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Comparative HPV genotype distribution among women with normal and abnormal cervical cytology in Yaoundé, Cameroon

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Abstract:

Background: The epidemiology of human papillomavirus (HPV) infection and the pattern of HPV genotype distribution are parameters needed to assess the risk of cervical cancer. Oncogenic HPV types are well-known pathogen for lower genital tract neoplasias, representing the primary cause of cancer death in Africa and the second in Cameroon. This study was conducted to identify the various genotypes particularly the high-risk HPV types in normal and abnormal cervical cytology from women in Yaoundé, Cameroon.
Methodology: This was a hospital-based, analytical cross-sectional study carried out on 226 symptomatic women wherein cervico-vaginal samples were obtained during gynaecological examination for Pap smears, HPV-DNA and genotype detection with linear array HPV strip, conducted from November 2019 to January 2021.
Results: From the 226 women whose cervical samples were collected for Pap smears, 71 (31.4%) had abnormal cytology results while 155 (68.6%) had normal results. The overall HPV prevalence in the study population was 34.1% (77/226). The HPV prevalence in women with abnormal Pap smears was 100% (71/71) and are distributed in following descending order; LSIL (21.1%, 15/71), HSIL (21.1%, 15/71), ASC-US (19.7%, 14/71), ICC (19.7%, 14/71) and others (18.4%, 13/71). HPV-DNA was positive in 6 (3.9%) of the 155 women with normal cytology results, 4 (2.6%) of whom were high-risk HPV. There is statistically significant difference in the HPV prevalence between women with abnormal and normal Pap smear results (OR=3289, 95% CI=182.62-59235, p<0.0001). The frequently identified oncogenic HPV types were type 16 (31.2%, 24/77), type 45 (14.3%, 11/77) and type 18 (10.4%, 8/77).
Conclusion: It is evident from our study that symptomatic women with normal Pap smear can have HR-HPV infection and should therefore be screened for HPV and followed up with periodic Pap smears to detect any abnormal change in cervical cytology results, to prevent cervical cancer development. Women should be encouraged to take up cervical screening, through Pap smears, because it is a non-invasive and cost-effective method for early detection of pre-invasive lesions.

Keywords: human papillomavirus; genotypes; low risk; high risk; unclassified risk; cervical cytology

Répartition comparative des génotypes du VPH chez les femmes ayant une cytologie cervicale normale et anormale à Yaoundé, Cameroun

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Abstrait:

Contexte: L'épidémiologie de l'infection par le virus du papillome humain (VPH) et le schéma de distribution des génotypes du VPH sont des paramètres nécessaires pour évaluer le risque de cancer du col de l'utérus. Les types de VPH oncogènes sont des agents pathogènes bien connus des néoplasies des voies génitales inférieures, représentant la première cause de décès par cancer en Afrique et la deuxième au Cameroun. Cette étude a été menée pour identifier les différents génotypes, en particulier les types de VPH à haut risque dans la cytologie cervicale normale et anormale chez les femmes de Yaoundé, au Cameroun.

Méthodologie: Il s'agissait d'une étude transversale analytique en milieu hospitalier menée sur 226 femmes symptomatiques dans laquelle des échantillons cervico-vaginaux ont été obtenus lors d'un examen gynécologique pour les frottis Pap, l'ADN du VPH et la détection du génotype avec une bandelette VPH à réseau linéaire, menée à partir de novembre 2019 à janvier 2021.

Résultats: Sur les 226 femmes dont les échantillons cervicaux ont été prélevés pour les frottis Pap, 71 (31,4%) avaient des résultats cytologiques anormaux tandis que 155 (68,6%) avaient des résultats normaux. La prévalence globale du VPH dans la population étudiée était de 34,1% (77/226). La prévalence du VPH chez les femmes ayant des frottis de Pap anormaux était de 100 % (71/71) et est répartie dans l'ordre décroissant suivant ; LSIL (21,1 %, 15/71), HSIL (21,1%, 15/71), ASC-US (19,7%, 14/71), ICC (19,7%, 14/71) et autres (18,4%, 13/71). L'ADN du VPH était positif chez 6 (3,9%) des 155 femmes ayant des résultats cytologiques normaux, dont 4 (2,6%) étaient des VPH à haut risque. Il existe une différence statistiquement significative dans la prévalence du VPH entre les femmes ayant des résultats de frottis anormaux et normaux (OR=3289, IC à 95%=182,62-59235, *p*<0,0001). Les types de VPH oncogènes fréquemment identifiés étaient le type 16 (31,2%, 24/77), le type 45 (14,3%, 11/77) et le type 18 (10,4%, 8/77).

