Original Research Article

Variables affecting the development and progression of precancerous lesions in the Cameroon women population

Abstract

Introduction: Human papillomavirus (HPV) is the most common sexually transmitted infection worldwide. There are many different types of this virus; some types can cause health problems including genital warts, cervical cells lesions and sometimes cervical cancer. Generally, people can get HPV by having vaginal, anal or oral sex with someone who has the virus. The goal of this study was to find out what societal factors can encourage HPV infection to progress to carcinogenesis. **Methods:** This study covered 1443 women in three Cameroonian regions for cytological studies utilizing the Pap test. Multiplex PCR was used to characterize the presence of the human papilloma virus in positive pap smears. Low risk genotypes were shown to be prevalent in the study's findings (over 15%) **Results:** Women under 25 years presented 25% of high-risk genotypes. Genotype HPV 6 and 45 were more prevalent from women with early sexual exposure (before 15 years). HPV 6, HPV 11, and HPV 45, were quite common among women who had at least four sexual partners. When it came to contraceptive techniques, HPV 11 and 62 infections were absolutely absent in women who did not use them. The majority of HPV infection and cervical lesions were seen in women who used an oral contraceptive method plus an intrauterine device. More HPV, especially HPV 61, 45 were identified in smoking women with a frequency of more than 14%. Microbial and fungal infections were also examined according to HPV infections frequency. *Candida albicans* has been linked to the development of both high and low risk HPV infections. The presence of herpes simplex infection in HPV 6 genotype infection was significant. **Conclusion:** There is sufficient evidence that, in addition to papillomavirus infections, additional variables play a role in the transformation of normal epithelial tissue into malignant tissues in cameroonian women. Oral contraceptives, tobacco smoking, parity, number of pregnancies, early sexual sexual exposure, number of sexual partners, and microbial and fungal genital infections such as *Trichomonas vaginalis, Candida albicans, Herpes simplex*, *Gardnella vaginalis*, and *Aspergillus* were all investigated as risk factors.

Keys words: *Human papillomavirus, molecular epidemiology, risk factors, cervical cancer, Cameroon*

I- Introduction:

Cervical cancer is the most frequent cancer in the world after breast cancer, and it is caused by a variety of factors, including papillomaviruses [1]. It is a virus commonly found in individuals with cervical cancer or precancerous lesions. Most Human Papillomavirus (HPV) infections are known to regress without treatment, especially for infections caused by a low risk virus, high risk genotypes are involved and plays an important role in the pathophysiology of cancer, although it is not the only cause[2]. Co-factors have been widely researched in the progression of cervical HPV infection to high-grade CIN and invasive cervical cancer,cervical cancer screening is one of the most effective cancer prevention strategies, but most women in Africa have never been screened[3],[4]. in fact, Cervical cancer (CC) is the leading cause of cancer-related death among women in sub-Saharan Africa, primarily because of limited access to effective screening and preventive treatment[5]. Based on the pathogenic differences, HPV can be divided into high-risk and low-risk categories[6],[7]. The low-risk genotypes do not usually cause malignant lesions, resulting only in genital warts and low-grade cervical intraepithelial neoplasia grade 1 (CIN1), while the high-risk genotypes are a risk factor for cervical cancer[8]. Human papillomavirus genotypes vary by country in Africa, and some studies have found that subtypes of HPV vary by location and ethnicity; therefore, it is critical to try to risk factor understand the dynamics of this distribution. Understandingdistribution of virus genotypes based on socio-demographic information and factors that influence the progression of precancerous lesions into cancer;
is importantto better care for people who are infected with the human papillomavirusinvolved in cervical cancer development.

II- METHODS

Study design: This was a cross-sectional study that took performed at hospitals in three different Cameroon regions (the South, The Far North and the central regions),from February 2017 to September 2019.The study looked at all women, cancer-free or not, at various phases of development.

a- District of Niete

Niete is an agro-industrial locality located in southern Cameroon near the Atlantic coast in the Ocean Division and the Southern Region.

b- District of Mokolo

Mokolo is a city located in the Far-North region, near the border with Nigeria.

c- District of Yaoundé 1

The district of Yaounde III, Department of Mfoundi, Central Region.

Study settings and populations: Three regionswere purposely selected for the study. The reasons for their selection were the lack of sensitization campaign

Administrative and ethical considerations: Ethical statement

The National Ethics Committee of Cameroun issued an ethical clearance with the registration number 2014/08/485/CE/CNERSH/SP. Similarly, regional delegations issued administrative approval.

