Molecular Detection and Typing of Cervical Human Papillomavirus in a Selected Population in Bayelsa State: Comparison with the National and International Trend and Clinical Significance

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Authors’ contributions

This work was carried out in collaboration among all authors. Author YIO designed the study, performed the laboratory analyses, wrote the protocol and wrote the first draft of the manuscript. Authors AAN manage the histological analyses. Author YMT manage the molecular analyses. Author SIM manage the cytological reporting. Author IAI handle cervical sampling and literature search. Author SEI manage statistical analyses and literature searches. Author ABA handle editing of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

**Background:** The link between cervical lesions and human Papillomavirus (HPV) 16 and 18 is well established, but the magnitude of the risk of association and the importance of other high-risk HPV types is uncertain in Bayelsa state.

**Aims:** The study was aimed at detecting and typing of cervical HPV among selected subjects, establish the relationship between cervical dysplasia and HPV and also assess the level of knowledge of HPV, perception and attitude of women in Bayelsa State.

**Materials and Methods:** Questionnaires were used to assess the level of knowledge of HPV and cervical cancer. Papanicolaou stain for cervical cytology and Haematoxylin and Eosin stain used to study general tissue structure. Nested PCR was used to detect and multiplex PCR for typing.

**Results:** The prevalence of HPV spectrum among participants was 52% while high risk HPV was 24%. Five (5) subtypes were identified. The subtypes identified were HPV 52 (40.4%) most predominant, followed by 51 (1.9%), 45 (1.9%), 31 (1.9%) and HPV 30 (9.6%). The age-specific prevalence showed a peak prevalence of 44.2% in the ages of 25-34 years and lowest in the age group (15-24 years). Sequence alignment showed a single point mutation for HPV 45 and several points' mutation for HPV 52 at certain points of the sequenced nucleotides with Sequence no: 53CN12 and SeqH2011055303 and accession no: MG195999 and MG196000. HPV 52 was highest compared to previous studies, national and international. The level of knowledge on HPV infection and cancer of the cervix was low among subjects with a percentage score of 38.9%. Among other variables studied, life time sexual partners showed a statistically significant relationship in the prevalence of HPV (OR=0.02, P < 0.01). Perceived seriousness on HPV infection was high among HPV positive subjects compared with HPV negative participants ($\chi^2=16.39$, $p < 0.01$).

**Conclusion:** The emergence of HPV 52 in the study area requires public health attention and thus urgent need for local HPV vaccines production.

**Keywords:** HPV 52; Bayelsa; MG 195999; vaccine.

1. INTRODUCTION

Cervical cancer is one of the most common cancers that affect a woman's reproductive organs. It occurs when the cells of the cervix change in a way that leads to abnormal growth and invasion of other tissues or organs of the body. The cervix is the portion of the uterus that connects with the vagina. It is the gate of the uterus (womb).

Over the years, researches have principally focused on human Papillomavirus (hpv) serotypes 16 and 18 as purveyors of cervical cancer because it is believed to bring with them their oncogenes- E6 and E7 that bind and knock out p53 and Rb tumour suppressor genes respectively. This has assumed a central dogman when cervical cancer is mentioned vis a vis vaccine development. This gives a skew view of cervical cancer as researches have reviewed and identified several strains that are culprits in the lesion in this gateway of the womb.

Genital human Papillomavirus (hpv) contributions in invasive cervical cancer (ICC) ranges from 56.4% to 91.5% of all cervical cancer cases and this attributed majorly to HPV 16 and HPV 18 [1]. Previous studies has reported that HPV 35 was the fourth in the ranking before HPV 31 and 33 while HPV 18 and HPV 45 were considered second and third most frequent types identified. The percentages of HPV16 ranges from 38.5% in Mali [2] to 81.8% in Tanzania [3,4]. Previous study from Uganda reported HPV 16 as the highest (49%) followed by HPV 18 (23%), HPV 45 (13%), and HPV 35 (5%) [5]. Schmidt [6] has reported HPV pathology in women with ICC from South Africa, Ghana and Nigeria (2007 to 2010), prevalence stood at 93.7% of women with HPV positive and 86.8% with single type infection. In women infected with single HPV infection and those with squamous cell carcinoma (n = 447) were HPV16 (51.2%), HPV18 (15%), HPV35 (8.7%), HPV45 (7.4%). In women with adenocarcinoma (n = 27), HPV18 (29.6%) was the most common, followed by HPV16 (22.2%), HPV45 (18.5%) and HPV 35 (3.7%).

Furthermore in a meta-analysis by Guan [7] With a study population of 2,402, HPV positive cases with invasive cervical cancer (ICC) from Africa equally reported HPV16 (53.1%) as the most common, followed by HPV18 (19.8%), HPV 45...
(11.0%), and hpv 35 (4.9%) in all of the hpv positive invasive cervical cancer cases. The same study established that oncogenic potentials of the various hpv types over the disease spectrum among women showed that, hpv 35 was well represented in hpv-positive women with abnormal cytology (6.6%) and with either LSIL (7.9%)or HSIL (11.8%) [8]. Human Papillomavirus subtype 45 prevalence of 11.0% has been documented in Africa women, and was higher in invasive cervical cancer (ICC) studied in Africa. Comparing these findings, to other regions of the world, 3.0% in Eastern Asia to 6.1% in South/Central America HPV 45 was also higher in ICC, compared to 5.9% prevalence recorded in women with normal cytology [8,9].

In another study that evaluated the results of hpv DNA testing in 10,575 invasive cervical cancers (ICC) biopsies from 38 countries worldwide, 8,977 (85%) was positive for hpv [10]. Of the 691 cases from Africa continent, 544 (79%) were hpv positive; of these, 443 (81%) contained a single or undetermined hpv infection and 101 (19%) had multiple hpv types. Their study confirmed that hpv 16 is the most frequent type in ICC in Africa (48% among hpv-positive results), followed by hpv18 (23%), hpv 45 (10%), and hpv 35 (5%). Also, Bekele [10] reported that multiple infections were most commonly found in Sub-Sahara Africa (SSA). There is a wide regional variation of infection in SSA with multiple human Papillomavirus types between 2% in Ethiopia [11] and South Africa [12] and 34% in Mozambique [13]. Nugent [14] has documented 10 fold differences in multiple infection in HIV-positive women compared with HIV-negative women with Invasive cervical cancer.

