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Original Article **Open Access**

Co-infection of human papillomavirus and herpes simplex virus-2 with cervical dysplasia among women in Kaduna State, Nigeria

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Abstract:

Background: The epidemiology of human papillomavirus (HPV) and co-infections with herpes simplex virus type 2 (HSV-2) remains poorly characterized in Africa. High risk HPV (hrHPV) infection is the primary cause of 99.7% all cervical cancer especially in the presence of genital ulcer disease (GUD) which is usually caused by HSV-2. Herpes simplex virus-2 might interact directly with hrHPV to increase the risk of cervical cancer. Co-infection of HPV and HSV-2 in asymptomatic women could provide a clue to early diagnosis of cervical cancer and this could aid in the development of new strategies for effective management and improvement of the quality of life of women who are infected. If the synergy between HPV and HSV-2 can be prevented, there may be significant reduction of the incidence of cervical cancer.
Methodology: This was a descriptive cross-sectional study of 515 randomly selected apparently healthy women of reproductive age whose cervical samples were screened for HPV and HSV-2 in Kaduna State, Nigeria. The cervical samples were collected by liquid-based cytology (LBC) for detection of cervical epithelial cell abnormalities (CEA) and molecular detection of HPV and HSV-2 using conventional polymerase chain reaction (PCR) assay. Extracted viral DNAs from the samples were amplified by convectional PCR using specific hrHPV (HPV 16,18, 31 and 45) primer sets and a broad spectrum MY09/11 and GP5+/6+ primers for a wider range of HPV genotypes while HSV-2 DNA was detected by florescence-based PCR assay with HSV-2 gG primers and probes.
Results: PCR assay revealed that 14.7% (n=76) of the 515 selected women harbored HPV, HSV-2 or both. The prevalence of HPV, hrHPV, HSV-2 and HPV/HSV-2 co-infection among in the study participants are 11.8% (n=61), 9.3% (n=48), 5.6% 4% (n=29) and 2.3% (n=12) respectively. The frequency of HPV detection in HSV-2 infected participants (41.4%, 12/29) was significantly higher (OR=6.295, $p<0.0001$) than in HSV-2 negative participants (10.1%, 49/437), but only co-infections of HPV-31 ($p=0.004$) and HPV-18 ($p=0.040$) with HSV-2 were significantly associated. Cervical cytology reports on the smears revealed prevalence of cervical epithelial abnormalities (CEA) of 16.7% (n=86), with cervical dysplasia prevalence of 6.4% (n=33), consisting of high grade squamous intraepithelial lesion (HGSIL) of 1.6% (n=8), low grade squamous intraepithelial lesion (LGSIL) of 4.1% (n=21) and atypical squamous cells of uncertain significance (ASCUS) of 0.8% (n=4). The frequency of HPV detection in women with cervical dysplasia (93.9%, 31/33) was significantly higher (OR=14.22, $p<0.0001$) than in women without cervical dysplasia (6.2%, 30/482), and all HPV genotypes were significantly associated ($p<0.05$) with cervical dysplasia except HPV 16 ($p=0.051$). Also, the frequency of HPV/HSV-2 co-infection among women with cervical dysplasia (15.2%, 5/33) was significantly higher than among women without cervical dysplasia (1.5%, 7/482) (OR=12.12, $p<0.0001$). Comparing women with cervical inflammatory and atrophic changes (n=53), women with cervical dysplasia (HGSIL, LGSIL and ASC-US) had significantly higher frequency of HPV infection ($\chi^2=42.829$, $p=0.000$), HSV-2 infection ($\chi^2=25.140$, $p=0.001$) and HPV/HSV-2 co-infections ($\chi^2=66.602$, $p=0.000$).
Conclusion: Co-infection rate of hrHPV and HSV-2 among women in Kaduna State is 2.3%. Demographic factors significantly influencing the rate of co-infections included age and marital status. In spite of the screening method using the traditional Pap smear, GUD and cervical cancer continues to be a major public health problem, thus more sensitive and specific methods such as liquid based cytology technique and polymerase chain reaction for early detection of cervical dysplasia and hrHPV or HSV-2 in asymptomatic women should be adopted for routine use.

Keywords: HPV; hrHPV; HSV-2; Cervical dysplasia; Healthy women; Kaduna State; Nigeria

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Co-infection par le papillomavirus humain et le virus herpès simplex-2 avec dysplasie cervicale chez les femmes de l'État de Kaduna, au Nigéria

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Résumé:

Contexte: L'épidémiologie du virus du papillome humain (VPH) et des co-infections par le virus de l'herpès simplex de type 2 (HSV-2) reste mal caractérisée en Afrique. L'infection par le VPH à haut risque (VPH-2) est la principale cause de 99,7 % de tous les cancers du col de l'utérus, en particulier en présence d'ulcères génitaux (UG) qui sont généralement causés par le VPH-2. Le virus de l'herpès simplex de type 2 pourrait interagir directement avec le VPH-2 pour augmenter le risque de cancer du col de l'utérus. La co-infection par le VPH et le VPH-2 chez les femmes asymptomatiques pourrait fournir un indice pour un diagnostic précoce du cancer du col de l'utérus et cela pourrait aider au développement de nouvelles stratégies pour une gestion efficace et une amélioration de la qualité de vie des femmes infectées. Si la synergie entre le VPH et le VPH-2 peut être évitée, il pourrait y avoir une réduction significative de l'incidence du cancer du col de l'utérus.
Méthodologie: Il s'agit d'une étude transversale descriptive de 515 femmes apparemment en bonne santé en âge de procréer, sélectionnées au hasard, dont les échantillons cervicaux ont été examinés pour le VPH et le VHS-2 dans l'État de Kaduna, au Nigéria. Les échantillons cervicaux ont été collectés par cytologie en milieu liquide (LBC) pour la détection d'anomalies des cellules épithéliales cervicales (CEA) et la détection moléculaire du VPH et du VHS-2 à l'aide d'un test de réaction en chaîne par polymérase (PCR) classique. Les ADN viraux extraits des échantillons ont été amplifiés par PCR convectionnelle à l'aide d'ensembles d'amorces spécifiques du VPH hr (HPV 16, 18, 31 et 45) et d'amorces à large spectre MY09/11 et GP5+/6+ pour une gamme plus large de génotypes du VPH tandis que l'ADN du VHS-2 a été détecté par un test PCR basé sur la fluorescence avec des amorces et des sondes HSV-2 gG
Résultats: La réaction en chaîne par polymérase a révélé que 14,7% (n=76) des 515 femmes sélectionnées étaient porteuses du VPH, du VHS-2 ou des deux. Français La prévalence des co-infections VPH, VPH hr, VHS-2 et VPH/VHS-2 parmi les participants à l'étude est respectivement de 11,8% (n=61), 9,3 % (n=48), 5,6%, 4,0% (n=29) et 2,3% (n=12). La fréquence de détection du VPH chez les participants infectés par le VHS-2 (41,4%, 12/29) était significativement plus élevée (OR=6,295, p<0,0001) que chez les participants négatifs au VHS-2 (10,1%, 49/437), mais seules les co-infections du VPH-31 (p=0,004) et du VPH-18 (p=0,040) avec le VHS-2 étaient significativement associées. Les rapports de cytologie cervicale sur les frottis ont révélé une prévalence d'anomalies épithéliales cervicales (CEA) de 16,7% (n=86), avec une prévalence de dysplasie cervicale de 6,4% (n=33), composée de lésions intraépithéliales squameuses de haut grade (HGSIL) de 1,6% (n=8), de lésions intraépithéliales squameuses de bas grade (LGSIL) de 4,1% (n=21) et de cellules squameuses atypiques de signification incertaine (ASCUS) de 0,8% (n=4). Français La fréquence de détection du VPH chez les femmes atteintes de dysplasie cervicale (93,9%, 31/33) était significativement plus élevée (OR=14,22, p=0,0001) que chez les femmes sans dysplasie cervicale (6,2%, 30/482), et tous les génotypes du VPH étaient significativement associés (p<0,05) à la dysplasie cervicale, à l'exception du VPH 16 (p=0,051). De plus, la fréquence de co-infection VPH/VHS-2 chez les femmes atteintes de dysplasie cervicale (15,2%, 5/33) était significativement plus élevée que chez les femmes sans dysplasie cervicale (1,5%, 7/482) (OR=12,12, p<0,0001). Français En comparant les femmes présentant des modifications inflammatoires et atrophiques du col de l'utérus (n=53), les femmes atteintes de dysplasie cervicale (HGSIL, LGSIL et ASC-US) présentaient une fréquence significativement plus élevée d'infection par le VPH (χ^2=42,829, $\underline{p}$=0,000), d'infection par le VHS-2 (χ^2= 25,140, p=0,001) et de co-infections VPH/VHS-2 (χ^2=66,602, p=0,000).
Conclusion: Le taux de co-infection par le VPH et le VHS-2 chez les femmes de l'État de Kaduna est de 2,3%. Les facteurs démographiques influençant significativement le taux de co-infections comprenaient l'âge et l'état matrimonial. Malgré la méthode de dépistage utilisant le frottis traditionnel, l'ulcère gastroduodénal et le cancer du col de l'utérus continuent d'être un problème majeur de santé publique. Ainsi, des méthodes plus sensibles et spécifiques telles que la technique de cytologie en milieu liquide et la réaction en chaîne par polymérase pour la détection précoce de la dysplasie cervicale et du HPV-2 à haut risque ou du HSV-2 chez les femmes asymptomatiques devraient être adoptées pour une utilisation systématique.