Conclusion: Il ressort de notre étude que les femmes symptomatiques avec un frottis de Pap normal peuvent avoir une infection HR-HPV et doivent donc être dépistées pour le VPH et suivies de frottis de Pap périodiques pour détecter tout changement anormal dans les résultats de la cytologie cervicale, afin de prévenir le développement du cancer du col de l'utérus. Les femmes devraient être encouragées à entreprendre un dépistage cervical, par le biais de frottis vaginaux, car il s'agit d'une méthode non invasive et rentable pour la détection précoce des lésions pré-invasives.

Mots clés: virus du papillome humain; génotypes; faible risque; risque élevé; risque non classé; cytologie cervicale

Introduction:

Today, it is widely acknowledged that more than 20 human papillomavirus (HPV) genotypes are known to be sexually transmitted path ogen associated with malignancies of the reproductive organ of women, and is one of the main causal factors of cervical cancer (1). Cervical cancer is a gradual and continuous disease that advances from mild to more severe invasive disease caused by persistent infections with oncogenic strains of HPV (2). Among all cancers threatening women's health, cervical cancer remains one of the leading causes of morbidity and mortality worldwide (3), and the leading cause of cancer mortality in Africa (4).

In Cameroon, over 1474 women are diagnosed with cervical cancer each year and approximately 995 women die from the disease annually, rating it as the second most common cause of cancers in Cameroon predominantly affecting women due to poor access to standard health services (5). Cervical cancer is conside-

red a warning towards women's health and makes it a substantial public health problem.

In developed countries, HPV has been largely controlled by effective screening, prompt diagnosis and vaccination (6). Cervical HPV infections screening is done using Papanicolaou (Pap) smears that detect morphologic changes or by detecting viral DNA presence in cellular scrapes or biopsy tissues (7). The use of Pap smear screening and HPV prophylactic vaccines are effective in preventing cervical cancer (8) and has reduced the incidence of invasive cervical cancer by 70-90% in the developed world (9). In developing countries, merely 5% of women take up cervical screening due to lack of effective and organized cervical cytology screening programs (10).

Cervical cancer remains a major threat to women, especially in the developing countries. Fortunately, it is one of the most preventable cancers worldwide. With emphasis on primary and secondary preventive measures, the disease can be tackled and eradicated in the

ensuing decades with strong government policies since treatment is too expensive and tedious. This will need a collaborative effort of women, healthcare providers, families and the community at large. This study is aimed at determining the prevalence of HPV infections and characterizing the HPV types in cytological grade trends for better follow-up and strategic management.

Materials and method:

Study design and setting:

This was an analytical cross-sectional study carried out from November 2019 to January 2021 at the Yaoundé General Hospital and the Yaoundé Gynaeco-Obstetric and Paediatric Hospital, Cameroon.

Study population and participants:

The study participants consisted of 226 women who came for consultation, and were above 19 years of age, sexually active, with symptoms and signs of cervical pre-cancerous lesions, including women living with HIV (WLWH) who came for their routine gynaecology consultation. Women were excluded if they had had history of hysterectomy, abnormal bleeding, pregnant, had contraindications for Pap smear examination and unwilling to take part in the study.

Sample size:

A minimum sample size of 226 women was obtained using the statistical formula for proportion; $N = p(1-p) \times (Z\alpha^2/d^2)$, where N is the minimum number of participants, p is the prevalence of HPV of 39.0% (p=0.39) in Cameroonian women in 2016 (11), $Z\alpha$ is the 95% confidence interval ($Z\alpha$=1.96), and d is the error rate set at 5% (d=0.05).

Ethical consideration and informed consent:

The study was approved by the Research Ethics Committee of the University of Buea (Reference No. 2017/0491/UB/FS/HOD/MBP). Local ethical clearance was also obtained from the Institutional Ethics Committee for Research of Human Health (CIERSH) at the Yaoundé Gynaeco-Obstetric and Paediatric Hospital (Authorization No. 675/CIERSH/DM/ 2018) and Yaoundé General Hospital (Authorization No. 3616/017/HGY/DG). Informed consent was obtained after explaining to patients either in English, French or Pidgin (a local Lingua Franca) the purpose of the study.

Data collection:

After signing the consent form, a structure questionnaire was interviewer-administered to collect socio-demographic and other relevant data from each participant by trained laboratory technicians. Participants without proof of their HIV status were tested for HIV according to the Cameroon national testing guidelines. Those with negative results of more than four months prior to recruitment were re-tested for further confirmation.

Cervical and vaginal specimen collection:

Each participant underwent a gynaecological evaluation with a non-lubricated, clean and single-use speculum (Hybribio Biochemical Company Limited China) performed by a gynaecologist and two cervical specimens were collected for each participant. One specimen for oncotic cytological examination was collected using Ayre's spatula which was rolled onto the slide and immediately fixed in 95% ethanol, and allowed to air-dry for subsequent staining following Papanicolaou smear method.