The reported incidence of cervical cancer in Nigeria according to the information centre on hpv and cancer have been mere projection based on very few studies done among some selected population in Nigeria [15] and thus the need for a population-based study. The ranking of cervical cancer in Nigeria as the 2nd most frequent cancer among women between 15 and 44 years of age was also based on an estimate on the genital hpv prevalence in Ibadan, South western Nigeria [15]. In the Ibadan study, prevalence of hpv positive cases was 26.3% with the most prevalent high-risk hpv types were 16, 31, 35 and 58 [16]. Besides, in a study conducted in Abuja among HIV positive women revealed hpv 35 (8.7%) and hpv 56 (7.4%). The most prevalent high-risk hpv, hpv 52 and hpv 68 (2.8%, each) were the most prevalent types in human immunodeficiency syndrome negative women [17]. The commonest high-risk human Papillomavirus (hr-hpv) types seen among women in Lagos and Ogun State were types HPV 16, 35, 58 and 31 [18]. In another study, a rural community in Ondo state Nigeria, 10.0% prevalence of hpv was reported and it was also observed that 4.1% of these women were infected with single or multiple hpv genotypes with alpha-9 type which includes hpv 16, hpv 31, hpv 35, hpv 52 and hpv 58 been most common [19]. In another study done at Ille-Ife, Osun state, nine different hr- hpv types were identified with an hpv prevalence of 21.6% overall and 22.7% in women with cervical lesions. The predominant hr- types were hpv 16, 53, 18 and 52 [20]. The predominance of high-risk hpv types in Nigeria is trending, therefore, the need for this study to detect and type HPV among women attending two tertiary hospitals in Bayelsa state and make a comparison with that reported worldwide.

2. MATERIALS AND METHODS

Healthy sexually active female aged between 15 years and 65 years attending clinics at FMC Yenagoa and NDUTH Okolobiri Bayelsa State participated in the study. Cervical smears constituted 50% of the sample population while the remaining 50% were archived cervical tissue blocks (FFPE) previously diagnosed with various pathologies from 2010 to 2016 in the institution's histopathology laboratories. The research was both a retrospective and a cross-sectional.

2.1 Sample Size Determination and Sampling Methods

The prevalence reported by Gage [21] was used to determine the sample size for the study and a sample size of 100 samples were used for the study. Fadahunsi [22] has reported cervical cancer prevalence of 1.7% in Ille-Ife while Akarolo [19] had reported 14.7 % hpv prevalence in Irun, Nigeria. Also, Nugent [14] had reported worldwide hpv prevalence of 11.7%.

Therefore applying the formula $N = Z^2pq/d^2$ Where: $N = \text{the calculated sample size (for a population greater than 10,000)}$, $Z = \text{the standard (alpha) normal deviate usually set 1.96 which correspond to 95% confidence level}$, $p = \text{d degree of accuracy (precision) desired usually set at 0.05}$.

Snowball and convenience sampling technique was adopted, Snowball is a non-probability sampling technique based on referrals in which research participants' recruits
other participants hard to find. Convenience sampling is a non-probability sampling that involves the samples being drawn from that part of population that is close to hand [23].

2.2 Questionnaire Survey

The method of questioning was by Stephen [24] as modified by Udontre [25]. The questionnaires were structured in such a way to elicit an appropriate response from the respondents. The questions were divided into sections. This study adopted triangulation method of data generation.

2.3 Collection and Preparation of Cervical Tissues and Smears

Standard protocols were used in cervical tissues and smear preparations. Briefly, the participants’ vagina was dilated using plastic disposable speculum and sampled at the squamous columnar junction. The cervical smears were stained with Papanicolaou staining technique by Bancroft and Gamble [26] to identify cervical tissues morphology, lesions or dysplasia. The principle is based on selective absorption of the dye. The stain is a polychromatic stain containing Harris’s haematoxylin (staining nuclei blue), orange G-6 (staining keratinized cells-orange) and Eosin Azure (EA-50) which gave a subtle range of green, blue and pinks hues to the cytoplasm of epithelial cells. And The results was interpreted using the Bethesda system of classification. Smears were fixed in 95% isopropanol for 15 min. and rinsed in water for 1 min. The slides were then stained in Harris hematoxylin for 5 min. and rinsed in water until no stain comes out for 2 min and differentiated in 0.5% aqueous hydrochloric acid for 2 secs while observing the degree of differentiation microscopically and macroscopically. The slides were further rinsed in water for 2 min to stop the reaction. Blued in scot tap water for 2 min to restore the alkalinity of the cells and rinsed in water for 2 min. The smears were passed through ascending grades of alcohol (70%, 95%) alcohol for 2 min. Thereafter the second stain orange G-6 was applied for 2 min. aimed at staining of the keratinized cells orange. It was rinsed in two changes of 95% alcohol for 2 min. Stained with EA-50 for 3 min. and rinsed in absolute alcohol for 1 min. cleared in xylene, mounted in DPX and examined using X4 and X10 objectives.

