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Prevalence of Precancerous Lesions Based on Digital Cervicography with VIA/VILI among Women Positive for High-Risk Human Papillomavirus Serotypes: A Screening Center-Based Study in Cameroon

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Abstract

Background: Since 2021, high-risk Human Papilloma Virus (HR-HPV) testing has been the recommended screening test for cervical cancer for all settings; either used alone in a "test and treat" strategy, or with a triage test, with or without biopsy, before treatment. Cameroon has rolled out immunization against HPV 16 and 18, but studies show a higher prevalence of non-16/18 HR-HPV types. **Objectives:** Determine the prevalence of precancerous lesions, in women with HR-HPV infection and evaluate association of digital cervicography (DC) VIA/VILI positivity with HPV serotype, as a measure of their contribution to precancer and cancer incidence. **Methodology:** The study was cross-sectional, descriptive, and analytic. It took place at the Etoug-Ebe and Ekoudoum Baptist Hospitals in Yaoundé, during the period April-September 2022. We reviewed the records of women screened for cervical cancer between February 2020 and December 2021 and evaluated the prevalence of lesions on digital cervicography (DC) with VIA/VILI for wom-

en positive for HR-HPV serotypes. The data were analyzed using SPSS version 20.0 for Windows. P values < 0.05 were considered significant at 95% confidence interval. **Results:** We identified 315 cases with a positive HR-HPV deoxyribonucleic acid (DNA) test, 224 (71.1%) had a DC VIA/VILI triage test done. Of these, 30 (13.4%) women had a positive DC VIA/VILI, with five women (2.2%) having lesions suggestive of cancer. Out of 11 cases positive for HPV 16 alone, 05 (45.5%) had a positive DC VIA/VILI test. Of the 14 cases positive for HPV 18 alone, 03 (21.4%) had a positive VIA/VILI, meanwhile only 19 (10.7%) of the 177 cases positive for non-16/18 HPV had a positive VIA/VILI test. **Conclusion:** A high proportion of women (13.4%) with HR HPV had a positive DC VIA/VILI, with a significant proportion (2.2%) having lesions suggestive of invasive cervical cancer HR-HPV serotype was associated with DC VIA/VILI positivity; HPV 16 had the strongest association (45.5%), followed by HPV 18 (21.4%), and non-16/18 HR-HPV (10.7%), suggesting a decreasing order of oncogenicity.

Keywords

High-Risk, Human Papillomavirus, Precancerous, Digital Cervicography, VIA/VILI, Serotype

1. Background

Cervical cancer is a leading cause of mortality among women. The Global Cancer Observatory (GLOBOCAN) estimates that about 340,000 women died from this disease in 2020 [1]. Most of these deaths occur in sub-Saharan Africa, South America, and South-Eastern Asia [1]. Cervical cancer is a preventable malignancy because it has a long precancerous phase that can be detected and treated if women are screened [2]. There is also an effective vaccine against the human papillomavirus (HPV), recognized as the cause of the cancer. In high-income countries, screening programs based on cervical cytology (Pap smear) have resulted in 60% to 90% reduction in the mortality associated with the disease [3]. In low- and middle-income countries (LMICs), however, cytology-based programs have not been that successful due to the paucity of infrastructure, trained manpower and funds [3]. The consequences are late diagnosis and high mortality from invasive cervical cancer. In Cameroon, for example, 80% of cervical cancer cases are diagnosed at a late stage and a major part of these patients dies within 12 months [4].

In 2021, the World Health Organization (WHO) approved the use of HPV DNA tests that detect the presence of high-risk HPV (HR-HPV) serotypes, as the preferred screening method in all settings and recommended a rapid transition of all VIA (Visual Inspection with Acetic acid) based programs to HPV DNA testing programs [5]. According to WHO guidelines, HPV testing for cervical cancer screening can be used alone in a "test and treat" strategy or it can be used for primary testing, such that positive cases undergo a triage test such as

VIA, with or without VILI (Visual Inspection with Lugol's Iodine); cytology or colposcopy; with or without biopsy before treatment is considered [3]. Given the additional costs needed for the triage strategy and the risk of patient dropout, "test and treat" is considered a good opportunity for low- and middle-income countries.