Mots-clés: HPV; HPV-2 à haut risque; HSV-2; Dysplasie cervicale; État de Kaduna; Nigéria

Introduction:

Human papillomavirus (HPV) and herpes simplex virus type 2 (HSV-2) infections are among the most common human viral infections worldwide. Available data indicate that the worldwide prevalence of HSV-2 infection is high and deserving more attention (1). Both viruses share similar properties however, HSV-2 can cause more irritation and discomfort, and HPV often has a more serious impact on long-term health implications including cer-

vical dysplasia and cancer.

The cervix is the lower part of the uterus that opens at the top of the vagina. Cervical dysplasia is the development of abnormal cervical cells in the narrow neck of the uterus. It refers to abnormal changes in the endo and ectocervix but are not usually cancerous. Most of the time, there are no symptoms, however they are considered to be precancerous and can lead to cancer of the cervix if not treated. It is most often caused by high-risk human papilloma virus (hrHPV) infection and may lead to cancer.

Cervical cancer is an abnormal growth of cells arising from the cervix. It has the ability to invade or spread to other parts of the body. There is typically no sign at the early stage but later, symptoms may include abnormal post coital inter vaginal bleeding, pelvic pain, or pain during sexual intercourse (2). It constitutes a major public health threat to women in many low and medium resourced countries in South and Central America, sub-Saharan Africa, South and Southeast Asia where it is still the leading type of cancer among women (3). High risk HPV infection is the primary cause of virtually all cervical dysplasia. Although most HPV infections are sub-clinical and self-limiting within 1-2 years, the infection persists in about 5-10% of infected women, resulting in pre-cancerous lesions which can progress to invasive cancer 15-20 years later (4). HPV have been detected in up to 99.7% of cervical squamous cell carcinoma and 94 to 100% of cervical adenocarcinomas (5).

Studies have demonstrated that HSV-2 take part in some phase of cervical carcinogenesis and acts as a cofactor in the progression of cervical neoplasia to cervical cancer by inducing DNA damage and chromosomal abnormalities in cervical epithelial cells that are latently infected with HPV (6). Molecular evidence from *invitro* laboratory studies shows a possible synergism between HPV and HSV-2 infections (7). Specifically, an *invitro* data suggests that the XhoII sub-fragment of the HSV-2 genome induces the malignant transformation of HPV immortalized cervical cells and may be retained in cervical cancer tissue (8).

Many *invivo* studies have also demonstrated that induction of premalignant and malignant lesions in the mouse cervix occur after prolonged exposure to formalin or to ultraviolet (UV) inactivated HSV-1 or HSV-2 respectively (9). In these experimental studies, repeated exposure of the mouse cervix resulted in a sequence of pathological changes analogous to those that occur during human cervical carcinogenesis. The data obtained in the *invivo* model support conclusions derived from the *invitro* transformation of rodent cell by HSV-1 or HSV-2 (10). An effective intervention could be made available to women with pre-cancerous lesions caused by persistent hrHPV and HSV-2 infections after cervical screening.

The clinical significance of the association between HSV-2 and hrHPV infection remains to be elucidated. There is no documented information on HPV/HSV-2 co-infection in this region and Nigeria as a whole thus, there is need to explore the possibility of developing a system for rapid diagnosis and clinical screening of cervical cancer. Prevention and treatment of cervical cancer are crippled by lack of awareness and knowledge of the disease, sub-optimal public investment and competing health needs amongst others.

There is no adequate central database for cervical cancer in Nigeria, and no organized national screening programme, while the National Health Insurance Scheme (NHIS) has limited coverage for cancer treatment. The few existing cervical cancer screening programmes are based on Pap smear with its technological and human resource challenge, and visual inspection with acetic or Lugol's iodine (VIA/VILI), with its challenge of low-test characteristics. The polymerase chain reaction (PCR) assay has a potential to overcome the limitations of Pap smear and VIA or VILI.

The ideal strategy for prevention and treatment in Nigeria should have the potential to prevent HPV/HSV-2 infection, overcome the limitations of existing screening tools, and identify cases early. Appropriate vaccination of young girls and HPV-based cervical cancer screening methods has the potential to address these gaps. This study was carried out to determine the prevalence of hrHPV and HSV-2 co-infection types in Kaduna State, as well as to obtain a more robust measure of the vaccine-preventable proportion of cervical cancer in this region.

Materials and methods:

Study design and setting:

This was a hospital based descriptive cross-sectional study of apparently healthy women of reproductive age attending the Reproductive Health and Family Planning clinics of selected health care facilities. Across Kaduna State, Nigeria.

Ethical consideration:

Ethical approval for the study were obtained from the ethics committees of Kaduna State Ministry of Health, Barau Dikko Teaching Hospital (BDTH) and the Ahmadu Bello University Teaching Hospital Zaria (ABUTH). Informed consent was obtained from by each woman who participated in the study and the procedure for obtaining samples was explained to each woman before samples for investigations were taken. All findings as a result of the study were confidential and women whose results indicate any need for medical interven-

tion were counseled and appropriately referred for such.

Study participants, sample size and method of sampling:

The minimum sample size of 515 was determined using the Fisher's formula (11), $n=p^2q(1-p)/d^2$, and the prevalence rate of HPV reported by Magaji et al., (4) in a similar study carried out in Kaduna State, Nigeria. The study participants were apparently healthy women of reproductive age (15-65 years) irrespective of ethnicity, educational status and place of residence, and were randomly selected from the 9 Local Government Areas (LGAs) of Kaduna State. Exclusion criteria were women who were older than 65 years of age and those with previous operative or therapeutic history related to gynecologic diseases that may interfere with the results.