The cervical tissues were sectioned using rotary microtome into serial sections and were stained. The slides were dewaxed and hydrated in water, drained and transferred to haematoxylin solution (primary stain) for 20 min. with nuclei staining blue. Rinsed in water and immediately subjected to two seconds differentiation using 1% acid alcohol, rinsed to stop the reaction and blued in Scot's tap water to restore the nuclear stain. Then dehydrated in three changes of alcohol (70%, 95%, Absolute) and stained in 1% Eosin (cytoplasmic stain), dehydrated in two changes of alcohol (95% and Absolute) and cleared in xylene and mounted in DPX. Erhlich’s haematoxylin and Eosin stain was used for histological studies following the method of Bancroft and Gamble [26]. The slides were stained and read to confirm the previous diagnosis. The principle of reaction was based on the chemical theory of dye; where the acidic component of the tissue is stained by the basic dye (haematoxylin) blue and the basic component (cytoplasm) stained with the acidic dyes (Eosin) with help of mordant

2.4 DNA Extraction

Formalin-fixed Paraffin-embedded cervical tissue sections were deparaffinized using two changes of xylene and the tissue sections (scraped) and transferred to a 1.5 ml microcentrifuge tubes. One (1) ml of xylene was added to the sample tube, vortex and incubated at room temperature for 1 hr with gentle rocking and centrifuged at 10,000xg for 1 min. The supernatant was discarded and washed twice with descending grades of alcohol (absolute, 95% and 75%) for 5 min. each with gentle rocking. The samples were washed once with double distilled water for 5 min. with gentle rocking and the water removed as much as possible using a micropipette. The samples were then ready for extraction. To the deparaffinised tissue samples in a microcentrifuge tube, 45µl of water, 45µl of 2 x digestion buffers (pH 9.0) and 10µl of proteinase K was added and incubated at 55°C for 4 hrs. The microcentrifuge tubes were transferred to an incubator set at 94°C and incubated for further 20 min. Then 5µl of RNase A was added and incubated for additional 5 min at room temperature followed 350µl of genomic lysing buffer (pH 9.5) and mixed thoroughly by vortex. The mixture was centrifuged at 10,000xg for 1 min to remove insoluble debris and the supernatant transferred to a zymo-spin™ llc column and centrifuged, wash buffer (pH 8.0) was added and the spin column was then centrifuged at 14,000xg for 1 min. The zymo-spin™ llc was transferred to a clean labelled
2.5 Multiplex PCR Protocol

The method of Nishiwaki [27] was adopted. Seventeen (17) hpv genotypes (6, 11, 16L 16U, 18, 30 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66) were evaluated in single-tube PCR. Multiplex polymerase reaction is a technique by which different DNA primers are amplified in a single reaction and used for detection of a large number of mutation or organisms depending on their molecular size. The process amplifies DNA in a sample using multiple primers, 2X master mix and a temperature mediated DNA polymerase in a thermal cycler. Optimization of the primers was carried out to create the same annealing temperature during PCR. The PCR mixture included 2x multiplex master mix (which contains Taq polymerase, dNTPs, MgCl2) forward and reverse primers at a concentration of 0.1µl each and 3µl of the extracted DNA was used. Nuclease free water of 6.92µ was used to make up the PCR components to a final volume of 30 µl. Each PCR was carried out in a DNA thermal cycler (Gene Amp PCR system 9700, Singapore) with the following conditions initial denaturation step at 95°C for 5min, 40 cycles of denaturation step at 94°C for 30sec, annealing at 65°C for 60 sec and initial extension at 72°C for 90 sec with a final extension at 72°C for 5min. The PCR products were analyzed using electrophoresis tank on a 2% agarose gel stained with ethidium bromide with band sizes estimated by comparison result with Quick-load DNA molecular ladder, NECO England Biolabs Inc. Agarose gel electrophoresis was according to Sambrook and Russel [28], the principle is based on the separation of nucleic acid into sizes with the aid of an electric field where negatively-charged molecules migrate towards anode. The migration pattern was determined solely by the molecular weight of the proteins, where small weight molecules migrate faster than larger one. It is an initial step for further purification of a band of interest. Agarose powder (2g) was heated in Tris-Boris EDTA (TBE) 1x solution in a microwave until it had completely dissolved and was then allowed to cool in a water bath set at 50°C - 55°C. The required numbers of combs were placed on the gel tray; 1.5µl of the ethidium bromide was added to the cooled gel, which was poured into the gel tray. The gel was allowed to cool at room temperature, the combs removed and the gel placed in an electrophoresis chamber and covered with buffer (TBE). DNA bands were visualized under UV trans- illuminator using a gel imaging system as follows. The agarose gel was placed into the electrophoresis tank containing TBE 1x buffer and 10µl of each amplicon was loaded into each well of the agarose gel using a micropipette. An electric current of 120v was pass through the TBE 1x buffer in the electrophoresis tank for 20min. Following Sambrook and Russell, (2001). The band sizes were estimated by comparison with 100 bp and 1000 bp Quick-load DNA molecular ladder, new England Biolabs Inc.

3. RESULTS AND DISCUSSION

The relationship between cervical neoplasia and hpv infection was well established in our present
study. Also, high frequency of hpv DNA in biopsies and cervical smears is often anticipated in infection with HPV as it is documented in the present study. A total of one hundred cervical samples (DNA elutes) were analysed in the present study. This comprises of fifty (50) healthy females who voluntarily allowed the collection of cervical smears under aseptic technique and questionnaire survey conducted on them.

Also, fifty (50) formalin-fixed paraffin-embedded cervical tissue blocks previously diagnosed with various pathologies were confirmed and result presented. The subjects' demographic data were examined for their association with genital Human Papillomavirus (hpv) positive. Table 1 shows that more than half (60%) of the participants were from a monogamous family with 61% married, 14% divorced, 5% widows and 20% singles. Majority of the participants were unemployed (75.0%) while 25% were employed with equal distribution of place of residence, that is 50% urban and 50% rural dwellers. Christianity was the predominant religion (80%) followed by traditional worshippers (17%). Others factors such as participants age at primigravidae, parity, presence of co-wives, and family history of cancer, hormonal contraceptives, and condom use show no statistically significant relationship:

Among demographic factors studied, age of participants, types of family (monogamy versus polygamy), level of education, marital status, religion, residence and tribe of participants were considered in the present study. There was a statistically insignificant association between the above factor studied and hpv prevalence. This was in disagreement with previous studies or findings within and outside the African continent [28,29,30]. Using chi-square analysis, prevalent HPV infection was strongly associated (P<0.01) type of family whiles other socio-demographic factors: Age, tribe, level of education, marital status, employment status, religion and place of residence showed statistically insignificant association with hpv positive participants (Table 1) and our findings are in line with the report of Dunce [30].