In its global strategy to reduce the incidence of cervical cancer to 4 cases per 100,000 women, WHO aims at having 70% of women screened by a high-performance test by the age of 35 years and again by age 45 years. This is especially important for sub-Saharan African countries, such as Cameroon, with a high disease burden [6].

Unfortunately, Cameroon is far from reaching the WHO set target. According to the demographic and health survey of 2018, only 46% of women aged 15 to 49 had ever heard about cervical cancer and only 28% knew a screening test. Furthermore, only 4% of the women interviewed had ever done a cervical cancer screening test [7]. This low uptake is multifactorial including the absence of a national cervical cancer-screening program, limited availability outside the urban settings, and the need to pay user fees for screening, outside campaigns [8]. The most widely available screening method is visual inspection of the cervix with acetic acid. However, its low specificity and sensitivity make the switch to HR-HPV testing proposed by WHO imperative.

The HR-HPV tests have been introduced into Cameroon by some faith-based organizations, by a few government hospitals, where non-governmental organizations sponsor specific projects, and by major laboratories. The screening test for HR-HPV strains is superior to visual screening given that it is automated and less liable to human error. New devices like the AMPfire HPV by Atila Biosystems, used at the women's health programs of the Cameroon Baptist Convention Health Services (CBCHS), can test for 15 HR-HPV types while genotyping HPV 16 and 18 simultaneously. It can detect HR-HPV directly from a dry swab and is suitable for mass screening. The rationale for VIA/VILI after a positive HR-HPV test is to screen for the presence of a precancerous lesion and to orient eventual biopsy and treatment.

Knowing the prevalence of precancerous lesions, as detected by visual methods in HR-HPV positive women, may help to choose between a "test and treat" strategy, using HR-HPV testing alone, and a VIA/VILI-guided approach, for low resource setting. This may be tailored down to serotypes to discuss the rationale for routine treatment when specific serotypes are detected. Furthermore, in a context like ours with a recent report of a higher prevalence of non-16/18 HR-HPV types, not covered by the available bivalent and quadrivalent HPV vaccines [9], it is imperative to evaluate the aggressiveness of various HR-HPV types and their true contribution to the cervical cancer burden, beyond their prevalence in the community. The objective of this study, therefore, was to determine the prevalence of precancerous lesions, as detected through digital cervicography (DC)-aided VIA/VILI, in women positive for HR-HPV, and with respect to HR-HPV serotype.

2. Methodology

2.1. Study Design

The study was a cross-sectional descriptive and analytic study with retrospective data collection. It took place at the Women's Health Program departments of the Etoug-Ebe and Ekoudoum Baptist Hospitals during the period from April to September 2022. We reviewed the screening records of the Women's Health Programs of the two hospitals. All entries of women received between February 2020 and December 2021 for screening by HR-HPV DNA testing were selected. For those who were HR-HPV positive, their socio-demographic, and medical details, as well as their DC VIA/VILI results were consulted and recorded. We excluded records of women with HPV inadequate results and did exhaustive sampling.

2.2. Study Setting

The Women's Health Program (WHP) is a large cervical cancer prevention program in Cameroon run by a faith-based health organization called the Cameroon Baptist Convention Health Services (CBCHS). The CBCHS has a network of 94 health facilities located in seven of the 10 regions of Cameroon. Currently, the WHP has clinics in 12 of the CBCHS facilities, three sites in the North-west Region, two sites in the Southwest Region, two sites in the Littoral Region, one site in the West-Region, two sites in the Center Region, one site in the Adamawa, and one site in the South Region.

WHP is currently the largest cervical cancer prevention program in Cameroon and has screened an average of 8000 women for cervical cancer per year, since its inception in 2007. In WHP, cervical cancer screening is performed with HR HPV testing and triage with VIA/VILI, aided by a technique known as digital cervicography (DC). The latter uses a digital camera with a macro-conversion lens or a Samsung Galaxy S4 or S6 cell phone to take highly magnified real-time images of the cervix, which can be projected onto a television screen. Using this adjunct, the woman and the provider can see her cervix live on the screen at the same time; mimicking a video colposcopy.

