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

I.G. Nweke, A.A.F. Banjo, F.B. Abdulkareem and V.U. Nwadike

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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

- Consenting HIV positive women 18 years and above who were recently bled for CD4 counts and viral load estimations
- Consenting 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 paticipants 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 centrifuge, 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

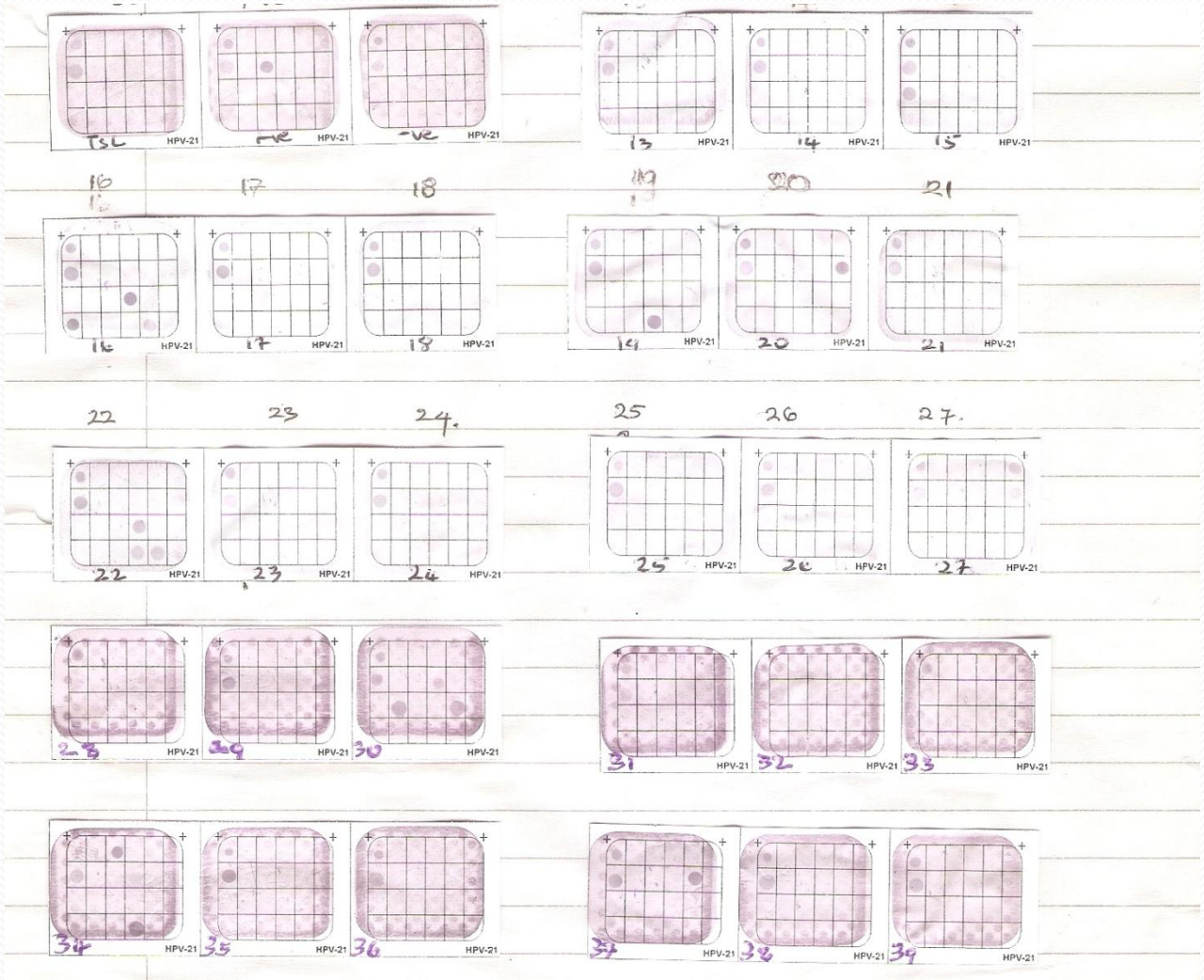