Data and sample collection:

A pre-designed and structured questionnaire which contains information on socio-demographic variables such as age, education, occupation, type of family, geographical location, annual income, and potential risk factors were administered to eligible women.

Cervical smears were collected by the attending physician assisted by a reproductive health nurse following standard procedures. Each study participant was positioned on the couch in dorsal position and a disposable sterile vaginal speculum (Cusco speculum) inserted in position to keep the labia separated. When the cervix was clearly seen, the blades of the speculum was locked in place and the vaginal walls and cervix was inspected for any abnormality.

Liquid based cytology (LBC) technique was used to collect the smear, carried out by using a cytology brush to take the sample. The larger end of the brush was inserted into the endocervix and gently rotated over the surface of the cervix, making sure that the squamo-columnar junction was well and correctly scrapped. The smeared brush was detached into a special vial containing the preservative liquid and labeled appropriately. The specimens were transported to the Pathology laboratory for analysis.

Detection of cervical epithelial abnormalities

The cervical smears were vortexed and strained to remove other elements such as mucus. An aliquot of the homogeneous mixture was frozen and stored at -20^{o}C for molecular studies while the remaining sample was centrifuged into layers based on density gradient. With the aid of a special polycarbonate filter, a thin layer of cells was placed on a clean grease free glass slide and the deposit was processed, fixed and stained with Papanicolaou technique. The stained slides were read and interpreted by a Pathologist using the Bethesda system (TBS) that provides a framework for consistent inter laboratory terminology for the reporting of cervicovaginal cytology specimen for diagnosis of cervical epithelial abnormalities (CEA).

Molecular detection of HPV and HSV-2 by PCR:

Genomic DNA was extracted from the LBC preserved cervical samples using Quick DNA viral extraction kit (Zymo Research Corp, South Africa) according to the manufacturer's instruction. Denaturation of viral particles and recovery of DNA was accomplished using a unique viral DNA buffer and zymo columns respectively.

The positive DNA samples were amplified by PCR to detect HPV DNA using highly sensitive MY09/MY11 and GP5+/GP6+ primer sets as previously described (12). The two pairs of consensus primers (HPV forward: 5'-GCiCAGGGiCACAATAATGG-3' and HPV reverse: 5'-GTiGTATCACAACAGTA ACAAA-3') were used to target a 65-base pair of the HPV L1 region of the viral genome which detects wider range of HPV genotypes by opening up the reading frame that enables the PCR amplification of at least 54 genital HPV types to detect HPV-DNA.

The PCR assay was performed in a 25µL reaction using One Taq Quick-load Master Mix (New England Biolab), which contained 1.25U Taq DNA polymerase in standard buffer (containing 1.8mM $MgCl_2$, 22mM NH_4Cl, 22mM KCl), 0.2µM each of forward and reverse primers and 200µM dNTPs. To each reaction, 2µL of extracted DNA (template DNA) was added. Amplification was carried out using the following thermocycler conditions; initial denaturation at 94°C for 3 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 1min and extension at 68°C for 1 min, followed by final extension at 68°C for 5min.

Different primer set and probes were used to amplify the HSV viral subtype using florescence-based PCR assay of HSV glycoprotein G (gG) gene as described by Ryncarz et al., (13). The forward HSV-2 primer used was 5′-TCCTG/CGTTCCTA/CACG/TGCCTCCC-3′ and the reverse primer was 5′-GCAGICAC/TACGT AACGCACGCT-3′ while the florescent labelled HSV-2 gG2 probe (HSV-gG2-P) used was 5′-FAM-CGACCAGACAAACGAACGCCGCCGT-3′ -TAMRA. Each 50µl PCR mixture contained 5µl of purified DNA, 833nM density per primer, and 100nM probe. After 2 mins of incubation at 95°C for denaturation, the 45 cycles of PCR were performed, where every cycle was 95°C for 20 sec and 58^{o}C for 1 min respectively. The fluorescent intensities were read during PCR cycling in a Corbett Rotor gene 6600 thermocycler. All viruses were detected in the range of 10^1-10^7 copies/reaction.

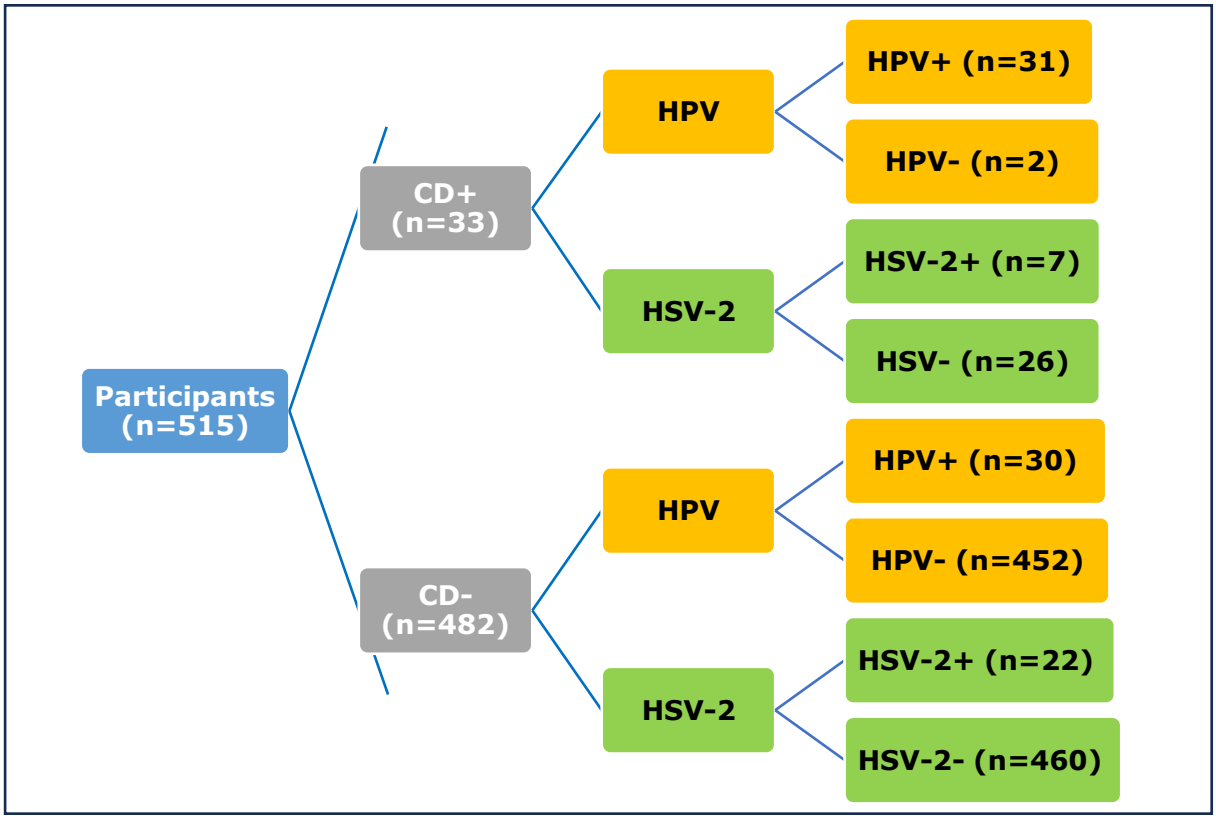
Statistical analysis:

The prevalence of different hrHPV genotypes, HSV-2 and HPV/HSV-2 co-infections were compared in each grade of cervical dysplasia by Chi square test (χ^2) and Fishers exact test which were used for categorical variables. Age categories were created as follows; 16-25, 26-35, 36-45, 46-55 and 56-65 years. Odd ratio (OR) was calculated at 95% confidence intervals (CI). The association between the various categories and variables were determined using the Statistical Package for the Social Sciences (SPSS) version 23.0 with $p<0.05$ considered statistically significant.

