Prevalence of Human Papilloma Virus DNA in HIV Positive women in Lagos University Teaching Hospital (LUTH) Lagos, Nigeria

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Introduction

- Persistent infections with high risk human Papilloma virus (HPV) is a well established cause of cervical cancer

- In Nigeria 23.7% of women harbour cervical HPV infection at any given point in time

- HIV positive women are more frequently infected with multiple HPV types due to their impaired immune status
Introduction

- HIV positive women with severe immunosuppression are 5 times more likely to have lower genital tract neoplasia.

- Treatment failure and recurrence are also more common among them.

- This necessitates routine screening for genital tract neoplasia and cancer among this group of women.
Aim of the study

- To determine the prevalence of genital human papilloma virus infection among HIV positive women at LUTH Lagos, Nigeria
- Relate HPV genotypes in the study population to commercially available HPV vaccine types
Study type

- The study design is a comparative cross-sectional analytic observational study.
- It was undertaken at the AIDS Prevention Initiative clinic (APIN) and the Gynecologic outpatient dept. of LUTH.
- Ethical clearance was obtained from the ethical committee of LUTH.
- Duration of study was between August 2011-August 2012.
Study Population

- Comprised of 100 HIV positive women within the reproductive age group attending APIN clinic

- The control group comprised of 100 HIV negative women coming for routine cervical cancer screening test.
Sampling Technique

- Systematic random sampling technique was used to select the study subjects.
- Average of 300 HIV positive women are bled every 2 weeks at the APIN clinic for CD4 counts and viral load estimations.
- Sampling interval: total population (300)/sample size (100) was set at 3.
- Similar sampling technique was applied in the selection of the control subjects.
Eligibility/Exclusion criteria

- Consentng HIV positive women 18 years and above who were recently bled for CD4 counts and viral load estimations
- Consentng HIV negative women 18 years and above.
- Those who were excluded were:
  - Females who were menstruating
  - Those who were pregnant
  - Those who have had hysterectomies performed on them.
  - Those who declined HIV testing.
HPV test collection

- Cervical samples were collected with disposable specimen collection kits (Hybribio Biochemical company Ltd. China)
- Stored at -20°C at the Anatomic and Molecular Pathology dept. of the CMUL, Lagos, Nigeria.
- All participants had VIA (visual inspection with acetic acid) performed on them
- Those with abnormal findings on VIA were referred to the Gynecology department of LUTH free of charge for colposcopy and when necessary biopsy and treatment.
HPV Serotyping

- Samples were screened for HPV infections using HPV Genoarray test kits (Hybribio Biochemical Company Ltd. China)

- Kits use a combination of both polymerase chain reaction (PCR) and flow through hybridization technology

- Twenty-one types of HPV DNA in cervical samples are genotyped qualitatively using this kit

- HPV types 6, 11, 42, 43, 44, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 53 and CP8304
HPV Serotyping

- This process involved:
  - DNA extraction
  - PCR amplification
  - Flow-through hybridization
  - Result interpretation
DNA extraction

- Aliquots of cervical samples were repeatedly centrifuged at 14,000 rounds per min. for 3 times each lasting 5 mins.

After each centrifugation, supernatant was discarded and buffer solutions added respectively to the remaining suspension.

- DNA was extracted by the lysis of cells, isolation, precipitation and purification.

- 1 ml of sample was then pipetted for PCR amplification.
PCR amplification

- All PCR reagents were spun for 5 minutes.
- PCR mastermix solution was prepared by mixing appropriate quantities of PCR-mix solution and DNA Taq polymerase for each reaction tube.
- One microlitre of DNA template was added to each PCR tube.
- The solution was centrifuged for a few seconds.
- Subsequently placed in a thermal cycler for DNA amplification.
- The primer used was MY09/11 primer system.
Flow-through Hybridization

- The PCR products were denatured at 95°C for 5mins.
- The HybriMem HPV-21 DNA microarray membrane marked with 21 HPV genotype probes was put in place.
- The PCR products and the prewarmed hybridization solution were mixed together and then added into sample wells.
- It was thereafter incubated for 20 mins and blocking solution added.
- The membrane was washed with hybridization solution and enzyme conjugate added to display the result.
Result interpretation

- Solution membranes were dried on absorbent paper.
- A positive result was indicated by a clearly visible indigo dot.
- The HPV genotype result was determined according to the position of specific probes on the HybriMem HPV-21 membrane.
- Multiple dots indicated multiple infections.
- Actual HPV types were determined by comparison of the position of the dots to known reference points.
HPV membrane result worksheet
HPV Serotyping

- For quality, positive and negative controls were included during the analysis
- Positive control was needed to demonstrate the efficiency and specificity of the PCR
- Negative control would indicate if the PCR reagents were contaminated
- To reduce contamination all equipments were sterilized by radiation before use.
Data presentation and test statistics

- Data processing was done using Epi info version 3.5.6 and Microsoft Excel
- Frequency distribution was used to determine the relationship between variables
- The student T-test was used for comparison of mean differences
- The Chi-square test was used to compare the differences between proportions
- All statistical analysis was at 5% level of significance $p \leq 0.05$ (95% confidence level)
Results

- Ninety-eight (98%) HIV positive and 97 (97%) HIV negative women participated in the study.