The endo-cervical brush (Cytyc, Montrouge, France) was used to collect the second cervical sample and the head of the cytobrush with the specimen was obtained by separation from its handle and then put into commercial aqueous buffered specimen collection and transport media (Roche Diagnostic Systems, Meylan, France), which came in 1.0mL aliquots in capped tubes that accommodated collection devices. The specimens were stored at -20°C pending DNA extraction and amplification. High vaginal specimens were collected from the participants for bacterial analysis using a sterile cotton swab.

Cytopathological examination:

The ethanol fixed slides were evaluated in the Cytopathology Laboratory of Yaoundé General Hospital and Yaoundé Gynaeco-Obstetrics and Paediatric Hospital by certified cytopathologists. Detection was done only on smears with adequate number of cells. All smears were reviewed independently by two senior pathologists who were blinded from the clinical or other laboratory findings to avoid bias. In the event of discrepant smear result readings, both pathologists reviewed the slides together and consensus was reached on final diagnosis and grading.

The cytology results were classified according to the 2001 Bethesda classification (12). All cytology results that were identified as abnormal by visual inspection and cytology were referred for colposcopy, and in case of any visible lesions, biopsy was recommended with a definitive diagnosis given to each study participant based on the results of visual inspection, cytology, colposcopy, and biopsy.

Detection of bacteria in vaginal samples:

Detection of bacterial vaginosis (BV) and

HPV genotype distribution among women in Cameroon

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Trichomonas vaginalis was carried out at the hospital routine laboratory. High vaginal swabs were used separately for direct wet preparation and then visualized under the light microscope for the presence of clue cells, yeast cells and motile *T. vaginalis*. BV was diagnosed using the Amsel's clinical criteria, which included the presence of any three of the following; homogeneous white vaginal discharge, vaginal pH $\geq$ 4.6, and release of fishy amine odour (Whiff's test) when 10% (w/v) of KOH was added to a vaginal fluid sample, presence of clue cells on Gram-stained microscopic examination represented about 20% of vaginal epithelial cells (13).

The bacterial types were quantified by calculating the average number of organisms counted in 10 non-consecutive microscopy fields at ×1000 magnification. Each slide was assigned a score of 0 to 10 using the Nugent score (14) based on the proportion of bacterial flora and morphology. This classification results were evaluated in an overall score in which 0–3 indicates a 'normal flora' lactobacilli-dominated, 7–10 was classified as 'BV-dominated flora', and the score of 4–6 was referred to as an 'intermediate or reduced *Lactobacilli* flora'.

Evaluation of cervical inflammation:
The inflamed cervical cells were analyzed by counting the number of neutrophils observed in microscope fields on the Pap slides. Pap-stained smears were evaluated at ×400 magnification initially to identify cervical mucus. Valid slides were observed at ×1000 magnification to identify multi-lobed nuclei neutrophils, which were counted in five non-adjacent fields and the average was quantified as 'normal' (0–5 neutrophils/field), 'intermediate' (6–30 neutrophils/field), and 'inflamed' (>30 neutrophils/field).

Molecular detection of HPV-DNA:
The cervical specimens for HPV-DNA detection were processed at the Centre for the Study and Control of Communicable Diseases (CSCCD) of the Faculty of Medicine and Biomedical Sciences, Yaoundé 1, Cameroon.

DNA extraction:
The DNA of cervico-vaginal samples was extracted using Qiagen DNeasy Kit ((Hamburg, Germany) following the manufacturers instructions. The extracted DNA was quantified using Nanodrop 2000C spectrophotometer (Thermo Scientific, Loughborough, UK). The optical density of 1 µL of the DNA sample was measured at 260/280 nm and the DNA concentration was calculated by the Nanodrop 2000 software. The extracted DNA was aliquoted in 50 µL aliquots in

sterile Eppendorf tubes and stored at -20°C until ready for use.

Linear Array HPV-DNA amplification:
Polymerase chain reaction assay for amplification of HPV DNA was performed according to the recommendations of the manufacturer. The reaction mixture contained 10µL of sample, 1x PCR buffer, 3.5mM $MgCl_2$, dNTPs mix (200µM of each deoxynucleoside triphosphate), GP5+ primers (5'-TTTGTTACTGTGGTAGATACT AC-3') and 5'-biotinylated GP6+ primers (5'-GA AAAATAAACTGTAAATCA TATTC-3') were used at 1µM each, 1.25U of Taq polymerase (Promega®) and sterile distilled water to a total reaction volume of 50µL. The primers used were synthesized in South Africa by Integrated DNA Technologies (IDT) Inc., and has been described in a previous published study (15).