For VIA, the program nurses apply 5 - 10 cc of 3% - 5% acetic acid with a 10 ml syringe on the cervix after insertion of a bi-valve vaginal speculum. A cotton swap is used with the aid of a ring-holding forceps to dab the cervix with the acetic acid for a few seconds. After two minutes, the acetic acid-soaked cotton swap is removed, and the cervix is examined for the appearance of whitish discoloration known as acetowhite epithelium, which may signify a precancer. After VIA, the nurses proceed immediately to perform the VILI, which entails the application of 1 - 2 cc of 5% Lugol's iodine into the cervix with a syringe and needle. The cervix is then inspected immediately for abnormal color changes.

In 2020, WHP introduced systematic screening of HPV for women aged 30 years and older. The test used for HPV screening is the Ampfire HPV test. This DNA PCR test requires dry cervical swabs that can be collected by the woman or a health care provider. It detects 15 types of HR HPV and reports 13 (31, 33, 35,

39, 45, 51, 52, 53, 56, 58, 59, 66 and 68) as a combined result while HPV 16 and HPV 18 are reported individually. The Etoug Ebe and Ekoudoum Baptist Hospitals are in Yaoundé, the capital of Cameroon.

2.3. Data Collection and Statistical Analysis

The data was collected using a data collection tool designed for the study. The data included socio-demographic characteristics, HPV test result, DC VIA/VILI result. The data were entered into an Epi-info Database and analyzed using SPSS version 20.0 for Windows. We used descriptive statistics to present the data and did bivariate analyses of variables, for association with DC VIA-VILI lesions, in women with HR-HPV. We used the Chi-square test to determine statistical significance. P values < 0.05 were considered significant at 95% confidence interval.

2.4. Ethical Consideration

We obtained ethical clearance from the Cameroon Baptist Convention Health Service Institutional Review Board and the Institutional Review Board of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé 1. We also obtained administrative authorization from the Directors of the Etoug-Ebe and Ekoumdoum Baptist Hospitals. No personal identifying information was included in the data collection tool and the computer used for data analysis was code protected.

3. Results

3.1. Target Population

We identified 315 cases with a positive HR-HPV deoxyribonucleic acid (DNA) test. The non-16/18 HR-HPV were present in 250 cases (79.5%), while HPV 16 and 18 were present in 13 (4.12%) and 18 (5.71%) cases, respectively. Coinfection with 16 and 18 was found in two women (0.06%).

3.2. Socio-Demographic Profile of Participants

The age range was 30 to 75 years, with a mean age of 41.2 ± 9.96 years (**Table 1**). The most represented groups in terms of occupation were traders (25.7%), teachers (18.7%), and housewives (15.2%) (**Table 2**)

3.3. Obstetric and Medical Parameters

Most women (183) (58.1%) had more than two children and 91 (28.9%) were HIV positive (**Table 3**).

3.4. HR HPV Types and Prevalence of DC VIA/VILI Lesions

Out of the 315 women who tested positive for HR-HPV, 224 (71.1%) had a DC VIA/VILI triage test done. Of these, 30 (13.4%) women had a positive DC VIA/VILI, with five women (2.2%) having lesions suggestive of invasive cervical cancer (**Table 4**).

Table 1. Socio-demographic data: Age, education, marital status, and age at first coitus.

Variables	Categories	Number	Proportion (%)
Age groups	30 - 34	93	29.5
	35 - 39	70	22.2
	40 - 44	54	17.1
	45 - 49	36	11.5
	50 - 54	25	7.9
	55+	37	11.8
Years of education	≤7	75	23.8
	8 - 12	78	24.7
	13 - 15	51	16.2
	>15	74	23.5
	No data:	37	11.8
Marital status	Married	209	66.3
	Single	83	26.4
	Divorced/Separated	9	2.8
	Widowed	14	4.5
Age at first coitus	≤17	91	28.8
	18 - 20	91	28.8
	21+	29	9.4
	No data	104	33

Table 2. Distribution of participants as per occupation.

Occupation	Number	Proportion (%)
Hairdresser	17	5.4
Sex worker	1	0.3
Housewife	48	15.2
Farmer	17	5.2
Health care worker	13	4.1
Secretary	7	2.2
Trader	81	25.7
Teacher	59	18.7
Student	4	1.3
Seamstress	19	6.0
Domestic worker	6	1.9
Others	38	12.1
No data	5	1.6

Others = administrative civil servants, Pastors, lawyers.

Table 3. Obstetric and medical characteristics of participants.