Selection criteria

Inclusion

The study was open to all Cameroonian women above the age of 18. They couldn't have had a hysterectomy, and they had to be ready to participate in the study, sign an informed consent form, and be sexually active.

Exclusion

All pregnant women;

All women with cervical cancer confirmed.

Sampling method: a minimum sample size of 763 was obtained using the sample formula

A simple sampling method in which possible participants were sequentially solicited at several locations.

Questionnaire

Each woman's data was collected through a 15-20 minute individual interview using a semi structured questionnaire administered by the investigator. The first section of the questionnaire was devoted to sociodemographic data such as age, occupation, and religion. The second section allowed for the collection of obstetric data as well as data on sex behavior.

Cervix sample collection

Participants were positioned in a gynecological posture on an examination table after counseling. For visual examination of the cervix, a clean, sterile, non-lubricated speculum was progressively placed into their vagina. 2 Exfoliated cells from the transformation zone of the cervix were collected using a cytobrush and an ayre spatula. The cells from the ayre

spatula were then placed straight onto a slide and fixed using the traditional method, whereas the cells from the cytobrush were used for PCR.

Cytological analysis

Pap smears were acquired utilizing an ayre spatula to collect exfoliated from the cervix's transition zone. The cells were directly transferred on a slide and fixed using standard methods. To interpret the outcomes of the slides, the Bethesda system was employed.

DNA analysis

The cells obtained with the cytobrush were utilized to isolate the virus. The QIAGEN extraction kit, which is widely available, was used to extract DNA from fresh cervical cells. Extraction was carried out in accordance with the manufacturer's instructions.

HPV genotyping strategy

a- Primers design

The targeted HPV genotypes' DNA sequences were received from Genbank. The primers were designed for each kind of HPV with a high prevalence in Cameroon, according to the literature. We were able to produce primers for 6 low risk genotypes (LR), (6, 11, 61, 62, 70, and 81), and 6 high risk genotypes (HR) (16, 18, 35, 45, 58 and 68) using the Primer 3 online application (https://primer3.ut.ee/).

b- Mix and PCR

On an Applied Biosystems Thermal Cycler, the following settings were utilized for PCR amplification: Denaturation for 3 minutes at 91°C, then 42 cycles of 27 seconds at 94°C, 45 seconds at 50°C, and 10 minutes at 64°C, followed by a 5-minute elongation step at 65°C. The reaction mixture was transferred to 8°C for electrophoresis after amplification.

The PCR result was analysed using agarose gel electrophoresis, which separates DNA products based on size and charge, allowing the presence and size of the PCR product to be determined. Transilluminator was used for visualization.

Control quality

The -globin gene was employed as a quality control gene for PCR amplification to show that the PCR was proceeding smoothly.

Data management and Statistical analysis

Data was entered into an Excel spreadsheet and checked for consistency before being analyzed with Graph Pad Prism version 5 and XLSTAT VERSION 2016.4,Significance was set at $P \leq 0.05$.

Descriptive statistics: mean and standard deviation were used for continuous variables and percentage for categorical variables. The chi-square test was used to assess the association between categorical variables. PCA analyses were used to correlate factors and virus variables.

III- Results

In this study, 1443 patients were evaluated, but only 149 women were maintained due to positive cervical smears. Despite the fact that our group was on average 35 years old, we discovered no HPV 58 in any of our samples. Women between the ages of 20 and 25 were the most representative group.

III-1- Characterization of HPV infection

III-1-1-According to age

Table 1 below, shows the various genotypes discovered in the community based on the age of the patients in the study. In the population study, both LR-HPV and HR-HPV were found, as shown in the table. We can observed, the distribution of genotypes by age demonstrates that genotype 6 is more prevalent in people over 35 years old, while HPV 21 is more common in people younger than 35.

Table 1: HPV distribution according to age

		[15-20 [	[20-25 [	[25-30 [	[35-40[	[40-45[	[45-50[	>=50	
HPV Low risk	**HPV 6**	0	0.67	0.67	0	0.67	0.67	0.67	**< 0.005**
	HPV 11	1.34	1.34	0.67	0.67	0	0.67	0.67	
	HPV 61	0	1.34	0	0	1.34	0	0.67	
	HPV 62	0.67	0	0.67	0.67	0	0.67	0	
	HPV 70	1.34	0.67	0	0	0.67	0.67	0.67	
	HPV 81	1.34	0.67	0	0	0.67	1.34	0.67	
HPV High risk	**HPV 16**	0.67	6.04	1.34	5.37	0.67	6.04	1.34	**<0.001**
	HPV 18	6.04	2.01	2.68	0.67	3.36	3.36	1.34	
	HPV 35	2.68	1.34	2.01	1.34	2.01	0.67	4.7	
	HPV 45	1.34	0.67	2.68	2.68	0.67	1.34	1.34	
	HPV 58	0	0	0	0	0	0	0	
	HPV 68	2.01	1.34	2.68	0	0.67	2.01	1.34	