Amplification was carried out in a thermocycler (MyCycler^{TM} (Biorad) and PCR conditions were as follows; one step of denaturation at 94°C for 4 minutes, followed by 40 amplification cycles each with the following steps; denaturation at 94°C for 1 minute, annealing at 40°C for 2 minutes and elongation at 72°C for 2 minutes. A final elongation step was prolonged by 4 minutes. Amplified PCR products were identified on 2% agarose gel electrophoresis for subsequent genotyping.

Linear Array HPV-DNA genotype detection:
HPV-DNA genotype detection on the amplicons was performed using the Linear Array HPV genotyping test (Roche Molecular Systems, Pleasanton, CA, USA). The working hybridization buffer, working ambient wash buffer and working citrate buffers were prepared ahead of time. Briefly, the required number of Linear Array HPV Genotyping strips were removed from the HPV Strip pouch using clean forceps, and placed upward into the appropriate well of the 24-well tray, and 4mL of pre-warmed working hybridization buffer was added. Using a pipette with an aerosol barrier tip, 75µL of denatured amplicon was added into the appropriate wells containing labeled strips, and the 24-well tray with the lid was placed in a 56°C shaking water bath for 30 minutes. The working ambient wash buffer (4 mL) was added to each well containing a strip. The 24-well tray was gently rocked 3 to 4 times to rinse the strips, and the buffer was immediately vacuum aspirated.

Pre-warmed working stringent wash buffer (4mL) was added to each well containing a strip, and the cover was placed on the 24-well tray and then returned to the 56°C shaking water bath for 15 minutes. The tray was removed

from the shaking water bath, and the wash buffer removed from the wells by vacuum aspiration; 4mL of conjugate were added to each well containing a strip, and the tray incubated for an additional 30 minutes. The conjugate was then removed, and the strips were washed by gentle rocking in the presence of the wash buffer. Citrate buffer (4mL) was then added to each well, and the tray was incubated for 5 minutes. After aspiration of this buffer, 4mL of working substrate was added to each well, and the tray was incubated for 5 minutes before aspiration. Deionized water (4mL) was then added to each well, and the strips were removed to a clean surface to dry at room temperature. The HPV genotyping results were interpreted according to the recommendation of the manufacturer using detectable hybridization bands.

Classification of HPV types and cervical lesions:
        HPV genotypes were classified based on the report of Muñoz et al., (16). For the purpose of analysis and oncogenic potential classification analysis used in this present study, eleven HPV genotypes were classified as high risk (types 16, 18, 31, 33, 35, 45, 51, 52, 58, 68, and 82), five as low risk (types 11, 13, 54, 62 and 72) and one as unclassified risk type (type 97). The total HPV and type-specific HPV (total high-risk type, low risk type and unclassified risk type) were scored following the cytology results that were graded as 'normal' and 'abnormal'.
        The 'abnormal' cytology results were graded as; atypical squamous cells of undetermined significance (ASCUS); atypical glandular cells (AGC); low-grade squamous intraepithelial lesions (LSILs); atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H); high-grade squamous intra-epithelial lesions (HSILs); and intra-epithelial cervical carcinoma (ICC).

Statistical analysis of data:
        Data were entered into a Microsoft Excel spreadsheet and analyzed using SPSS Version 23.0. Categorical explanatory variables were cross-tabulated against dichotomous outcomes. Continuous variables were summarized using proportions (percentages), means (± standard deviation) and frequency distribution tables. The data were analyzed by Fisher's exact test, and the prevalence rate and descriptive statistics were calculated as required. The distribution and prevalence of HPV type-specific infection among cytological 'normal' and 'abnormal' samples were compared (Odds ratio with 95% confidence interval were computed), and the probability (*p*) type I error was set at 0.05.

Results:

Socio-demographic characteristics of the study participants:
        A total of 226 women, 62 women living with HIV (WLWH) and 164 HIV-negative women, were enrolled in the study over a period of 14 months and screened for cervical abnormality by Pap smears analysis. The age of the participants ranged from 20-79 years with a mean age of 44 ± 10.65 years. Women aged 40-49 years constituted the highest number of the participants (37.2%, 84/226), amongst whom 131 (57.9%) were married and 68 (30.1%) had not attained at least a secondary level education. Most (151, 66.8%) have been exposed to tobacco and 110 (48.7%) have been pregnant 1 to 5 times previously.
        The social characteristics showed that majority of the participants (70.4%, 159/226) had sexual exposure on or before 19 years, with exposure in 77.4% (48/62) of the WLWH and 67.7% (111/164) of HIV-negative women, but most (88.1%, 199/226) do not have multiple sexual partners, as 75.8% (47/62) of WLWH and 92.7% (152/164) of HIV-negative women responded not having multiple sexual partners. A high frequency of participants (75.5%, 171/226) had experienced signs and symptoms of HPV infection 1 to 3 times, and more than half (64.2%, 145/226) reported they had never used any form of contraceptives (Table 1).