Of the thirty-nine (39) participants that had early sexual debut (above 18 years of age), 28 (82.9%) were HPV positive as against 10 (66.7%), positive participants who started below 18 years of age. Among other variables studied such as lifetime sexual partners showed a statistically significant relationship in the prevalence of hpv (OR=0.02, P<0.01) as seen in Table 2. These findings suggested that the acquisition of hpv infection is linked to the sexual lifestyle of an individual and may not necessarily be influenced by the level of education and residence of participants. A study in Lagos Nigeria also reaffirmed the statistical significance in the lifetime sexual partners as a risk factor for hpv infection, Manga [31].

Furthermore, our result showed that the level of knowledge of our subjects on hpv vaccination was low (32.8%) as shown in Table 3. The finding (low level of knowledge) in our present study was consistent with previous works in Taiwan where students exhibited moderate knowledge regarding hpv vaccine [32,33,34] and in Columbia were only 7.8% of subjects knew [35]. However, the present finding is different from that of [36] who reported 51% knowledge of hpv vaccination among respondents and [37] who reported 85% in their study. The present study analysis on the knowledge about hpv infection and cervical cancer was equally low among participants (38.9%). The mean score of the present study is much higher compared with the works of Hanisch [37] were merely 15% of their respondents were aware that hpv causes cervical cancer. Ezat and Hod [38] reports reaffirmed a higher percentage of 92.2% in the level of knowledge of hpv causing cervical cancer among 200 nurses in Lagos University, Nigeria. The mean scores for knowledge of genital hpv infection and cancer of the cervix was 19.0±9.2 with maximum scores of 11 questions. Also, the mean scores for knowledge on HPV vaccination and cervical cancer prevention was 17.0±6.3 out of a total of 7 questions. Table 4 reveals the perception and attitude of participants towards hpv vaccination. Of the fifty (50) participants that assessed questionnaires, 40.0% thought they can be easily infected with hpv while 10.0% were uncertain with a statistically non-significant mean score of (χ² = 5.9, P=0.41) in their level of perceived susceptibility between hpv positive participants compared with the negative participants. Again there was a statistically significant level of perceived seriousness on hpv infection among hpv positive participants when compared with the negative participants (χ² = 16.39, p = 0.01). Also, there exists no statistically significant relationship between perceived benefits, perceived barriers and clue to action.

Multiplex PCR was used to subtype hpv genotypes in the present study see table 5. Out of the 52% hpv positive participants subjected to genotyping using multiplex PCR only 29 reacted. Different disease conditions presented with
different hpv genotypes either as a single infection or multiple hpv types, 52, 51, 45, 31, 30. Hpv 52 was observed in 40.4% (21/52) of participants followed by hpv 30 (9.6%), while HPV 51, 45 and 31 showed 1.92% respectively. Women with normal results for histology and cytology presented a genotype distribution of 68.9% (20/29), followed by atypical squamous cell of unidentified significance (17.2%), LSIL (10.3%), and least in cervicitis and 3.4% for others (Table 5). Out of the 52% hpv positive participants subjected to genotyping using multiplex PCR only 29 reacted. Different disease conditions presented with different hpv genotypes either as a single infection or multiple hpv types, 52, 51, 45, 31, 30. Hpv 52 was observed in 40.4% (21/52) of participants followed by hpv 30 (9.6%), while HPV 51, 45 and 31 showed 1.92% respectively. Women with normal results for histology and cytology

Table 1. Association between socio-demographic factors of participants and cervical human papillomavirus

| Age Groups (years) | % Positive | % Negative | Total | $\chi^2$ | p-value |
|-------------------|------------|------------|-------|---------|---------|
| 24                | 0(7.6)     | 06(12.5)   | 010   | 0.04    | 0.08    |
| 25-34             | 23(44.2)   | 15(31.3)   | 038   | 0.82    | 0.41    |
| 35-44             | 11(21.1)   | 12(25.0)   | 023   | 0.02    | 0.89    |
| 45-54             | 07(13.5)   | 07(14.6)   | 014   | 0.02    | 0.89    |
| ≥55               | 07(13.5)   | 08(16.6)   | 015   | 0.03    | 0.86    |
| Total             | 52(100)    | 48(100)    | 100   | 0.00    | 0.00    |

| Type of Family | Monogamous | Polygamous | Total | $\chi^2$ | p-value |
|----------------|------------|------------|-------|---------|---------|
|                | 20(38.5)   | 40(83.3)   | 060   | 20.94   | 0.01    |
| Total          | 52(100)    | 48(100)    | 100   | 0.00    | 0.00    |

| Level of Education | Primary | Secondary | Tertiary | Others | Total | $\chi^2$ | p-value |
|--------------------|---------|-----------|----------|--------|-------|---------|---------|
|                    | 14(26.9)| 12(23.1)  | 20(38.5) | 06(11.5)| 52(100)| 20.94   | 0.01    |
|                    | 08(16.6)| 10(20.8)  | 20(41.7) | 10(20.8)| 40(100)| 20.94   | 0.01    |
|                    |         | 022       | 040      | 016    | 100   | 0.00    | 0.00    |