Results:

A total of 515 apparently healthy women were randomly enrolled from 9 Local Government Areas (LGAs) across the Senatorial Zones of Kaduna State, Nigeria for the study. The social demographic characteristics of participants as presented in Table 1 shows that the mean and modal age of the women were 41.8 ±10.9 years and 40.0 years respectively. The most and least frequently recruited age groups of the women were 36-45 years (33.6%) and 16-25 (6.0%) years respectively.

The distribution pattern of HPV and HSV-2 with respect to cervical cytology reports among the 515 women participants is shown in Fig 1. There was a total of 61, 29, 12 and 33 cases of HPV, HSV-2, HPV/HSV-2 co-infection and cervical dysplasia detected in the 515 cervical samples, giving the respective prevalence of 11.8%, 5.6%, 2.3% and 6.4%. Among women with cervical dysplasia, HPV was detected in 93.9% (n=31/33) while 6.2% of women without cervical dysplasia, had HPV infection (n=30/482) (OR=14.218, 95% CI= 6.65-30.39, $p<0.0001$). Also, HSV-2 was detected in 21.2% (7/33) and 4.6% (22/482) among women with and without cervical dysplasia respectively (OR=7.025, 95% CI=2.83-17.43, $p<0.0001$). Furthermore, among women with cervical dysplasia, HPV/HSV-2 co-infection was detected in 15.2% (n=5/33) while among women without cervical dysplasia, HPV/HSV-2 co-infection was detected in 1.5% (n=7/482) (OR=12.12, 95% CI=3.62-40.62, $p<0.0001$).



CD=cervical dysplasia; HPV=Human papillomavirus; HSV-2 = Herpes simplex virus type 2; n=number

Fig 1: Distribution pattern of HPV and HSV-2 infection among apparently healthy women with and without cervical dysplasia in Kaduna State, Nigeria

Table 1: Prevalence of HPV and HSV-2 infection in relation to socio-demographic factors among apparently healthy women participants in Kaduna State, Nigeria

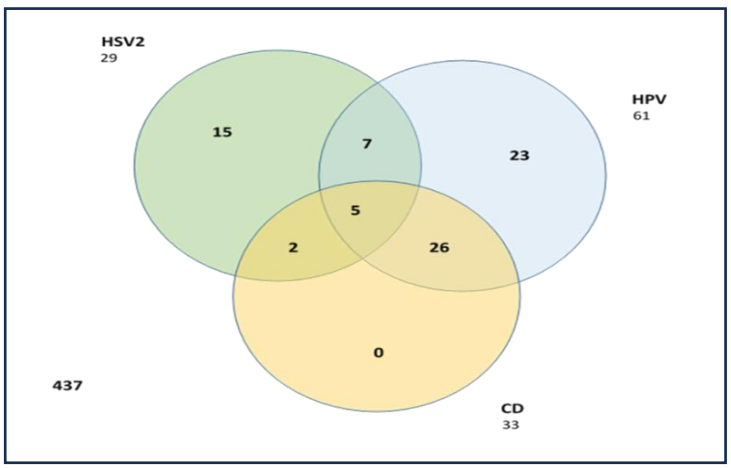
Variable	No examined (%)	No HPV positive (%)	*p* value	No HSV-2 positive (%)	*p* value	No HPV/HSV-2 positive (%)	*p* value	No cervical dysplasia (%)	*p* value
Age group (years)									
16-25	31 (6.0)	2 (6.5)	0.171	0	0.003*	0	0.021*	0	0.001*
26-35	119 (23.1)	15 (12.6)		4 (3.4)		0		7 (5.9)	
36-45	173 (33.6)	28 (16.2)		6 (3.5)		2 (1.6)		5 (2.9)	
46-55	116 (22.5)	9 (7.8)		8 (6.9)		6 (5.2)		17 (14.7)	
56-65	76 (14.8)	7 (9.2)		11 (14.5)		4 (5.3)		4 (5.2)	
Educational status									
None	66 (12.8)	4 (6.0)	0.606	3 (4.5)	0.048*	1 (1.5)	0.415	5 (7.5)	0.983
Primary	74 (14.4)	9 (12.2)		8 (10.8)		3(4.1)		4 (5.4)	
Secondary	144 (28.0)	20 (13.9)		2 (1.4)		2 (1.4)		10 (6.9)	
Tertiary	199 (38.6)	24 (12.1)		14 (7.0)		4 (2.0)		12 (6.0)	
Qur'anic	32 (6.2)	4 (12.5)		1 (3.1)		2 (6.3)		2 (6.3)	
Marital status									
Single	34 (6.6)	3 (8.8)	0.476	4 (11.8)	0.075	3 (8.8)	0.021*	3 (8.8)	0.271
Married	422 (81.9)	54 (12.8)		21 (5.0)		7 (1.7)		23 (5.5)	
Divorced	33 (6.4)	3 (9.0)		4 (12.1)		2 (6.0)		4 (12.1)	
Widowed	26 (5.0)	1 (3.8)		0 (0.0)		0 (0.0)		3 (11.5)	
Type of marriage									
Monogamy	332 (64.5)	40 (12.0)	0.634	16 (4.8)	0.154	7 (2.4)	0.080	18 (5.4)	0.421
Polygamy	142 (27.6)	18 (12.7)		8 (5.6)		2 (1.4)		11 (7.7)	
Not Married	41 (8.0)	3 (7.3)		5 (12.2)		3 (4.9)		4 (9.8)	

*Statistically significant association

The association of social-demographic characteristics such as age, education, marital status and type of marriage on HPV, HSV-2, HPV/HVS-2 co-infection and cervical dysplasia among the study participants is presented in Table 1. The results shows that the prevalence of HPV infection is highest among women between 36-45 years (16.2%, 28/173) although there was no statistically significant difference in the prevalence of HPV infection with respect to age group in the study (p=0.171), while women in age group 56-65 years had significantly higher rate of HSV-2 infection (14.5%, 11/76) than other age groups (χ^2= 16.06, p=0.003). Women in age groups 56-65 and 46-55 years had significantly higher prevalence of HPV/HSV-2 co-infections than other age groups (p=0.021). The prevalence of cervical dysplasia was significantly higher among women in the age group of 46-55 years (14.7%, 17/116) than other age groups (χ^2= 19.07, p=0.001).

With respect to the level education of the women, 38.6% (199/515) had tertiary education while 12.8% had no formal education. The prevalence rates of HPV (12.1%), HSV-2 (7.0%) and cervical dysplasia (6.0%) are higher among women who had tertiary education compared to women who are not educated, however, only 2.0% of the women with the tertiary education had co-infection of both viruses. Among the 32 women who had Qur'anic education, 6.3% had cervical dysplasia and 6.3% had HPV/HSV-2 co-infection.

Age was significantly associated with prevalence of HSV-2 infection (p=0.003), HPV/HSV-2 co-infection (p=0.021) and cervical dysplasia (p=0.001). Education was significantly associated with only HSV-2 infection (p= 0.048) and marital status was only significantly associated with on HPV/HSV-2 co-infection (p=0.021) (Table 1).