Table 1: Socio-demographic characteristics of the women participants attending Gynaeco-Obstetrics and Paediatric Hospitals in Yaoundé, Cameroon

Sociodemographic characteristics	HIV negative (%) (n = 164)	WLWH (%) (n = 62)	Total (%) (n = 226)
Age group (years)			
20 – 29	7 (4.3)	4 (6.5)	11 (4.9)
30 – 39	40 (24.4)	16 (25.8)	56 (24.8)
40 – 49	64 (39.0)	20 (32.3)	84 (37.2)
50 – 59	32 (19.5)	15 (24.2)	47 (20.8)
60 – 69	19 (11.6)	5 (8.1)	24 (10.6)
70 – 79	2 (1.2)	2 (3.2)	4 (1.8)
Mean age ± SD (years)	44.93 ± 10.39	46.27 ± 11.34	44 ± 10.65
Marital status			
Married	91 (55.5)	40 (64.5)	131 (57.9)
Single	54 (32.9)	14 (22.6)	68 (30.1)
Divorced	1 (0.6)	1 (1.6)	2 (0.9)
Widow	18 (10.9)	7 (11.3)	25 (11.1)
Educational level			
Primary school or less	48 (29.3)	20 (32.3)	68 (30.1)
Secondary	84 (51.2)	31 (50.0)	115 (50.9)
University	32 (19.5)	11 (17.7)	43 (19.0)
Tobacco use			
No	53 (32.3)	22 (35.5)	75 (33.2)
Yes	111 (67.7)	40 (64.5)	151 (66.8)
Age group of first sexual exposure (years)			
10 – 19	111 (67.7)	48 (77.4)	159 (70.4)
20 – 29	52 (31.7)	14 (22.6)	66 (29.2)
≥ 30	1 (0.6)	0	1 (0.4)
Mean age ± SD (years)	18.28 ± 3.17	17.13 ± 2.84	17.71 ± 3.005
Had multiple sexual partners			
No	152 (92.7)	47 (75.8)	199 (88.1)
Yes	12 (7.3)	15 (24.2)	27 (11.9)
No of times pregnant			
0	12 (7.3)	9 (14.5)	21 (9.3)
1 – 5	84 (51.2)	26 (41.9)	110 (48.7)
6 – 10	57 (34.8)	25 (40.3)	82 (36.3)
> 10	11 (6.7)	2 (3.2)	13 (5.8)
No of times experienced HPV signs and symptoms			
0	8 (4.9)	2 (3.2)	10 (4.4)
1 – 3	127 (77.4)	44 (70.9)	171 (75.7)
> 3	29 (17.7)	16 (25.8)	45 (19.9)
History and types of contraceptives used			
None	110 (67.1)	35 (56.5)	145 (64.2)
IUCD	0	2 (3.2)	2 (0.9)
Natural	2 (1.2)	0	2 (0.9)
Condom	9 (5.5)	6 (9.7)	15 (6.6)
Injection	10 (6.1)	3 (4.8)	13 (5.8)
Norplant	12 (7.3)	3 (4.8)	15 (6.6)
Tablets	21 (12.8)	13 (20.9)	34 (15.0)

WLWH: Women living with HIV; HIV: Human immuno deficiency virus; HPV: Human papilloma virus; IUCD: Intra uterine contraceptive device; SD: Standard deviation

Vaginal microbial flora of the study participants:
A total 121 (53.5%) of the 226 women had vaginal infection on microscopic examination with the most frequent infection being bacterial vaginosis caused by *Gardnerella vaginalis* 42 (34.7%) and the least frequent being genital wart 4 (3.3%) (Table 2).

Cervical inflammatory lesions in the study participants:
A total 212 (93.8%) of the 226 participants had evidence of cervical inflammation and/or lesion while 14 (6.2%) had no evidence

(Table 3). Fifty-eight (93.5%) of 62 WLWH had cervical inflammation/lesion compared to 154 (93.9%) of 164 HIV-negative women, with no statistically significant difference (OR=1.062, 95% CI=0.3204-3.521, *p*=1.000). Of the 62 WLWH, 21 (33.9%) tested positive for HPV-DNA while 56 (34.1%) of the 164 HIV negative women tested positive for HPV. There was no statistically significant difference in the prevalence of HPV in WLWH and HIV-negative women (OR=0.9878, 95% CI=0.5329-1.831, *p*=1.00). One (25.0%) of 4 WLWH with no cervical inflam-

mation was positive for HPV-DNA while 1 (10%) of 10 HIV-negative women with no cervical inflammation was also positive for HPV-DNA showing no statistically significant difference (OR=3.000, 95% CI=0.1399-64.310, p=0.5055).