Variables	Categories	Number	Proportion (%)
Parity	≤2	132	41.90
	3 - 4	94	29.84
	5 - 6	70	22.22
	7+	19	6.04
HIV status	Positive	91	28.89
	Negative	211	66.98
	No data	13	4.13
HIV positive on treatment	Yes	68	74.72
	No	9	9.89
	No data	14	15.38

Table 4. DC VIA/VILI results in HPV positive women.

DC VIA/VILI result	Frequency	Percent (%)
Negative	194	86.6
Positive-ablation-eligible	17	7.6
Positive-LEEP-eligible	8	3.6
Positive-suspicious for cancer	5	2.2
Total	224	100.0

3.5. Factors Associated with Positive DC VIA/VILI in HR-HPV Infection

Considering HR-HPV type, there were 11 cases positive for HPV 16 alone and five of these (45.5%) had a positive DC VIA/VILI test. Of the 14 cases positive for HPV 18 alone, 03 (21.4%) had a positive VIA/VILI, while only 19 (10.7%) of the 177 cases positive for non-16/18 HR HPV had a positive VIA/VILI test (Table 5). Among the five women with cervical lesions suspicious for invasive cancer, three were positive for HPV 16 and two for non-16/18 HR HPV.

Considering sociodemographic and medical characteristics, age at sexarche < 18, parity ≤ 2, positive HIV status, having < 8 years of formal education and not being married did not reveal any statistically significant association with DC VIA/VILI positivity in HR-HPV positive women (Table 6).

4. Discussion

This study assessed the prevalence and characteristics of lesions by DC VIA/VILI in women who tested positive for HR-HPV at two screening centers for cervical cancer, in Yaoundé.

Socio-demographic characteristics of HR-HPV-positive women

Forty-three percent of HPV-positive women had their first sexual intercourse before 18 years. This is higher than the 33% reported by Nejo *et al.* in 2018 in

Table 5. Distribution of DC VIA/VILI lesions with respect to HR-HPV type.

HR-HPV type	DC VIA/VILI positive	DC VIA/VILI Negative	Total	Proportion positive on DC VIA/VILI (%)
HPV 16	5	6	11	45.5
HPV 18	3	11	14	21.4
Non 16/18 HR-HPV	19	158	177	10.7
Non 16/18 HR-HPV + HPV 16	2	7	9	22.2
Non 16/18 HR-HPV + HPV 18	1	7	8	12.5
HPV 16 + HPV 18	0	2	2	0.0
HPV 16 + 18 + Non 16/18 HPV	0	3	3	0.0
Total	**30**	**194**	**224**	**13.4**

HR = High Risk, HPV = Human Papillomavirus, VIA = Visual Inspection with Acetic Acid VILI = Visual Inspection with Lugol's Iodine.

Table 6. Sociodemographic and medical parameters in relation to DC VIA/VILI positivity in HR-HPV infection.

Predictor Variable	Categories	VIA/VILI positive	VIA/VILI Negative	RR (95%CI)	p-Value
Age at first coitus (n = 212)	<18 years	12 (13.48%)	77 (86.52 %)	1.11 (0.54 - 2.24)	0.7812
	18 years +	15 (12.19%)	108 (87.81%)		
Parity (n = 224)	≤2	14 (12.07%)	102 (87.93%)	0.81 (0.41 - 1.58)	0.5465
	3+	16 (14.81%)	92 (85.19%)		
HIV Status (n = 224)	Positive	10 (14.71%)	58 (85.29%)	1.15 (0.56 - 2.31)	0.7032
	Negative	20 (12.82%)	136 (87.18%)		
Marital status (n = 224)	Married	20 (13.19%)	132 (86.84%)	0.95 (0.46 - 1.91)	0.8806
	Single	10 (13.89%)	62 (86.11%)		
Level of education (n = 224)	<8 years	8 (12.12%)	58 (87.88%)	0.87 (0.40 - 1.85)	0.7179
	≥8 years	22 (13.92%)	136 (86.61%)		

VIA: Visual Inspection with Acetic acid, VILI: Visual Inspection with Lugol's Iodine, RR: relative risk.

Southwest Nigeria [10]. However, Simo *et al.* in Yaoundé in 2021 found that 40% of HPV-positive women in their study had their first sexual intercourse before 16 years of age [11]. We found that some 66% of these women were married. This is close to the 58.7% reported in Tanzania, by Swai *et al.* 2022 [12] but lower than the 73% observed by Simo *et al.* in Yaoundé in 2021 [11]. Some 60% of HR-HPV-positive women had three or more term births. This suggests a lower fecundity in the urban setting of Yaoundé when compared to the finding by Tebeu *et al.* in Baham, western region of Cameroon, where 83% of HR-HPV-positive women had four or more term deliveries [13].