CD=cervical dysplasia; HPV=Human papillomavirus; HSV-2 = Herpes simplex virus type 2

Fig 2: Venn diagram showing the distribution pattern of HPV, HSV-2, HPV/HSV-2 co-infection and cervical dysplasia among apparently healthy women participants in Kaduna State, Nigeria

The Venn diagram (Fig 2) shows the distribution of HPV, HSV-2, HPV/HSV-2 co-infection and cervical dysplasia among the women who participated in the study. It showed that 41.6% (n=5/12) of those with HPV/HSV-2 co-infection had cervical dysplasia 7 of 29 (24.1%) women with HSV-2 infection had cervical dysplasia. The distribution of hrHPV and other HPV genotypes among women with HSV-2 infection is shown on Table 2. The frequency of HPV/HSV-2 co-infected participants (41.4%, 12/29) was significantly higher (OR=6.295, p<0.0001) than in HSV-2 negative participants (10.1%, 49/437). The HPV genotype most frequently associated with HSV-2 is HPV-31 (20.7%, 6/29), and the least is HPV-45 (3.4%, 1/29). Multiple infection of hrHPV is significantly associated with HSV-2 infection (p=0.004) as shown on Table 2.

Table 3 displayed the distribution of HPV genotypes among women with and without cervical dysplasia. The frequency of HPV detected in women with cervical dysplasia (93.9%, 31/33) was significantly higher (OR= 14.22, p<0.0001) than in women without cervical dysplasia (6.2%, 30/482). The frequency of other unidentified hrHPV among women with cervical dysplasia was 33.3% (11/33) while the least frequent was HPV-16 (6.1%, 2/33). The HPV genotypes significantly associated with cervical dysplasia are HPV-18 (p= 0.004), HPV-31 (p=0.002), HPV-45 (p=0.039) and other hrHPV types (p<0.0001) while HPV 16 was not significantly associated with cervical dysplasia (p=0.1081). Women with multiple HPV infection were 27.3% (9/33).

The characteristics of cervical dysplasia and epithelial cell abnormalities (CEA) reported among women infected with hrHPV and HSV-2 is presented in Table 4. The most frequent cervical dysplasia type is low grade squamous intraepithelial lesion (LGSIL) (63.6%, 21/33) followed by high grade squamous intraepithelial lesion (HGSIL) (24.2%, 8/33) and atypical squamous cells of undetermined significance (ASC-US) (12.1%, 4/33). Four of the 8 HGSIL (50.0%) were infected with HPV, and 37.5% (3/8) were co-infected with HSV-2. Of the women with LGSIL, 38.1% (8/21) were infected with HPV while only 9.5% (2/21) had HPV/HSV-2 co-infection. Two of the 4 (50.0%) women with ASC-US were infected with HPV while 25% (1/4) each were co-infected with HSV-2.

Of the 31 women with inflammation of the cervix, 16.1% (5/31) were infected with HPV, 6.5% (2/31) were infected with HSV-2, and 3.2% (1/31) were co-infected with HPV/ HSV-2. Only 2 of 22 (9.1%) women with atrophic changes had HPV infection, 3 of 22 (13. 6%) had HSV-2 infection, and 2 of 22 (9.1%) had HPV/HSV-2 co-infection. Compared to women with cervical inflammatory and atrophic changes, women with HGSIL, LGSIL and ASCUS had significantly higher frequency of HPV infec- tion (χ^2=42.829, p=0.000), HSV-2 infection (χ^2=25.140, p=0.001) and HPV/HSV-2 co-infections (χ^2=66.602, p=0.000).

Table 2: Distribution of HPV genotypes among apparently health women participants with and without HSV-2 co-infection

HPV genotype		No examined (%)	No HSV-2 positive (%)	No HSV-2 negative (%)	χ^2	OR (95% CI)	p value
HPV 16							
	Pos	9 (1.7)	2 (6.9)	7 (1.4)	2.099	5.069	0.086
	Neg	506 (98.3)	27 (93.1)	479 (98.6)		(1.00-25.59)	
HPV 18							
	Pos	6 (1.2)	2 (6.9)	4 (0.8)	4.286	8.926	0.040*
	Neg	509 (98.8)	27 (93.1)	482 (99.2)		(1.56-50.9)	
HPV 31							
	Pos	30 (5.8)	6 (20.7)	24 (4.9)	9.672	5.022	0.004*
	Neg	485 (94.2)	23 (79.3)	462 (95.1)		(1.87-13.49)	
HPV 45							
	Pos	21(4.1)	1 (3.4)	20 (4.1)	0.031	0.832	0.666
	Neg	494 (95.9)	28 (96.6)	466 (95.9)		(0.108-6.4)	
Other HPVs							
	Pos	38 (7.3)	1 (3.4)	37 (7.6)	0.2186	0.433	0.874
	Neg	477 (92.6)	28 (96.6)	449 (92.4)		(0.057-3.28)	
Multiple HPVs							
	Pos	30 (5.8)	6 (20.7)	24 (4.9)	9.672	5.022	0.004*
	Neg	485 (94.2)	23 (79.3)	462 (95.1)		(1.87-13.49)	
Total		**515**	**29 (5.6)**	**486 (94.4)**			

HPV = Human papillomavirus; HSV-2 = Herpes simplex virus type 2; x^2 = Chi square; OR = Odd ratio; CI = Confidence interval

Table 3: Distribution of HPV genotypes among apparently healthy women participants with and without cervical dysplasia

HPV genotype		No examined (%)	Cervical dysplasia		x^2	OR (95% CI)	*p* value
			Positive (%)	Negative (%)			
HPV16							
	Pos	9 (1.7)	2 (6.1)	7 (1.5)	1.608	4.378	0.2048
	Neg	506(98.3)	31 (93.9)	475 (98.5)		(0.87-21.98)	
HPV18							
	Pos	6 (1.2)	3 (9.1)	3 (0.6)	12.584	15.967	0.004*
	Neg	509 (98.8)	30 (90.9)	479 (99.4)		(3.09-82.53)	
HPV31							
	Pos	30 (5.8)	7 (21.2)	23 (4.8)	12.368	5.373	0.002*
	Neg	485 (94.2)	26 (78.8)	459 (95.2)		(2.11-13.67)	
HPV45							
	Pos	21 (4.1)	4 (12.1)	17 (3.5)	3.842	3.733	0.039*
	Neg	494 (95.9)	29 (87.9)	465 (96.5)		(1.19-11.94)	
Other hrHPVs							
	Pos	38 (7.4)	11 (33.3)	27 (5.6)	30.816	8.426	0.000*
	Neg	477 (92.6)	22 (66.7)	455 (94.4)		(3.71-19.16)	
Multiple HPVs							
	Pos	30 (5.8)	9 (27.3)	21 (4.4)	25.535	8.232	<0.0001*
	Neg	485 (94.2)	24 (72,7)	461 (95.6)		(3.41-19.89)	
Total HPVs							
	Pos	61 (11.8)	19 (57.6)	42 (8.7)	66.018	14.22	<0.0001*
	Neg	454 (88.2)	14 (42.4)	440 (91.3)		(6.65-30.39)	
		515	**33 (6.4)**	**482 (93.6)**			

HPV = Human papillomavirus; hr = high-risk; x^2 = Chi square; OR = Odd ratio; CI = Confidence interval