Distribution of HPV genotypes with respect to cervical lesion grades:

Of the 226 cervical samples analyzed, 77 (34.1%) tested positive for any HPV type while 149 (65.9%) tested HPV negative. One hundred and fifty-five (68.6%) samples had 'normal' cytology result, from which 6 (3.9%) tested positive for HPV-DNA, while 71 samples had 'abnormal' cytology result with all (100%) testing positive for HPV-DNA, with a statistically significant difference (OR=3289, 95% CI=182.6 -59235, p<0.0001). From the 71 samples with 'abnormal' Pap smear results, HR-HPV was detected in 71.8% (51/71), while from 155 samples with 'normal' Pap smear results, HR-HPV was detected in 2.6% (4/155), showing a statistically significant difference (OR=96.263, 95% CI=31.417-294.95, p<0.0001) (Table 4).

The most prevalent HPV types in the study were high risk type 16 (31.2%, 24/77), followed by type 45 (14.3%, 11/77) and type 18 (10.4%, 8/77), although type 16 predominated in all the other cervical cytological abnormalities. In women with observed cytological abnormality, low risk types were frequently identified in women with ASCUS (57.1%, 8/14) and LSIL (53.3%, 8/15), while high risk types were identified in the following ascending order; AGC (66.6%, 4/6), ASC-H (85.7%, 6/7), ICC (92.6%, 13/14) and HSIL (93.3%, 14/15) respectively.

The prevalence of HPV infection in participants with 'abnormal' cervical results is almost the same for ASCUS (19.7%, 14/71), LSIL (21.1%, 15/71), HSIL (21.1%, 15/71) and ICC (19.7%, 14/71). The oncogenic HPV type 16 is the most prevalent types in both normal (33.3%, 2/6) and abnormal Pap smear results (30.9%, 22/71). In general, HPV type 16 predominated in women with ASCUS (21.4%, 3/14), AGC (33.3%, 2/6), LSIL (26.7%, 4/15), ASC-H (42.9%, 3/7) and HSIL (46.7%, 7/15) but for ICC, type 45 was predominant (21.4%, 3/14) (Table 3).

Table 2: Vaginal microbial flora women attending Gynaeco-Obstetrics and Paediatric Hospitals in Yaoundé, Cameroon

Cytology results	Frequency (n)	Percentage (%) at 95% CI
Trichomonas vaginalis	35	28.9
Candida albicans	40	33.1
Genital warts	4	3.3
Bacterial vaginosis (*Gardnerella vaginalis*)	42	34.7
Total	**121**	**100**

Table 3: Cervical inflammatory lesions of women attending Gynaeco-Obstetrics and Paediatric Hospitals in Yaoundé, Cameroon

Cervical examination	HIV negative (n=164)*			HIV positive/WLWH (n=62)*		
	No HPV -ve (%)	No HPV +ve (%)	Total (%)	No HPV -ve (%)	No HPV +ve (%)	Total (%)
Normal	9 (5.5)	1 (0.6)	10 (6.1)	3 (4.8)	1 (1.6)	4 (6.5)
Inflamed	45 (27.4)	18 (11.0)	63 (38.4)	14 (22.6)	5 (8.1)	19 (30.6)
Lesions	34 (20.7)	20 (12.2)	54 (32.9)	21 (33.9)	3 (4.8)	24 (38.7)
Inflamed + Lesion	20 (12.2)	17 (10.4)	37 (22.6)	3 (4.8)	12 (19.4)	15 (24.2)
Total	**108 (65.9)**	**56 (34.1)**	**164 (100)**	**41 (66.1)**	**21 (33.9)**	**62 (100)**

*: No significant difference in the prevalence of HPV in HIV positive and HIV negative (WLWH) participants (OR=0.9878, 95% CI=0.5329-1.831, p=1.000);
WLWH: Women living with HIV; HPV: Human papilloma virus; HIV: Human immunodeficiency virus

Table 4: Prevalence and distribution of HPV genotypes with respect to cervical lesion grade in women attending Gynaeco-Obstetric and Paediatric Hospitals, Yaoundé, Cameroon