| Marital Status | Married | Single | Divorced | Widow | Total | $\chi^2$ | p-value |
|----------------|---------|--------|----------|-------|-------|---------|---------|
|                | 31(59.6)| 11(21.2)| 07(13.5)| 03(5.7)| 52(100)| 20.94   | 0.01    |
| Employment status | % Positive | % Negative | % Positive | % Negative | Total | $\chi^2$ | p-value |
| Employed       | 20(38.5)| 02(4.0)   | 12(25.0) | 02(4.0) | 44(100)| 20.94   | 0.01    |
| Unemployed     | 32(61.5)| 08(16.6)  | 08(18.0) | 08(16.6)| 56(100)| 20.94   | 0.01    |
| Total          | 52(100)| 48(100)   | 100      | 100    | 100   | 0.00    | 0.00    |

| Religion | Christianity | Islam | Traditional | Total | $\chi^2$ | p-value |
|----------|--------------|-------|-------------|-------|---------|---------|
|          | 41(78.8)     | 01(1.9)| 10(19.2)    | 52(100)| 75.56  | 0.86    |
|          | 39(81.3)     | 02(4.2)| 07(14.5)    | 48(100)| 75.56  | 0.86    |
| Residence | Rural | Urban | Total | $\chi^2$ | p-value |
|-----------|-------|-------|-------|---------|---------|
|           | 30(57.7)| 22(42.3)| 52(100)| 20.94   | 0.01    |
| Religion  | 30(61.5)| 20(41.7)| 50(100)| 20.94   | 0.01    |
|           | 39(81.3)| 20(41.7)| 50(100)| 20.94   | 0.01    |
| Tribe     | Iton  | Ibo   | Hausa  | Others | Total | $\chi^2$ | p-value |
|-----------|-------|-------|-------|--------|-------|---------|---------|
|           | 30(57.7)| 09(17.3)| 02(3.8)| 11(21.2)| 52(100)| 20.94   | 0.01    |
|           | 30(61.5)| 11(22.9)| 01(2.1)| 06(12.5)| 48(100)| 20.94   | 0.01    |
|           |         | 02(4.2)| 07(14.5)| 07(14.5)| 48(100)| 20.94   | 0.01    |
|           |         | 08(16.6)| 08(16.6)| 08(16.6)| 48(100)| 20.94   | 0.01    |
|           |         | 02(4.2)| 07(14.5)| 07(14.5)| 48(100)| 20.94   | 0.01    |
|           |         | 08(16.6)| 08(16.6)| 08(16.6)| 48(100)| 20.94   | 0.01    |
|           |         | 02(4.2)| 07(14.5)| 07(14.5)| 48(100)| 20.94   | 0.01    |
|           |         | 08(16.6)| 08(16.6)| 08(16.6)| 48(100)| 20.94   | 0.01    |
|           |         | 02(4.2)| 07(14.5)| 07(14.5)| 48(100)| 20.94   | 0.01    |
|           |         | 08(16.6)| 08(16.6)| 08(16.6)| 48(100)| 20.94   | 0.01    |
|           |         | 02(4.2)| 07(14.5)| 07(14.5)| 48(100)| 20.94   | 0.01    |
|           |         | 08(16.6)| 08(16.6)| 08(16.6)| 48(100)| 20.94   | 0.01    |
|           |         | 02(4.2)| 07(14.5)| 07(14.5)| 48(100)| 20.94   | 0.01    |
|           |         | 08(16.6)| 08(16.6)| 08(16.6)| 48(100)| 20.94   | 0.01    |
|           |         | 02(4.2)| 07(14.5)| 07(14.5)| 48(100)| 20.94   | 0.01    |
|           |         | 08(16.6)| 08(16.6)| 08(16.6)| 48(100)| 20.94   | 0.01    |
|           |         | 02(4.2)| 07(14.5)| 07(14.5)| 48(100)| 20.94   | 0.01    |
|           |         | 08(16.6)| 08(16.6)| 08(16.6)| 48(100)| 20.94   | 0.01    |
|           |         | 02(4.2)| 07(14.5)| 07(14.5)| 48(100)| 20.94   | 0.01    |