Table 4: Characteristics of cervical dysplasia and epithelial cell abnormalities among apparently healthy women with HPV, HSV-2 and HPV/HSV-2 co-infections in Kaduna State, Nigeria

Variable		Cervical dysplasia (n=33)			Other epithelial abnormalities (n=53)		X^2	*p* value
		HGSIL (%)	LGSIL (%)	ASCUS (%)	Inflammation (%)	Atrophic changes (%)		
HPV								
	Pos	4 (50.0)	8 (38.1)	2 (50.0)	5 (16.1)	2 (9.1)	42.829	0.000*
	Neg	4 (50.0)	13 (61.9)	2 (50.0)	26 (83.9)	20 (90.9)		
HSV-2								
	Pos	3 (37.5)	2 (9.5)	1 (25.0)	2 (6.5)	3 (13.6)	25.140	0.001*
	Neg	5 (62.5)	19 (90.5)	3 (75.0)	29 (93.5)	19 (86.4)		
HPV/HSV-2								
	Pos	3 (37.5)	2 (9.5)	1 (3.2)	1 (3.2)	2 (9.1)	66.605	0.000*
	Neg	5 (62.5)	19 (90.5)	3 (96.8)	30 (96.8)	20 (90.0)		
Total		**8**	**21**	**4**	**31**	**22**		

HPV = Human papillomavirus; HSV-2 = Herpes simplex virus type 2; HGSIL = High grade squamous intraepithelial lesion; LGSIL = Low grade squamous intraepithelial lesion; ASCUS = Atypical squamous cell of undetermined significance; x^2 = Chi square

Discussion:

Both HPV and HSV-2 are a major global health concern with high morbidity globally and Africa. HSV-2 plays a role as a co-factor in the acquisition of HPV infection (1). To the best of our knowledge, this is the first study on the co-infection of HPV and HSV-2 among women in Kaduna State and Northern Nigeria as a whole. The prevalence of total HPV, hrHPV and HSV-2 among the study participants is 11.8%, 9.3% and 5.4% respectively and co-infection of HPV and HSV-2 is 2.3%.

In Nigeria, there are inadequate data on the prevalence of HPV infections and studies on co-infection of HPV and HSV-2 are sparse. Different HPV rates have been reported in the different parts of the country. In Kano, northwest Nigeria, a high HPV seroprevalence of 76% was reported by Auwal et al., (14), and a study in Gombe, northeast Nigeria by Manga et al., (15) reported a 48.1% prevalence of cervical HPV infection among women with mean age of 39.6±10.4 years. The prevalence of 11.8% in our study is lower than the rate from these studies, probably because of the difference in the methodology used; serology in those studies compared to molecular method in ours. In southwest Nigeria, cervical HPV was detected in 26.3% of sexually active women above 15 years in Ibadan and 14.7% among 1282 women in Irun (16). In Okene,

northcentral Nigeria, HPV prevalence of 21.6% among 231 women was reported, and in Abuja, the Federal Capital of Nigeria, the prevalence was 37.0% among 275 women studied (17).

The prevalence of HSV-2 in our study was 5.4% (29/515). This rate is lower than those of previous studies in Zaria, Kaduna State (18,19), which reported HSV-2 IgG seroprevalence of 53.8% and 82.2% respectively. The wide difference in these rates from ours is likely due to the methodology used in the Zaria studies (serology) compared to the molecular method (PCR) used in our study. In another similar study carried out by Sani et al., (20) in Dutse, Jigawa State, the prevalence of HSV-2 among women of reproductive age attending General Hospital was 30.9%. The prevalence of HSV-2 among pregnant women in Edo State, south-south Nigeria, was reported by Kalu et al., (21) to be 44.3% and among women with cervical dysplasia in southwest Nigeria, it was 75.2% (22). These variations might also be due to changes in culture and sexual behaviors of women in different communities and geographic locations.

The low prevalence of co-infection of HPV with HSV-2 of 2.3% in our study indicates that co-infection rate is low among women of reproductive age in Kaduna State, Nigeria. This co-infection rate is lower compared to the study by Okoye et al., (22) who reported co-infection of HPV and HSV-2, and HPV with HIV among women in southwest Nigeria to be 45.7% and 26.7% respectively. In Ghana, Oksana et al., (23) reported 44.4% in a similar co-infection study of HPV and HSV-2. However, these studies were purely serological, with ELISA technique used to detect viral antibodies, which is different from the molecular technique (PCR) used to detect the viral DNA in our study.

The prevalence 6.4% for cervical dysplasia in our study is slightly higher than the study of Oguntayo et al., (24) who reported 4.8% in a 5-year review of patients who were screened for cervical cancer at Ahmadu Bello Teaching Hospital, Zaria, Nigeria between 2005 and 2010, with rates of cervical intraepithelial neoplasia (CIN) I, II and III of 3.6%, 0.8% and 0.4% respectively. This is probably because liquid-based cytology technique used in our study has better precision compared with Pap smears which was used the Zaria study. The prevalence of cervical dysplasia is however lower in our study when compared to the prevalence reported in other parts of Nigeria, which include 11.8%, 12.0% and 12.2% in Ibadan, Uyo and Enugu respectively (25,26) but agrees with 6.2% and 6.0% reported in studies in Kaduna (27) and Kano (14) respectively. This difference could be attributed to the variation in the socio-cultural lifestyles of women in the different geographic regions. The trend of cervical dysplasia among Nigerian women should be a concern to researcher and policy makers for a deliberate action to cub the menace in our society.

The prevalence of cervical epithelial cell abnormalities (CEA) in our study was 23.1% (119/515), which includes all women who had cervical dysplasia (6.4%, 33/515), those who had cervical inflammation (6.0%, 31/515) and those who had atrophic changes (4.3%, 22/515). The prevalence of CEA of 23.1% in our agrees with the study by Bassey et al., (25) who reported 23.4% of CEA among women in Uyo, south-south Nigeria with 11.5% cervical dysplasia and 10.0% inflammatory changes. Inflammatory changes are relatively frequent findings in cervical smears and are generally believed to be a consequence of genital infection (28). The presence of inflammatory cells on cervical smears is not necessarily due to infection but could be due to reactive or reparative cellular changes of the cervix following injury, radiation, intrauterine devices or atrophy. However, in their study, there was no significant difference in the resolution of cytologic inflammatory changes after treatment with antibiotics. It has been reported that the prevalence of cervical dysplasia is high among women with inflammatory smear after colposcopy and biopsy. It is therefore imperative to consider inflammatory cervical cytology smear as being abnormal smears because it could mask the dysplastic cells giving false negative result, hence the importance of treating women with inflammatory smears and repeating the test within 3–6 months of treatment (28).

This study shows that age was a significant risk factor for co-infection of HPV/HSV-2 (p=0.021) and cervical dysplasia (p=0.001). These findings are consistent with previous reports (20). Women in the 46-55years age group had the highest incidence of HPV/HSV-2 co-infection (5.2%) and cervical dysplasia (14.7%). Age is an important factor because the chances of a woman developing cervical dysplasia increases with increasing age. The mean age for developing dysplasia and carcinoma in situ has been reported to range from 34.7 to 38.6 years and 39.6 to 43.5 years, respectively (28). The prevalence of HPV infection was highest among women in 36-45 years age category (16.2%, 28/173) in our study, which agrees with the study of Oguntayo et al., (24) who reported the same peak age-specific prevalence rate of CIN in their study. Our finding however differs from those of Ogah et al., (29) and Akarolo-Anthony et al., (16) in similar HPV studies in Lokoja and Abuja Nigeria, which reported high HPV infection rates among younger women of less than 30 years with decrease in infection rate with age. This may probably be due to the fact that younger women including teenagers are more sexually active with

higher number of partners compared to older women.