HPV type	Total participants (n=226)	Normal cytology (n=155)	Abnormal cytology types (n=71)						
			Total (n=71)	ASC-US (n=14)	AGC (n=6)	LSIL (n=15)	ASC-H (n=7)	HSIL (n=15)	ICC (n=14)
Any HPV	77 (34.1)	6 (3.9)	71 (100)	14 (100)	6 (100)	15 (100)	7 (100)	15 (100)	14 (100)
HR-HPV	55 (24.3)	4 (2.6)	51 (71.8)	6 (42.8)	4 (66.6)	7 (46.7)	6 (85.7)	14 (93.3)	13 (92.6)
LR-HPV	18 (8.0)	2 (1.3)	16 (22.5)	8 (57.1)	1 (16.7)	8 (53.3)	0	0 (0)	0
UR-HPV	4 (1.8)	0	4 (5.6)	0	1 (16.7)	0	1 (12.3)	1 (6.7)	1 (7.1)
High-risk HPV types (n=55)									
16	24 (31.2)	2 (33.3)	22 (31.0)	3 (21.4)	2 (33.3)	4 (26.7)	3 (42.9)	7 (46.7)	2 (14.3)
18	8 (10.2)	0	8 (11.3)	1 (7.1)	1 (16.7)	1 (6.7)	1 (14.3)	3 (20.0)	1 (7.1)
31	1 (1.3)	0	1 (1.4)	0	0	0	0	1 (6.7)	0
35	1 (1.3)	0	1 (1.4)	0	0	0	0	0	1 (7.1)
45	11 (14.3)	1 (16.7)	10 (14.1)	1 (7.1)	1 (16.7)	1 (6.7)	2 (28.6)	3 (20.0)	3 (21.4)
51	1 (1.3)	0	1 (1.4)	0	0	0	0	0	1 (7.1)
52,33,35,58	6 (7.8)	0	6 (8.5)	1 (7.1)	0	1 (6.7)	0	0	3 (21.4)
58	1 (1.3)	1 (16.7)	0	0	0	0	0	0	0
68	1 (1.3)	0	1 (1.4)	0	0	0	0	0	1 (7.1)
82	1 (1.3)	0	1 (1.4)	0	0	0	0	0	1 (7.1)
Total	55 (71.4)	4 (66.6)	51 (71.8)	6 (42.8)	4 (66.6)	7 (46.7)	6 (85.7)	14 (93.3)	13 (92.6)
Low-risk HPV types (n=18)									
11	4 (5.2)	1 (16.7)	3 (4.2)	2 (14.3)	1 (16.7)	1 (6.7)	0	0	0
13	4 (5.2)	0	4 (5.6)	2 (14.3)	0	2 (13.3)	0	0	0
54	6 (7.8)	1 (16.7)	5 (7.0)	2 (14.3)	0	3 (20.0)	0	0	0
62	2 (2.6)	0	2 (2.8)	2 (14.3)	0	0	0	0	0
72	2 (2.6)	0	2 (2.8)	0	0	2 (13.3)	0	0	0
Total	18 (23.4)	2 (33.3)	16 (22.5)	8 (57.1)	1 (16.7)	8 (53.3)	0	0	0
Unclassified risk HPV types (n=4)									
97	4 (5.2)	0	4 (5.6)	0	1 (16.7)	0	1(14.3	1 (6.7)	1 (7.1)

ASC-US: Atypical squamous cells of undetermined significance; AGC: Atypical glandular cells; LSILs: Low-grade squamous intraepithelial lesions; ASC-H: Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; HSILs: High-grade squamous intraepithelial lesions; ICC: Intra-epithelial cervical carcinoma; HPV: Human papilloma virus

Discussion:

Over the last three decades, the role of HPV infections in the development of cervical cancer has acquired fundamental health significance and HPV testing in addition to Pap smears has become a relevant diagnostic and prognostic tool that has reduced the widespread and incidence of cervical cancer. In view of the proven HPV vaccination efficacy, cervical cytology screening for the detection of pre-invasive cervical lesions, is considered an important public health strategy to assess the impact of HPV, the burden and distribution of the major oncogenic HPV genotypes in different geographical regions of Cameroon.

The result of this study on microbial flora was contrary to the clinical study conducted by Desruisseau (17) on the epidemiology of HPV in fertile women in Cameroon where a significant number of *T. vaginalis*, *Candida albicans* and BV were not detected. Mogtomo et al., (18) in their study on the incidence of cervical diseases associated with HPV in HIV-infected women reported that the main microbial infectious condition was BV as similarly observed in our present study. The high rate of vaginal microbial infections may be attributed to the sexual behavior of the sampled women, poor hygiene, stress, immunosuppression and unhealthy living conditions of the women.

In our study, cases of cervical inflammation and lesions were detected in women who tested negative for HPV infection. Although this is uncommon, other factors, such as chemical irritants and microbial infections caused by *T. vaginalis*, *C. albicans* or BV may be responsible for cervical lesions (19). Inflammatory process may result in the production of non-specific protective antimicrobial oxidants which may be responsible for the damage to host DNA, leading to neoplasias (20). The association of inflammation with many cancers suggests that inflammation may be considered as a risk factor for carcinogenesis.