III-1-2- HPV distribution according to first sexual exposure

The figure 1 below shows the diverse genotypes found in the population based on the age of the patients' first sexual encounter. Girls who had intercourse before the age of 20 had the highest frequency of high-risk genotype 45; this frequency is relatively rare, while it is also seen in women between the ages of 20 and 24. All of our samples were negative for HPV 58, and patients over the age of 50 had moderate high-risk genotypes.

Patients and their age of first sexual exposure, as well as their capacity to present a viral genotype, exhibited relationships (R=0.7) in the Pearson correlation analysis.

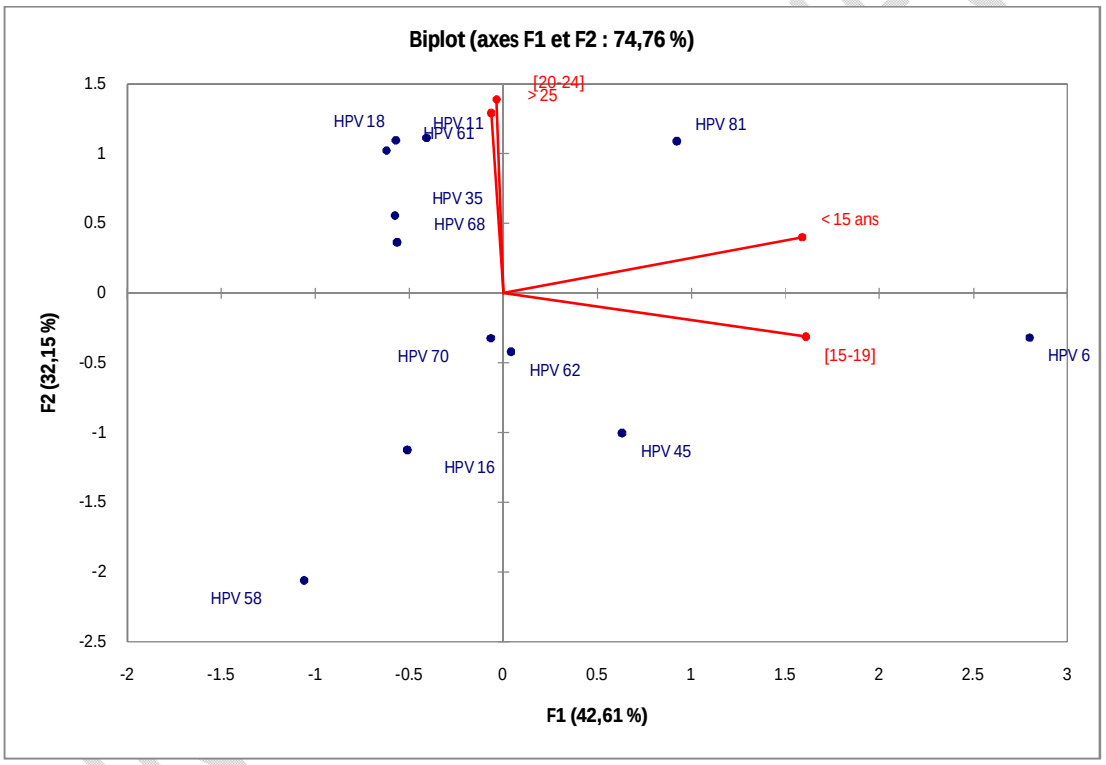

Figure 1: Observation diagram of HPV and first sexual exposure

III-1-3- HPV infection and number of sexual partners

The figure 2 below, presents the genotype of the viruses and the number of sexual partners. Infection with the HPV 6 genotype develops positively in women with less than 5 unprotected ratios with three separate persons. It would be common among women with high sexual potential, a pattern that would also be attributable to infection with the HPV 45

genotype.Women who have had unprotected sexual exposure with 1 or 2 HPV genotypes are more likely to develop infection with HPV 81. Infected women with four sexual partners had a higher chance of developing HPV 18 infection.