- Mean ages of the participants were 36.8±9.0 years and 43.8±10.5 years for the test and control groups respectively.
Figure 1: showing HPV distribution for the different age ranges
HPV test result (HIV positive women)

- A total of 19 different HPV types were identified from 45 (44.90%) of the women
- Thirty-seven women (37.75%) were infected with the high risk types
- Eleven women (11.20%) had multiple HPV high risk HPV infections involving between 2 and 7 HPV types
- Five females (5.10%) were infected with the low risk group
HPV test result (HIV positive women)

- Commonest high risk types detected were:
  - Type 31 (16.80%)
  - Type 52 (15.20%)
  - Type 53 (9.10%)
  - Type 35 (7.60%)

- Commonest low risk types detected were:
  - Types 6 and 11 (3.0%) each
  - Type 44 (1.5%)
HPV test result (HIV positive women)

- Overall single genotypes were found in 27 females (27.55%)

- Both high and low risk genotypes were found in 4 females (4.0%)
HPV test result (HIV negative women)

- Eleven females (11.34%) tested positive for HPV infections
- All HPV infections detected were of the high risk types
- Three females (3.0%) tested positive for multiple HPV types
- Commonest high risk type detected was type 18 (23.10%) followed by 16, 52 and 56 (15.40%) respectively.
Figure 2: showing distribution of HPV genotypes among the respondents
Discussion

- The prevalence of HPV in this study among HIV positive and negative women was 44.90% and 11.20% respectively.
- This is comparable with a prevalence of 57.10% seen in HIV positive West African immigrants resident in Southern Italy (mostly Nigerians).
- A prevalence of 26.30% was also discovered among the general population of Ibadan, Nigeria.
Discussion

- Reasons for the increased prevalence seen in HIV positive women include:
- A more efficient HPV replication in immunodeficient host leading to increased detection rate, treatment failures and recurrence.
- There is also a higher chance of developing persistent HPV infections (arbitrarily defined as 2 or more positive HPV tests in one year)
- Persistence is the first step towards the development of high grade SILs and cancer
Discussion

- Previous studies carried out in other parts of Africa have shown a higher HPV prevalence depending on how the women were selected and HPV tested for:
  - Burkina Faso (66.10%)
  - Zambia (97.2%)
- This may be attributable to the exhaustive nature of the HPV detection strategy
- Studies have shown that by using a primer pair alone, HPV types 26, 35, 42, 45, 52, 54, 55, 59, 66, 68 and 73 might be missed, leading to erroneously low results.
Discussion

- The commonest high risk HPV types detected among HIV positive women were types 31, 52, 53 and 35 in decreasing order of prevalence.
- This finding is similar to what was found in Burkina-Faso: types 52, 35 and 58.
- But sharply contrasts with a world wide prevalence of HPV 16 and 18 and our control group.
- This findings may have important implications:
Discussion

- If cross immunity is not induced across viral types by existing vaccines,
- Efficacy of existing prophylactic HPV vaccines may be limited in immunosuppressed women in these regions
- Among the control group, high risk type 18 was the commonest followed by 16, 52 and 56
- This is not unusual since the behavioral and socioeconomic characteristics of HIV infected women may differ from the normal population
Discussion

- The incidence of multiple HPV types among the HIV positive women (11%)
- This is similar to the incidence discovered in a cohort of HIV positive women in the US (12%)
- Much lower than 45% and 78.6% seen in Brazilian and Zambian HIV positive women
- Variability in the genotype method used may account for these differences
Discussion

- Older women 25-34yrs age range were more likely to be infected with high risk HPVs
- unlike <25yrs and >55yrs
- This is also similar to what was discovered in HIV positive Rwandan women
- May be due to the time taken for persistence to dev in those 25-34 yrs and the decreased sexual activity seen in those >55yrs
Discussion

- HIV negative women 45-54 yrs had 2 folds increased risk of having high risk HPV than 25-34yrs.
- Similar to what was discovered in the general population of Ibadan, Nigeria.
- A fraction of the spouses of these women may continue to have multiple sexual partners thereby reinfecting themselves and these women.
Conclusion

- Due to the high prevalence and diversity of HPV genotypes found in the HIV positive women,
- There should be adequate protocols for cervical cancer screening in this group of women
- Bilateral and Multilateral donor programmes in developing countries should be linked to cervical cancer screening strategies among these women
- Studies should also be carried out to determine the efficacy of existent HPV vaccines on this group of patients
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References


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