the study population. High risk HPV 16 was the commonest genotype found and it accounted for 30.8% followed by HPV 53 (28.2%) and HPV 18 (23.1%) respectively. The least common HPV genotypes were 33, 35, 45, and 68 each representing 2.6% each (Figure 2). A total of 14 (58.3%) women had single HR- HPV infection and 10 (41.7%) had multiple genotypes. The most commonly found HPV types in multiple infections were HR- HPV 16, 53(47.1%), 16, 18 (23.5%) and 18, 53 (17.6%). Table 2 shows the relationship between the HR-HPV positivity and age. HR-HPV positivity prevalence was not different in the age groups considered, ranging between 33.3% among women younger than 25 years, 25.6% among those in the 35-44 years age group,12.5% in 55-64 years age group, 20% in 25-34 years age group and 20.6% in the 45-54 years age group. There is no significant relationship between age of women and HR-HPV. Nulligravida women account for 8.3% of women with HR-HPV, while 54.2% women with HR-HPV had five pregnancies or more (Table 3). Women with 1-2 pregnancies and 2-3 pregnancies were responsible for 8.3% and 29.1% of women with HR-HPV positivity respectively. There is a significant relationship between parity and HR-HPV (P<0.05).

<table>
<thead>
<tr>
<th>Age group</th>
<th>NILM (%)</th>
<th>ASCUS (%)</th>
<th>LSIL (%)</th>
<th>INADEQUATE (%)</th>
<th>TOTAL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;25</td>
<td>6 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>6 (5.1)</td>
</tr>
<tr>
<td>25-34</td>
<td>12 (75)</td>
<td>0 (0)</td>
<td>1 (6.3)</td>
<td>3 (18.8)</td>
<td>16 (13.6)</td>
</tr>
<tr>
<td>35-44</td>
<td>39 (84.5)</td>
<td>4 (8.7)</td>
<td>2 (4.3)</td>
<td>1 (2.7)</td>
<td>46 (40)</td>
</tr>
<tr>
<td>45-54</td>
<td>26 (70.3)</td>
<td>1 (2.7)</td>
<td>10 (27)</td>
<td>0 (0)</td>
<td>37 (31.4)</td>
</tr>
<tr>
<td>55-64</td>
<td>6 (75)</td>
<td>0 (0)</td>
<td>2 (25)</td>
<td>0 (0)</td>
<td>8 (6.8)</td>
</tr>
<tr>
<td>&gt;65</td>
<td>3 (60)</td>
<td>0 (0)</td>
<td>2 (40)</td>
<td>0 (0)</td>
<td>5 (4.2)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>92 (78)</td>
<td>5 (4.2)</td>
<td>17 (14.4)</td>
<td>4 (3.4)</td>
<td>118 (100)</td>
</tr>
</tbody>
</table>

P=0.012
NILM: Negative for Intraepithelial Lesion or Malignancy
ASCUS: Atypical Squamous Cell of Undetermined Significance
LSIL: Low Grade Squamous Intraepithelial Lesion

Table 1: Cytological diagnoses (Bethesda) among different age groups.
Table 2: Age group distribution of women with HR-HPV.

<table>
<thead>
<tr>
<th>Age group</th>
<th>HR-HPV</th>
<th>HR-HPV</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;25</td>
<td>4 (66.7)</td>
<td>2 (33.3)</td>
<td>6 (5.4)</td>
</tr>
<tr>
<td>25-34</td>
<td>12 (80)</td>
<td>3 (20)</td>
<td>15 (13.5)</td>
</tr>
<tr>
<td>35-44</td>
<td>32 (74.4)</td>
<td>11 (25.6)</td>
<td>43 (38.7)</td>
</tr>
<tr>
<td>45-54</td>
<td>27 (79.4)</td>
<td>7 (20.6)</td>
<td>34 (30.6)</td>
</tr>
<tr>
<td>55-64</td>
<td>7 (87.5)</td>
<td>1 (12.5)</td>
<td>8 (7.2)</td>
</tr>
<tr>
<td>&gt;65</td>
<td>5 (100)</td>
<td>0 (0)</td>
<td>5 (4.5)</td>
</tr>
<tr>
<td>Total</td>
<td>87 (78.4)</td>
<td>24 (21.6)</td>
<td>111 (100)</td>
</tr>
</tbody>
</table>

P VALUE = 0.928

Table 3: Relationship between parity and HR-HPV positivity.

<table>
<thead>
<tr>
<th>NO. of pregnancy</th>
<th>HR-HPV Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>2 (8.3)</td>
</tr>
<tr>
<td>1-2</td>
<td>2 (8.3)</td>
</tr>
<tr>
<td>3-4</td>
<td>7 (29.1)</td>
</tr>
<tr>
<td>5-6</td>
<td>10 (41.7)</td>
</tr>
<tr>
<td>&gt;7</td>
<td>3 (12.5)</td>
</tr>
<tr>
<td>Total</td>
<td>24 (100)</td>
</tr>
</tbody>
</table>

P = 0.036

The prevalence of HR-HPV positivity found among women during routine Pap smear in Ile-Ife, Nigeria is 21.6%. This is similar to a study done by Scharzt et al. in Okene central Nigeria which found a prevalence of 19.7% [6]. The prevalence of HR-HPV in the study done in Ibadan by Thomas et al. was 16.6%. This is lower than the prevalence found in our study, probably due to the fact that their study was a population based study while ours was a community-hospital based study [7]. In a hospital based study done in rural India by Aggarwai et al. a prevalence of 8.2% was reported [8].