Flow-through Hybridization

- 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

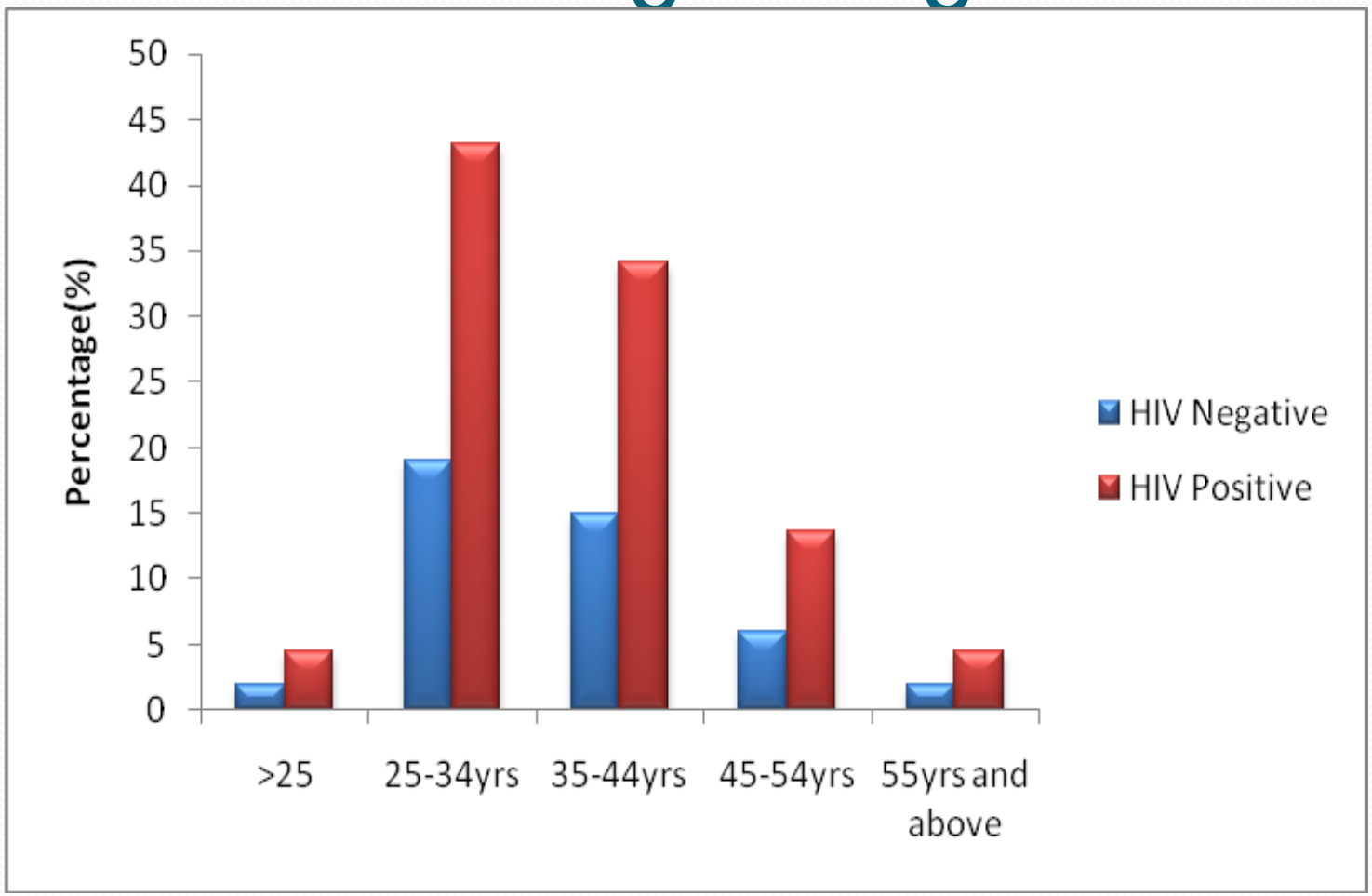
- Data processing was done using Epi info version 3.5.6 and Microsoft Excel
- Frequency distribution was used to determine the relationship between variables
- The student T-test was used for comparison of mean differences
- The Chi-square test was used to compare the differences between proportions
- 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 was 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)