The mean age at first sexual intercourse among women infected with HR-HPV

was 18.09 (SD ± 2.9) years. This is similar to the 17.9 years reported by Akaaboune *et al.* in 2018 in Yaoundé [14]. According to the American Cancer Society, becoming sexually active at an early age (especially younger than 18 years) is a risk factor for HPV infection [15].

Out of the 315 women with HR-HPV infection, 64% had eight or more years of education and 23.4% had more than 15 years of education. Akaaboune *et al.* described that 60% of women with HR-HPV infection in Yaoundé had secondary education and 14% had university education [14]. This higher level of education in women infected with HR-HPV can be explained by the fact that the most educated women tend to be more aware of cervical cancer and tend to get screened.

DC VIA/VILI positivity in women with HR-HPV

We observed a prevalence of Positive DC VIA/VILI of 13.4% among women with HR-HPV infection. This is similar to the 9.9% obtained by Cholli *et al.* in Mutengene (a rural setting), and Douala (an urban setting), in Cameroon [16]. A higher figure (19%) was reported by Poli *et al.* in India [17], and a much higher one (50%), by Levy *et al. at* Dschang, in Cameroon [18]. These differences in the prevalence of VIA/VILI lesions could be due to differences in positivity criteria but could also suggest differences in serotypes and their oncogenicity. In the latter study, any acetowhite lesions in the transformation zone were considered positive. Some of these could be due to metaplasia, inflammation, leukoplakia, and condyloma. According to the Geneva Foundation for Medical Education and Research, the expected rate of CIN2+ lesions in HPV-positive women is between 8 and 15% [19].

DC VIA/VILI positivity with respect to HR HPV types

The highest prevalence of DC VIA/VILI lesions was seen with HPV 16 (45.5%), followed by HPV 18 (21.4%). The non-16/18 HR-HPV were least associated with DC VIA/VILI positivity (10.7%). Kunckler *et al.* in Dschang found that VIA test was positive in 80% of women with HPV 16 infection [20]. These findings and the observation that three of the five lesions suggestive of invasive cervical cancer on DC VIA/VILI suggest the highest oncogenicity for HPV 16, followed by HPV 18 and the non-16/18 HR-HPV.

It is acknowledged that HPV 16 and 18 are present in about 70% of cervical cancers [21]. Their detection in DNA-based screening tests must increase suspicion for associated premalignant or invasive lesions and result in further evaluation and management, as appropriate for the setting.

Other determinants of DC VIA/VILI positivity in HR-HPV infection

It is suggested that immunosuppression related to HIV infection may play a greater role during HPV natural history, namely acquisition, persistence, and progression to precancerous lesions [22]. Although our study did not find a significant difference in VIA/VILI positivity between HIV-positive and HIV-negative participants, we must maintain a high suspicion index for precancerous or cancerous lesions when HIV-positive women present with HR-HPV infection.

The limits of the study

This study was screening center-based and the findings may not be a replica of the situation in the community due to selection bias. The retrospective nature of the study made it difficult to obtain complete records, especially for some variables like age at sexarche. These weaknesses may have affected analysis. Assessment of precancerous lesions was limited to DC VIA/VILI, because histology results were not available for many of the cases. The grouping of non-16/18 serotypes as one item does not enable identification of other particular serotypes that may need to be covered, imperatively, by HPV vaccination.

5. Conclusions

A high proportion of women (13.4%) with HR-HPV had a positive DC VIA/VILI, with a significant proportion (2.2%) having lesions suggestive of invasive cervical cancer. Only HR-HPV serotype was associated with DC VIA/VILI positivity, with HPV 16 having the highest prevalence of lesions (45.5%), followed by HPV 18 (21.4%), and non-16/18 HR-HPV (10.7%), suggesting a decreasing order of oncogenicity.

There is a need to do a nationwide study for a detailed mapping of HR-HPV serotype prevalence and evaluation of their contribution to the cervical cancer burden. This would enable the elaboration of a rational cost-effective national cervical cancer control strategy.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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