Hpv = human Papillomavirus, P<0.05, %= percentage, \(x^2\) = chi square, N = Number, Values expressed as positive and negative respectively, *Significant difference observed, p< 0.05
| S/no | HPV Primer | Forward and reverse primer sequence | Size (bp) | Length | GC % | Tm°c |
|------|------------|-------------------------------------|-----------|--------|------|------|
| 1    | pp x6/F, PPx6/R | GCTAAGGTGTCCTGTTCGACTGCGGCTA, GCGAGCGACCTCCCTACGTAAT | 283       | 27     | 55.56/55.56 | 69.16/69.16 |
| 2    | pp x11/F, pp x11R, PP x11L/F, PP x11L/R | GCAGCGGATCCTCTACGACGTGAC, TGGGAGCCCTCTTCCTCAATTGAC | 472       | 25     | 52/52 | 66.22/66.22 |
| 3    | pp x16L/F, pp x16L/R | CTGGAGGCTGCTCTCCACCTAGTG, TGGGAGGTACACCTACGATGAGG | 217       | 24     | 67.08/67.08 | 58.33/58.33 |
| 4    | PP x16U/F, PP x16U/R | CTGGAGGTTACCAATGGGAGAGGG, GGCCATACCCGCTGTCTTCGCTTT | 397       | 26     | 59.67/59.69 | 69.32/69.32 |
| 5    | PP x18/F, PP x18/R | AACGAGTCTAGGAGGGAGGGAGGGCGTGA, TGCCGGAATGCTGAGTGTGGTCG | 187       | 26     | 59.69/69.69 | 69.32/69.32 |
| 6    | pp x30/F, pp x30/R | GCAGCGGATGTCCTCTACGACGTGAC, TGGGAGGTACACCTACGATGAGG | 249       | 25     | 60/60 | 69.5/69.5 |
| 7    | PP x31/F, PP x31/R | GCTGGCTCAAGCTCTACAAAAACGCACT, CCAGGGGAGCTCCCATCATCACAAT | 360       | 28     | 53.57/53.57 | 69.01/69.01 |
| 8    | pp x33/F, pp x33/R | ACAGAGGAGCAGCCCGGGTCCTTTT, GTGGGAGGTACACCTACGATGAGG | 139       | 26     | 57.69/57.69 | 69.32/69.32 |
| 9    | PP x35/F, PP x35/R | CCATAACCTGAGGGGAGGAGGGAGGG, CGGGTTCGAGACAGCTCCAGCAAC | 434       | 27     | 59.26/59.26 | 70.88/70.88 |
| 10   | PP x39/F, PP x39/R | CCACGCCCTGCTGCTGCTGCAGAG, CCAGCGGATGTCCTCTACGACGTGAC | 229       | 27     | 59.26/59.26 | 70.68/70.68 |
| 11   | PP x45/F, PP x45/R | TGTTGAGACATCACACTACCATACGTGGA, TCAGAGGAGCAGCCCGGGTCCTTTT | 205       | 25     | 52/52 | 66.22/66.22 |
| 12   | pp x51/F, pp x51/R | CAACATGCAAGGAGCGTAGGTCTG, CACCCCTCTACCTCGACAGTG | 299       | 23     | 56.52/56.52 | 66.33/66.33 |
| 13   | pp x52/F, pp x52/R | CTCCGAGGTGCTGCTGCTGCTGCT, GGAGGCTAGGATGAGGTGCTAGG | 517       | 25     | 52/52 | 66.22/66.22 |
| 14   | PP x56/F, PP x56/R | TGTTGAGACATCACACTACCATACGTGGA, TCAGAGGAGCAGCCCGGGTCCTTTT | 330       | 32     | 40.63/40.63 | 65.93/65.93 |
| 15   | pp x58/F, pp x58/R | ACCACCGAGCCACCAACATCGAAGAGG, CTGGGAGGTACACCTACGATGAGG | 128       | 27     | 55.56/55.56 | 67.21/67.21 |
| 16   | PP x59/F, PP x59/R | CCAGGGAGGAGCAGCTCCAGCAGCT, TGAGAGGAGCAGCTCCAGCAGCAGC | 169       | 27     | 55.56/55.56 | 69.16/69.16 |
| 17   | PP x66/F, PP x66/R | GGCGGGGAGCTCCATCACCTCCTCTTCCTTC, GCAGAGGAGCAGCTCCAGCAGCAGC | 277       | 27     | 66.87/66.87 | 73.71/73.71 |
|      |                  |                                      | 29     | 58.62/58.62 | 71.69/71.69 |
presented a genotype distribution of 68.9% (20/29), followed by atypical squamous cell of unidentified significance (17.2%), LSIL (10.3%), and least in cervicitis and 3.4% for others.

Cervical human Papillomavirus worldwide prevalence has been estimated at 11.7% [15] with 24.0% documented in Sub-Saharan Africa across all ages ranging from 17.4% in southern Africa to 33.6% in Eastern Africa and 31% in Nigeria [39]. The current prevalence of human Papillomavirus across all ages in our present study was 52.0%. This may be due to the sensitivity of the MY09/11nested GP5+/6+ PCR method used in the present study. Sub-Saharan Africa harbours the highest prevalence of hpv with a certain degree of variation based on the sample types and methods of detection used by various researchers. Human Papillomavirus typing provides a soft landing for vaccination and management. [40] had earlier in his publication debunked the antenna trials focusing only on human Papillomavirus 16 and 18 to identify women at high risk for high-grade cervical cancer and equally proposed that hpv type-specific screening should focus not only on hpv 16 and hpv 18 alone but also hpv 45 and others. In this present study hpv type 52, 51, 45, 33, and 31 were subtype despite the inclusion of predominant type hpv16 and 18 in the primer synthesis (Table 6). Therefore women in Bayelsa state should be screened for these variants despite the evolutionary relatedness of some of these variants with hpv 18 reported worldwide. Previous studies [41,42,43] observed that hpv types 16, 18 and 45 are the most common types of hpv associated with cervical cancer and accounted for 75% squamous cell carcinoma and 94% of adenocarcinoma in their studies. Several other studies have raised the possibility of certain hpv types more common in sub-Saharan Africa women than those common in another continent [44].

The current cervical human Papillomavirus prevalence of 52.0% reported in the present study is the highest known to be reported in Nigeria among women as shown in Table 7. This is followed by that of Dunce [30] who reported 48.1% among normal women presenting for cervical cancer screening at the Federal Teaching Hospital Gombe (FTHG) Gombe state, North-Eastern Nigeria. [16] had equally reported a prevalence of 26.3% in Ibadan, Nigeria. Other prevalence reported in Nigeria is 19.6% among HIV infected population

| Variables                        | Prevalence of HPV DNA | Total | OR     | Fexact |
|----------------------------------|-----------------------|-------|--------|--------|
| Age at sexual debut              |                       |       |        |        |
| <18 years                        |                       |       |        |        |
| ≥18 years                        |                       |       |        |        |
| Lifetime sexual partners         |                       |       |        |        |
| Single                           |                       |       |        |        |
| Multiple                         |                       |       |        |        |
| Age at primigravidae             |                       |       |        |        |
| >18 years                        |                       |       |        |        |
| 18-28years                       |                       |       |        |        |
| ≥28years                         |                       |       |        |        |
| Parity                           |                       |       |        |        |
| Nulliparous                      |                       |       |        |        |
| Primiparous                      |                       |       |        |        |
| Multiparous                      |                       |       |        |        |
| Presence of co-wives             |                       |       |        |        |
| No other wife                    |                       |       |        |        |
| One other wife                   |                       |       |        |        |
| Family history of cervical cancer|                       |       |        |        |
| Yes                              |                       |       |        |        |
| No                               |                       |       |        |        |
| Hormonal contraceptives          |                       |       |        |        |
| Yes                              |                       |       |        |        |
| No                               |                       |       |        |        |
| Use of condom                    |                       |       |        |        |
| Yes                              |                       |       |        |        |
| No                               |                       |       |        |        |
in Lagos [18]. Fourteen point seven (14.7%) in Irun [19] 25.0% in Abuja [16]; 19.7% in Okene [45] and 21.6% in Ile-Ife [20]. This variation or disparity among the different study populations may be explained by variation in exposures to different risk factors based on diverse socio-economic, cultural and geographical differences and to a large extent the nature of the sample and methods of detection. However, studies done in other parts of sub-Saharan Africa among similar population (Normal women and other abnormal women) groups have shown a higher prevalence compared with the present study in Bayelsa State, Nigeria. A prevalence of 66.1% was reported among high-risk women in Burkina Faso [46] slightly higher than the present study.