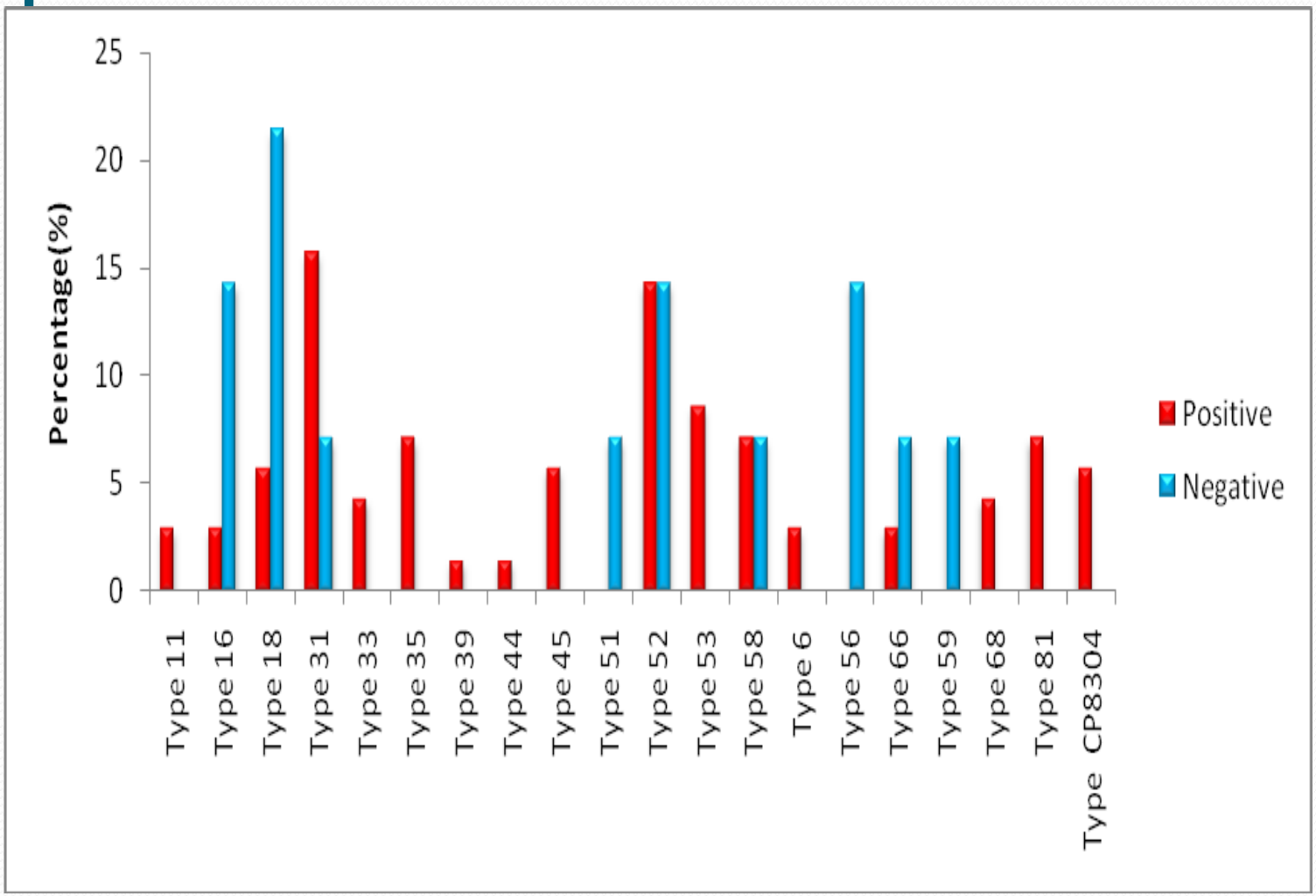
- Overall single genotypes were found in 27 females (27.55%)

- 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 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 errornously 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
- Zambia: types 53, 31, 51 and 45
- 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

Discusssion

- 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 Brazillian 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

Co- authors

- Banjo A A F
- Professor of Anatomic Pathology. College of Medicine, University of Lagos, Nigeria.
- Abdulkareem F B
- Professor of Anatomic Pathology. College of Medicine, University of Lagos, Nigeria
- Nwadike V U
- Consultant Microbiologist, Federal Medical Centre Abeokuta, Ogun state.

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