The educational status of the participants in our study shows that 38.6% (n=199) of the women attended tertiary institution and 27.9% (n=144) were educated at secondary level. The frequency of participants who were infected with HPV, HSV-2 among the women with tertiary education were 12.1% (24/199) and 7.0% (14/199) respectively. These values are higher compared to the infection rate of the viruses among the women without formal education with 6.0% (4/66) and 4.5% (3/66) respectively. The rate of HPV/HSV-2 co-infection was highest (6.3%, 2/32) among women with Qur'anic education. However, education was not a statistically significant risk factor for HPV/HSV-2 infection but was a significant factor for HSV-2 infection (p=0.048).

A study on level of education and cervical cancer awareness observed that high literacy level among women does not translate to adequate knowledge of HPV and HSV-2 prevention (30). This implies that more awareness campaign is required to educate women on preventive measures and management of CEA. It is expected that women who have acquired western education especially at tertiary level should be adequately informed on the preventive measure of cervical diseases and cancer vaccines. Higher educational level among females can help reduce rate of these viral infections and CEA since lower educa tional status may leave the women with low-income jobs which can expose them to infection and cervical diseases. However, in our study, there was no statistically significant association between level of education and prevalence of cervical dysplasia (p=0.983)

In this study, the frequency of women from monogamous family was 64.5% (n=332) and polygamous family was 27.6% (n=142) while the frequencies of women in monogamous family with HPV/HSV-2 co-infection and cervical dysplasia were 2.4% (7/322) and 5.4% (18/332) respectively, which are higher than the respective rates in women from polygamous families. The higher rates among the women from monogamous families could be attributed to the fact that spouses of the women in monogamous setting may have multiple sexual partners while some of the women may already have contracted HPV or HSV-2 before getting married. Women who were exposed to sexual intercourse before marriage have greater tendency to having multiple sexual partners which increase their exposure risks to HPV and HSV-2 infection.

Muhammed et al., (31) reported that 87.4% of apparently healthy women who had cervical dysplasia were married and 66.9% were from polygamous settings, implying that marital status and type of marriage play a significant role in rate of cervical dysplasia among women. On the contrary, our study revealed that only 5.5% (23/515) of the married women and 7.7% (11/142) of those from polygamous homes had cervical dysplasia, but the association of marital status with cervical dysplasia was not statistically significant (p= 0.271). Our study also shows that HPV/HSV-2 co-infection was relatively high among single (8.8%) and unmarried (4.9%) women, however, HPV infection rate was higher among the married women (12.8%) while HSV-2 infection rate was higher among the singles (11.8%) and divorced (12.1%) women but no significant association between marital status and HPV (p=0.476) or HSV-2 (p=0.075) infection observed. Nevertheless, HPV/HSV-2 co-infection was significantly associated with marital status (p=0.021) in the study.

Our study showed that women with multiple HPV infections were more susceptible to HSV-2 infection, which may be due to the reduced immunity of such women from multiple HPV infections. According to Kim et al., (32), multiple HPV infection is common among patients with cervical dysplasia and cancer. In their study, they observed that the association between multiple HPV infection and HGSIL was significantly stronger (p=0.002) compared to that of single HPV infection and HGSIL, with about half (56.3%) of the women with HPV infection in their study having multiple HPV. This is not too different from the result of our study as 49.2% (30/61) of the participants with HPV had either double or triple HPV infections.

Kabir et al., (33) reported that HPV-DNA was prevalent in majority of the examined cervical cancer tissues and that HPV 16, HPV 18, HPV 45, HPV 51 and HPV 52 were the predominant HPVs detected in both single and multiple HPV infections in Maiduguri, northeast Nigeria.The characteristics of CEA among the participants in our study showed that HPV, HSV-2 and HPV/HSV-2 co-infection were detected in women with cervical dysplasia (HGSIL, LGSIL and ASC-US), and those with infla mmatory and atrophic changes, but the prevalence rates were significantly higher in women with cervical dysplasia than in women with cervical inflammatory and atrophic changes (p<0.0001). Although our study was among healthy individuals, HPV and HVS-2 infections were established in all cases of CEA which implies that co-infection of HPV and HSV-2 play significant role in the development of all forms CEA.

Conclusion:

The prevalence of cervical dysplasia, HPV, hrHPV, HSV-2 and HPV/HSV-2 co-infection among apparently healthy women in Kaduna State, northwest Nigeria were 6.4%, 11.8%, 9.3%, 5.6% and 2.3% respectively.

This suggests that molecular screening for hrHPV genotypes and HSV-2 could prove highly effective in identifying cervical pathology at a significantly highly treatable level among women in Kaduna State.

Despite the tremendous progress that has been achieved in the screening and management of women with HPV related cervical disease, there is still a need for clinically robust biomarkers such as HSV-2 to further refine the screening, triage, and management of women. High incidence of genital ulcer diseases, cervical dysplasia and cancer with considerable mortality in this region is evidenced of abundant hrHPV and HSV-2 infection in Kaduna State especially in the absence of routine screening for these viruses and low public awareness.

In addition, development of effective treatment and prevention interventions will be needed, particularly in the area of HPV and HSV-2 vaccines. These vaccines should be made available, affordable and introduced among young girls and women to enhance protection against HPV, HSV-2 infection and subsequently cervical epithelial disease, cervical dysplasia and cancer.

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Contributors of authors:

ADS was involved in study conceptualization, project administration, molecular extraction, polymerase chain reaction assay of HPV genotypes and writing of the original draft; AM was involved in study supervision, validation of data, review and editing of the manuscript; EEE was involved in study supervision, methodology, visualization, investigation, review of statistics and editing of manuscript; OAO was involved in supervision, investigation, selection/validation of participants and review of manuscript; AOK was involved in investigation, cytology validation and review of literature and manuscript; AM was involved in cytology validation and review of operational procedures (Bethesda system); SBB was involved in validation of participants, review of literature and editing of the manuscript; and ERA was involved in molecular extraction and detection of HSV-2.

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No conflict of interest is declared.

References:

1. Looker K. J., Magaret, A. S., Turner, K. M., Vickerman, P., Gottlieb, S. L., and Newman L. M. Global estimates of prevalent and incident herpes simplex virus type 2 infections in 2012. PLoS One. 2015; 10: e114989. doi: 10.1371/journal.pone.0114989
2. National Cancer Institute (NCI), Defining Cancer, National Institute of Health. USA, 2015
3. Parkin, D. M., Whelan, S. L., Ferlay, J., and Storm, H. Cancer Incidence in Five Continents, Volumes I to VIII IARC Cancer Base No. 7, Lyon, 2005.http://www.iacr.com.fr/statist.htm (Accessed February 11, 2012).
4, Magaji, S. J., Aminu, M., Inabo, H. I., and Oguntayo, A. O. Spectrum of high-risk human papillomavirus types in women in Kaduna State, Nigeria. Annals of Afri Med. 2019; 18 (1): 30-35 doi: 10.4103/aam.aam_10_18
5. Okunade, K. S. Human Papillomavirus and Cervical Cancer. J Obstetr Gynaecol. 2020; 40 (5): 602-608. doi:10.1080/01443615.2019.1634030
6, Raju, K. Virus and Cervical Cancer: Role and implication: Rev Biomed Res Ther. 2015; 2 (3): 220-230.
7. Jones, C. Cervical Cancer. Is Herpes Simplex Virus type 2 a Co-factor? Clin Microbiol Rev. 1995; 8: 549-556. doi: 10.1128/cmr.8.4.549
8. Guidry, J. T., and Scott, R. S. The interaction between human papillomavirus and other viruses. Virus Res. 2017; 231:139–147. doi: 10.1016/j.virusres.2016.11.002
9. Phillip, M., Donald, A., and Schreiber, H. Inflammation as a Tumor promoter in Cancer Induction. Sem Cancer Biol. 2004; 14 (6): 433-439. doi: 10.1016/j.semcancer.2004.06.006
10. Niller, H. H., and Minarovits, J. Viral hit and run oncogenesis; Genetic and epigenetic scenarios. Cancer Lett. 2011; 305 (2): 200-217. doi: 10.1016/j.canlet.2010.08.007
11. Niang, L., Winn, T., and Rushi, B. N. Practical Issues in Calculating the Sample Size for Prevalence studies. Arch Orofacial Sci. 2006; 1 (3): 9-14
12. Adejo, D. S., Aminu, M., Ella, E. E., Oguntayo, O. A., and Obishakin, O. F. Prevalence of high-risk human papillomavirus genotypes among apparently healthy women with normal and abnormal cervical cytology in Kaduna State, Nigeria. Afr J Clin Exper Microbiol. 2024; 25 (1): 17–27.https://dx.doi.org/10.4314/ajcem.v25i1.3
13. Ryncarz, A. J., Goddard, J., Wald, A., Huang, M. L., Roizman, B., and Corey, L. Development of a high-throughput quantitative assay for detecting herpes simplex virus DNA in clinical samples. J Clin Microbiol. 1999; 37(6): 1941-1947. doi:10.1128/JCM.37.6.1941-1947.
14. Auwal, I. K., Aminu, M., Atanda, A. T., Tukur, J., and Sarkinfada, F. Prevalence and Risk Factors of High-Risk Human Papillomavirus Infection among Women Attending Gynaecology Clinics in Kano, Northern Nigeria. Bayero J Pure Appl Sci. 2013; 6(1):67-71.doi:10.4314/bajopas.v6i1.14
15. Manga, M. M., Fowotade, A., Abdullahi, Y. M., et al. Epidemiological Pattern of Cervical Human

Papillomavirus infection among women presenting for cervical cancer screening in North-Eastern Nigeria. Infect Agents Cancer. 2015; 10: 39. doi: 10.1186/s13027-015-0035-8

16. Akarolo-Anthony, S. N., Famooto, A. O., Dareng, B. O., et al. Age-Specific Prevalence of Human Papillomavirus Infection among Nigerian Women BMC Publ Health. 14: 656-662 doi: 10.1186/1471-2458-14-656
17. Ezebialu, C., Ezebialu, I., Ezeifeka, G., Nwobu, R., Okani, C., and Chukwubuike, C. Prevalence of Cervical Human Pappillomavirus Infection in Awka, Nigeria. J Biosci Med. 2020; 8: 37-47. doi: 10.4236/jbm.2020.83005
18. Aminu M, and Bodam, O. B. Seroprevalence of Herpes simplex Virus-2 among Patients' presenting with fever at the University Health Service Ahmadu Bello University Main Campus Zaria, Nigeria. J Infect Dis Dev Ctries. 2014; 1 (1): 37-42. doi:10.3855/jidc.567
19. Ameh, E. R., Aminu, M., and Ella, E. E. Seroprevalence of HSV-2 among Women of Reproductive Age in Zaria, Kaduna State. Biol Med. 2016; 8(7):1-6.doi:10.4172/0974-8369.1000338
20. Sani, N. M., Rukayya, H. M., Ubandoma, M. S., Mujahid, N. S., Oni, M. A., and Isaka, I. Seroprevalence of Anti HSV-2 IgG among Women of Reproductive age attending General Hospital Dutse. J Microbiol Res. 2019; 4: 1-5 doi:https://doi.org/10.47430/ujmr.1941.001
21. Kalu, E. I., Ojide, C., Adeola, F., and Ugochukwu, N. Sexual Behaviour correlates with HSV-2 Seroprevalence among pregnant women in Nigeria. J Infect Dev Ctries. 2014; 8 (8): 1006-1012. doi: 10.3855/jidc.4336.
22. Okoye, J. O., Ngokere, A. A., Onyenekwe, C. C., Omotuyi, O., and Dada, D. I. Epstein-Barr virus, Human Papillomavirus and Herpes Simplex Virus 2 co-presence severely dysregulates miRNA expression. Afr J Lab Med. 2020; 10 (1): a975. doi: 10.4102/ajlm.v10i1.975
23. Oksana, D., Agyemang-Yeboah, F., Asmah, R. H., et al. Sero-prevalence of herpes simplex virus type 1 and type 2 among women attending routine Cervicare clinics in Ghana. BMC Infect Dis. 2018; 18: 378. doi: 10.1186/s12879-018-3288-1
24. Oguntayo, A. O., and Samaila, M. O. A. Prevalence of cervical intraepithelial neoplasia in Zaria. Ann Afr Med. 2010; 9 (3): 194-195. doi: 10.4103/1596-3519.68351
25. Bassey, E. A., Ekpo, M. D., Abasiattai, A. M., and Ekanem M. I. Cervical Cancer Screening in Uyo, South-South Nigeria. Trop J Obstetr Gynaecol. 2008; 25 (1): 1- 5.
26. Chukwali, L. I., Onuigbo, W. I. B., and Mgbor, N. C. Cervical Cancer Screening in Enugu, Nigeria. Trop J Obstetr Gynaecol. 2003; 20 (2): 109-112.
27. Magaji, S. J., Aminu, M., Inabo., H. I., et al. Prevalence of squamous intraepithelial lesion among women in Kaduna State, Nigeria. Ann Trop Pathol. 2017; 8: 94-98. doi:10.4103/atp.atp_5_17
28. Mosuro, O. A., Ajayi, I., Odukogbe, A. T. A., et al. Prevalence of Cervical Dysplasia and Associated Risk Factors among Women Presenting at a Primary Care Clinic in Nigeria. J Basic Clin Reprod Sci. 2015; 4 (2): 70-79
29. Ogah, J., Kolawole, O., Awelimobor, D. High risk human papilloma virus (HPV) common among a cohort of women with female genital mutilation. Afr Health Sci. 19 (4):2985-2992.
30. Agida, T. E., Akaba, G. O., Isah, A. Y., and Ekele, B. Knowledge and perception of human papilloma virus vaccine among the antenatal women in a Nigerian tertiary hospital. Nig Med J. 56:23–27.
31. Muhammad, Z., Usman, I. H., Datti, Z. A., et al. Incidence and risk factors of cervical dysplasia among human immune deficiency virus positive and human immune deficiency virus negative women at Aminu Kano Teaching Hospital. Sahel Med J. 20:160-167
32. Kim, M., Park, N. J., Jeong, J. Y., and Park, J. Y. Multiple Human Papilloma Virus (HPV) Infections Are Associated with HSIL and Persistent HPV Infection Status in Korean Patients. Viruses. 2021; 13(7): 1342. doi:10.3390/v13071342.
33. Kabir, A., Bukar, M., Nggada, H. A., Rann, H. B., Gidado, A., and Musa, A. B. Prevalence of human papillomavirus genotypes in cervical cancer in Maiduguri, Nigeria. Pan Afr Med J. 2019 Aug 5; 33:284. doi:10.11604/pamj.2019.33.284.18338.