| Indices of Knowledge                                                                 | Total   | HPV+   | HPV-   | Summary          |
|-------------------------------------------------------------------------------------|---------|--------|--------|------------------|
| HPV can cause cervical cancer                                                       | 19(38)  | 15(78) | 04(22) | $x^2=13.1, p=0.04$ |
| Most people with genital HPV have no visible signs or symptoms                     | 10(20)  | 08(80) | 02(20) | $x^2=7.2, p=0.07$  |
| HPV can cause genital warts                                                         | 11(22)  | 10(90) | 01(10) | $x^2=1.5, p=0.01$  |
| I can transmit H to my partner even if I have no HPV symptoms                      | 23(46)  | 22(95) | 01(05) | $x^2=38, p=0.01$    |
| Having one type of HPV means that you cannot acquire a new type                    | 13(26)  | 08(61) | 05(39) | $x^2=1.4, p=0.23$   |
| A vaccine exists to prevent HPV infection                                           | 10(20)  | 04(40) | 06(60) | $x^2=0.8, p=0.37$   |
| A negative test for HPV means that you do not have HPV                              | 11(22)  | 09(81) | 02(19) | $x^2=8.9, p=0.02$   |
| HPV can be passed from mother to her baby during birth                               | 32(64)  | 29(90) | 03(10) | $x^2=42, p=0.01$    |
| Pap smear will almost always detect HPV                                              | 35(70)  | 30(85) | 05(15) | $x^2=36, p=0.01$    |
| Changes in Pap smear may indicate that a woman has HPV                               | 24(48)  | 20(83) | 04(17) | $x^2=21, p=0.01$    |
| If a woman’s Pap smear is normal, she does not have HPV                             | 26(52)  | 21(80) | 05(20) | $x^2=20, p=0.01$    |
| HPV vaccine protects against cervical cancer                                         | 14(28)  | 10(70) | 04(30) | $x^2=5.1, p=0.01$   |
| HPV vaccine did not protect against all types of the virus strain that causes cervical cancer | 10(20)  | 07(70) | 03(30) | $x^2=3.2, p=0.04$   |
| HPV vaccine did not protect against all sexually transmitted infections              | 15(30)  | 12(80) | 03(20) | $x^2=11, p=0.05$    |
| HPV vaccine is only available for a woman currently in Bayelsa                        | 17(34)  | 15(88) | 02(12) | $x^2=19.8, p=0.01$  |
| Women who receive HPV vaccine still need frequent pelvic examination                 | 30(60)  | 25(83) | 05(17) | $x^2=2.7, p=0.01$   |
| Women who receive HPV vaccine still have to get Pap smear                             | 16(32)  | 12(75) | 04(25) | $x^2=8, p=0.00$     |
| Currently, HPV vaccine is available in Bayelsa                                       | 19(38)  | 13(68) | 06(32) | $x^2=5.2, p=0.02$   |

**Table 3. Knowledge on HPV infection and cervical cancer, HPV vaccination and prevention of cervical cancer and their percentage scores**

- HPV+ = Human Papillomavirus positive, HPV- = Human Papillomavirus negative. Significant difference observed, $p<0.05$

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**Table 4. Perception and Attitude towards HPV vaccination among subjects studied**

| Perception and attitude of subjects | Total% | HPV+% | HPV-% | p-value |
|-------------------------------------|--------|-------|-------|---------|
| **Perceived susceptibility**        |        |       |       |         |
| Do you think you can be easily infected with HPV |        |       |       |         |
| Yes                                 | 20(40.0) | 18(90.0) | 2(10.0) | \(x^2 = 5.94, p =\) |
| No                                  | 25(50.0) | 19(76.0) | 6(24.0) | 0.11 |
| Uncertain                           | 05(10.0) | 02(40.0) | 03(60.0) |       |
|                                      | 50(100) | 39(78.0) | 11(22.0) |       |
| **Perceived seriousness**           |        |       |       |         |
| Do you think HPV infection is a serious disease? |        |       |       |         |
| Yes                                 | 41(82.0) | 39(96.0) | 02(4.0) | \(x^2 = 16.39\) |
| No                                  | 08(10.0) | 06(60.0) | 02(40.0) | \(p = 0.01\) |
| Uncertain                           | 03(6.0) | 01(25.0) | 02(75.0) |       |
|                                      | 50(100) | 43(86.0) | 07(14.0) |       |
| **Perceived benefits**              |        |       |       |         |
| Would you be vaccinated against HPV if HPV infection is preventable? |        |       |       |         |
| Yes                                 | 43(86.0) | 37(86.0) | 06(14.0) | \(x^2 = 5.42\) |
| No                                  | 04(8.0) | 03(75.0) | 01(25.0) | \(p = 0.14\) |
| Uncertain                           | 03(6.0) | 01(33.0) | 01(67.0) |       |
|                                      | 50(100) | 41(82.0) | 09(18.0) |       |
| **Perceived barriers**              |        |       |       |         |
| If you do not want to be vaccinated, what are the barriers for having an HPV vaccine? |        |       |       |         |
| Yes                                 | 15(30.0) | 12(80.0) | 03(20.0) | \(x^2 = 4.94\) |
| No                                  | 20(40.0) | 11(55.0) | 09(45.0) | \(p = 0.18\) |
| Uncertain                           | 15(30.0) | 13(87.0) | 02(13.0) |       |
|                                      | 50(100) | 36(72.0) | 14(28.0) |       |
| **Clue to action**                  |        |       |       |         |
| Whose recommendation would be most effective in encouraging you to get an HPV vaccine? |        |       |       |         |
| Doctor recommendation               | 20(40.0) | 19(95.0) | 01(5.0) | \(x^2 = 3.37\) |
| Family opinion                      | 10(20.0) | 08(80.0) | 02(20.0) | \(p = 0.64\) |
| Mass media campaign                 | 05(10.0) | 04(80.0) | 01(20.0) |       |
| Self-decision                       | 03(6.0) | 02(67.0) | 01(33.0) |       |
| Others                              | 12(24.0) | 09(75.0) | 03(25.0) |       |
|                                      | 50(100) | 42(84.0) | 08(16.0) |       |