From this study conducted in Yaoundé, 226 women were screened for cervical abnormality based on Pap smear analysis out of which 71 (31.4%) had abnormal cervical cytology while 155 (68.6%) had normal cervical cytology results. From the women with normal cytology results, 6 (3.9%) tested positive for HPV, out of which 4 (66.6%) were high-risk types. These results are in accordance with those obtained by Doh et al., (21) in a systemic review of oncogenic HPV prevalence among women with normal cytology results in Cameroon where the researchers observed that the most prevalent HPV types in normal cervical cytology smear were high-risk types. This could be due to the fact that the samples were collected from women referred from other health institutions with complaints of signs and symptoms from suspected HPV infection and cervical lesions. Another plausible reason could be that these tests (PCR based reverse-line strip test and reverse-blot

hybridization to detect HPD-DNA) may vary in their sensitivity and specificity.

Among the 71 participants with abnormal cervical grade results, most reported almost the same trend as follows; LSIL 21.1% (15/71), HSIL 21.1% (15/71), ASCUS 19.7% (14/71), and ICC 19.7% (14/71) but for ASC-H and AGC with almost the score rate of 9.9% (7/71) and 8.5% (6/71) respectively. These results are in accordance with those of Tagne et al., (22) study on the prevalence of precancerous cervical lesions and high-risk HPV types in the same town where this present study was conducted, where the authors reported almost the same trend of prevalence for abnormal cervical cytology results with LSIL (49.6%), HSIL (15.3%), ASC-US (3.7%) and AGC (0.7%) in that order. Desruisseau et al., (17) in a clinical pilot study conducted on the epidemiology of HPV in fertile women in Cameroon reported a contrary result and could not offer any plausible explanation on why participants were not diagnosed with HSIL cervical grade results. In this present study, LSIL precancerous lesions were also common (21.1%, 15/71) possibly due to the fact that it is a mandatory passage for all higher-grade precancerous lesions and cancers. In addition, many high-grade lesions (HSIL, AGC) would regress to LSIL before returning to normal, provided the subject is not exposed to risk factors that would significantly increase the risk of a malignant progression (23).

In our study, all the 71 (100%) participants with 'abnormal' cervical cytology were HPV positive and the most prevalent HPV type among them was the oncogenic with HPV type 16, detected in 22 of 71 (31.0%) samples, followed by HPV type 45 in 10 of 71 (14.1%) samples and HPV type 18 in 8 of 71 (11.3%) women. A high frequency (93.3%, 14/15) of the high-risk or oncogenic HPV types was detected in cervical samples of women with HSIL. Our findings are in concordance with Sarma et al., (24) where HSIL women were mostly infected with high-risk HPV types 16 and 18. A similar trend of results was noted in a study on HPV genotypes in high grade cervical lesions conducted in Cameroon by Sando et al., (25) who reported that majority (89.7%) were the high-risk types in the following descending order; HPV-16 (30.7%), HPV-18 (28.2%) and HPV45 (15.3%).

The distribution of HPV types in our study is also quite similar to a retrospective study conducted by Pirek et al., (26) with the three most common high-risk types noted in descending order as HPV 16 (88%), HPV 45 (32%) and HPV 18 (14.8%). Our study also confirmed the statistically significant association of abnormal cervical cytology and HPV infec-tions. Compared to women with 'normal' cervical cytology, women with 'abnormal' cervical cytology were 3289 times more likely to have any HPV infections (OR=3289, 95% CI=182.62-59235, *p*<0.0001) and 96 times more likely to have HR-HPV infections (OR=96.263, 95% CI= 31.417-294.95, *p*< 0.0001). It is well-established that HPV 16 and 18 are the most common HPV types worldwide, accounting for more than 70% of cervical cancer cases, as well as other cancers associated with HPV infections (27,28). The predominance of HPV 16 infections over other HPVs may result partly from the special ability of this HPV type to escape immune surveillance and become more virulent (29). Almost all cervical cancers contain traces of HPV, which infect basal cells within the cervical epithelium or at the squamo-columnar junction causing squamous cell abnormalities and bringing about ASC-US, LGSILs and HGSILs (30,31).

Conclusion:

Our results agree with the global data that HPV types 16 and 18 are the most common HR-HPV types. In Cameroon, these HR-types including type 45 are the most prevalent in women with normal and abnormal cervical cytology smear, confirming these HPV types as important risk factor for cervical cancer progression. Other high-risk genotypes were also identified in our study, which offers the baseline data for future research with significant implications on the need for raising awareness of women, government authorities and stakeholders about the role of HPV in cervical cancer management and for timely and effective implementation of HPV vaccination campaigns in Cameroon.

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Contributions of authors:

CMM conceptualized the study, performed the analysis, interpreted the data and wrote the manuscript; DMT drafted, analyzed data and reviewed the manuscript; GMI performed laboratory and data analyses and reviewed the manuscript; DNA contributed to draft manu-

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