The age pattern shows peak of HR-HPV positivity in women of age 35-44 years (39.0%) while middle aged women (45-54 and 55-64 year age groups) account for 38.2% of HR-HPV. This is similar to the study done in Ibadan which showed a higher prevalence among older women and this has been associated with a higher incidence of cervical cancer [7].

The prevalence of HPV in women with normal cytology is 22.4% which is similar to the report of World Health Organization (WHO) information center of HPV and related cervical cancer in Nigeria that reported a prevalence of 23.7% of HPV in normal cytology [8]. The prevalence of HR-HPV among women with abnormal cytology is 22.7%. The prevalence amongst women with normal and abnormal smears were similar due to the fact that cytological abnormalities caused by HPV do not manifest immediately after being infected with the virus.

Previous HPV surveys in sub-Saharan Africa have generally shown relatively high prevalence with some variations, depending on how women were selected and how HPV was tested for. Using the Hybrid Capture (HC) assay, 17% prevalence of HR-HPV types was found in rural Uganda, while a prevalence of 8.2% was found in women in rural India [9,10]. Polymerase chain reaction (PCR)-based assays showed HPV prevalence of 40% in rural Mozambique, 31% in Harare, Zimbabwe, 18% in Dakar and Pikene, Senegal, and 44% in Nairobi, Kenya. The higher prevalence in PCR-based studies is due to the higher sensitivity of the test [11-14].

International study shows a prevalence of 27% among women receiving treatment in an STD clinic in America in a study done by Datta et al. The prevalence was highest among person aged 14-19 and it was observed that it decreased with increasing age [15].

The prevalence of high risk HPV in cervical smears was found to be higher in African American (45.1%) compared to white woman (27.3%) in a study done by Bansal and Zhao in Pittsburgh, USA [16]. Multiple HR-HPV infection shows a high level in several studies done worldwide [17,18].

Out of the total women with HR-HPV, 41.7% have multiple infections in our study. Didelot-Rousseau et al., in a study done in Burkina Faso found a multiple infection rate of 50.1% while a multiple HR –HPV infection rate of 33.5% was found in Ibadan [7,19].

In this study, HPV 16 is the most common HR-HPV which is consistent with previous studies done in Africa [17-23]. It is followed by HPV 53 which was also reported by Franco et al. in Brazil [24]. In Mozambique HPV 35 was found to be slightly higher than HPV16 [11]. HPV 52 was noted to be slightly more than HPV 16 or 35 in Kenya [12]. In Senegal, HPV16 and 58 were the most common in women with cervical lesions [13]. In the study done by Thomas et al. the commonest HR-HPV is HPV 16 followed by HPV 31 while HPV 31 was not found in our study [7]. Presently there are no vaccines against common HR-HPV (31, 33, 52 and 53) found in Africa, studies done in Africa may promote the production of vaccines targeted at HR-HPV genotypes prevalent in the continent.

The reported age pattern of HPV prevalence differs from one country to another, but the predominant reported pattern shows an early peak in the young age groups, soon after the start of sexual intercourse [25-28], followed by a steady decline in middle age, after clearance of a large proportion of acquired infections, and a steady state in the age group 40 and above [17,29-32].

In two other sub-Saharan African studies, HPV prevalence showed no significant decline with age, and indeed, in one of these two studies high-risk HPV were more frequently detected in older than younger women [7,13]. The age-specific point prevalence of cervical HPV infection in our study group was notable, with a peak among women less than 25 years of age, followed by women in 35-44 year group. The explanation for the persistent high prevalence of HPV in middle and older aged women, specifically, in the Ghanaian and Nigerian societies where polygamy is generally accepted, a fraction of men and women (mainly men) may continue to have multiple sexual partners throughout the year and this can lead to multiple sexual partners and thus increased risk of HPV infection.
their life and therefore re-infect themselves and their spouses [7]. Additionally, as previously suggested, women in developing countries, like Ghana, Nigeria and India, may have decreased ability to clear HPV infections, possibly due to concomitant genital infections or nutritional deficiencies, since the development of an efficient immune response against HPV acquired over age is the generally accepted reason for the decline in HPV prevalence observed in other populations [7]. The groups of women who remain persistent carriers of HPV by middle age are now considered the high-risk group for cervical cancer [32,33].

It was observed in this study that women with high parity (three pregnancies or more) accounted for 83.7% of women with high risk HPV and this is consistent with a study done in Washington D.C, USA by Hildesheim et al. [34]. It was found in these group of low income earners that the prevalence of HPV increases with the number of pregnancies, although a study done by Lazcono-Ponce et al. showed no association between numbers of pregnancies and HPV infection [35]. There is still insufficient data to give final conclusions about the effect of number of births on the risk of HPV infections.

Majority of the women had normal cytological diagnosis (81.4%). This is consistent with the study done in Ibadan which showed 90.6% normal cytology among the women studied. Abnormal cytology smear was seen in the older age group while women less than 25 years have no cervical lesion; this may be explained by the fact that it takes about an average of 10 to 15 years for infection with HR-HPV to transform into malignancy [36].

The prevalence of HR-HPV is not significantly different in the women generally (21.6%) as compared with women with abnormal cytology (22.7%) and this is consistent with WHO report on HPV and related cancer in Nigeria [8].

This study shows that HPV 53 is the second most common type after HPV 16. High prevalence of HR-HPV in all age group may be a distinctive feature of a population where HPV transmission continues into the middle age and cervical cancer incidence is very high. It is advocated that HPV vaccine made available in the country should take into cognizance the peculiarities of HPV genotypes present in the community. Population based study need to be done for further characterization of HR-HPV genotypes in our environment.

References

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