\(HPV+ = \text{Human Papillomavirus positive}, \ HPV- = \text{Human Papillomavirus negative}, \ x^2 = \text{Chi-square}\)

**Table 5. Cervical HPV genotype typing and combination in cervical lesions studied using multiplex PCR**

| HPV types | Normal | ASCUS | LSIL | SCC | ADC | Cervicitis/ others | Total |
|-----------|--------|-------|------|-----|-----|--------------------|-------|
| HPV 52    | 15(71.40) | 02(9.50) | 03(14.30) | 0(0.00) | 0(0.00) | 01(4.80) | 21(40.38) |
| HPV 51    | 0(0.00) | 01(10.00) | 0(0.00) | 0(0.00) | 0(0.00) | 01(4.80) | 01(1.92) |
| HPV 45    | 01(100.0) | 0(0.00) | 0(0.00) | 0(0.00) | 0(0.00) | 0(0.00) | 01(1.92) |
| HPV 31    | 01(100.0) | 0(0.00) | 0(0.00) | 0(0.00) | 0(0.00) | 0(0.00) | 01(1.92) |
| HPV 30    | 03(60.0) | 02(0.00) | 0(0.00) | 0(0.00) | 0(0.00) | 0(0.00) | 05(9.6) |
| Total     | 20(68.9) | 05(17.20) | 03(10.30) | 0(0.00) | 0(0.00) | 01(3.4) | 29(55.7) |
| No of genotype combination         | HPV 52 | HPV 52 | HPV 52 | -   | -   | HPV 52 | HPV 52 |
|                                      | 5      | 51    | 30    | 30  | 30  | 31    |       |

\(ASCUS= \text{Atypical squamous cell of undetermined significance}, \ LSIL= \text{low grade squamous intraepithelial neoplasia}, \ SCC= \text{squamous cell carcinoma}, \ ADC= \text{adenocarcinoma}. \text{Values are expressed as percentages}\)
Table 6. Age and human Papillomavirus type Specific Genotype typing using 17 Gene Single Tube Multiplex PCR

| HPV types | NO (%) HPV in each age group (years) | 15-24 | 25-34 | 35-44 | ≥45 |
|-----------|-------------------------------------|-------|-------|-------|-----|
| HPV 30    | 0(0.0)                              | 03(21.4) | 02(22.2) | 0(0.0) |
| 31        | 0(0.0)                              | 01(7.1)  | 0(0.0)  | 0(0.0) |
| 45        | 0(0.0)                              | 01(7.1)  | 0(0.0)  | 0(0.0) |
| 51        | 0(0.0)                              | 01(7.1)  | 0(0.0)  | 0(0.0) |
| 52        | 04(100.0)                           | 08(57.1) | 07(77.7) | 02(6.8) |
| Total (29)| 04(13.8)                            | 14(48.3) | 09(31.0) | 02(6.8) |

HPV = Cervical human Papillomavirus. Value expressed percentage

Table 7. Cervical HPV prevalence by Age (years) among subjects studied

| Age (years) | Total examined (%) | HPV positive (%) | HPV negative (%) |
|-------------|--------------------|------------------|------------------|
| 15-24       | 10(10.0)           | 04(7.6)          | 06(4.2)          |
| 25-34       | 38(38.0)           | 23(44.2)         | 15(31.3)         |
| 35-44       | 23(25.0)           | 11(21.2)         | 12(29.2)         |
| 45-54       | 14(16.0)           | 07(13.4)         | 07(18.8)         |
| ≥55         | 15(15.0)           | 07(13.4)         | 08(16.7)         |
| Total       | 100(100.0)         | 52(52.0)         | 48(48.0)         |

HPV = Human Papillomavirus, % = percentage

Plate 1. Agarose gel electrophoresis showing the HPV detection using multiplex PCR (17 genotype primers). Lanes 1–15, 17 & 18 represent HPV positive (750bp). Lanes 19 & 20 represent HPV positive polymorphic gene (300bp) and (800bp) respectively. Lane M represents the Quick-Load 50bp DNA molecular ladder respectively.

4. CONCLUSION

Inadequate knowledge and awareness about human Papillomavirus in Bayelsa could be an explanation for the high prevalence of hpv prevalence recorded in the present study. HPV 52 was highest in the present study compared to previous studies, national and international. The emergence of hpv 52 in the study area requires public health attention and thus urgent need for local hpv vaccines production. Policies should be put in place towards improving future implementations on hpv vaccination uptake, increase awareness of hpv infection and reduced the morbidity and mortality from cervical cancer.

CONSENT AND ETHICAL APPROVAL

Ethical approval was obtained from the Research Ethics Committee, of the Niger Delta
University Teaching Hospital, Okolobiri ((NDUTH/REC/0003/2015) before proceeding with the study. Informed consent was sought from each respondent before questionnaires were administered and taking of samples for laboratory analysis. The privacy, dignity, and autonomy of the respondents were maintained accordingly throughout the conduct of the study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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