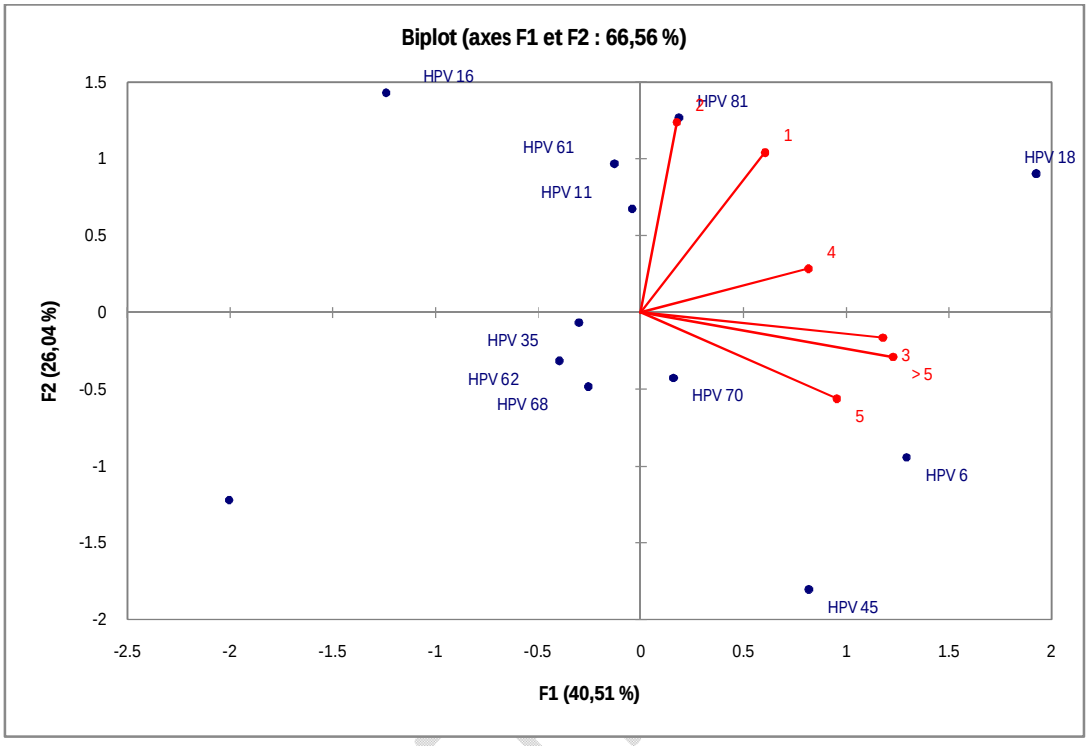

Figure 2: HPV and sexual partner number

III-1-4-HPV infection and delivery number

The table 2 below presents the different HPV genotypes of our study sample according to the number of pregnancies. Infections by low-risk genotypes have a high frequency of HPV 6 genotype infection in women who have had more than 6 pregnancies, or more than 4.5% onset. Infection with the HPV 11 genotype is also strongly represented with an overall frequency of more than 10%, with a strong representation in women with a single pregnancy.

Infection with the HPV 70 genotype also has a high overall occurrence rate with more than 11%; this infection is more represented in the class of women who had at least 2 pregnancies (more than 3%). Infections by other HPV are also well represented with moderate frequencies. Infections by high-risk genotypes showed a high frequency of infection in individuals, including the HPV 16 genotype, which had a frequency of more than 4.5% in women with more than 5 pregnancies as shown in figure 3 below. HPV 18 genotype infections are more common in women with 3 or more pregnancies. HPV 45 infection is absent in women with 4 pregnancies, and there are no cases of HPV 58 genotype; of the data to be checked by the correlation diagram and the correlation matrix.

Table 2: Distribution of genotypes of HPV-BR according to the deliveries number

		1	2	3	4	5	> 5	
HPV Low risk	**HPV 6**	2.01	1.34	2.01	0.67	1.34	4.7	**< 0.005**
	HPV 11	2.68	1.34	2.01	1.34	0.67	1.34	
	HPV 61	1.34	3.36	1.34	0	0.67	0.67	
	HPV 62	0.67	1.34	0.67	1.34	2.01	2.01	
	HPV 70	2.68	3.36	2.68	1.34	0.67	1.34	
	HPV 81	1.34	0.67	0.67	1.34	2.01	1.34	
HPV High risk	**HPV 16**	4.7	2.01	2.68	1.34	2.01	0	**<0.001**
	HPV 18	0.67	0.67	1.34	2.01	2.01	0.67	
	HPV 35	1.34	4.7	0.67	2.01	2.01	1.34	
	HPV 45	0.67	1.34	1.34	0	0.67	0.67	
	HPV 58	0	0	0	0	0	0	
	HPV 68	2.68	0.67	0.67	0.67	0.67	1.34	

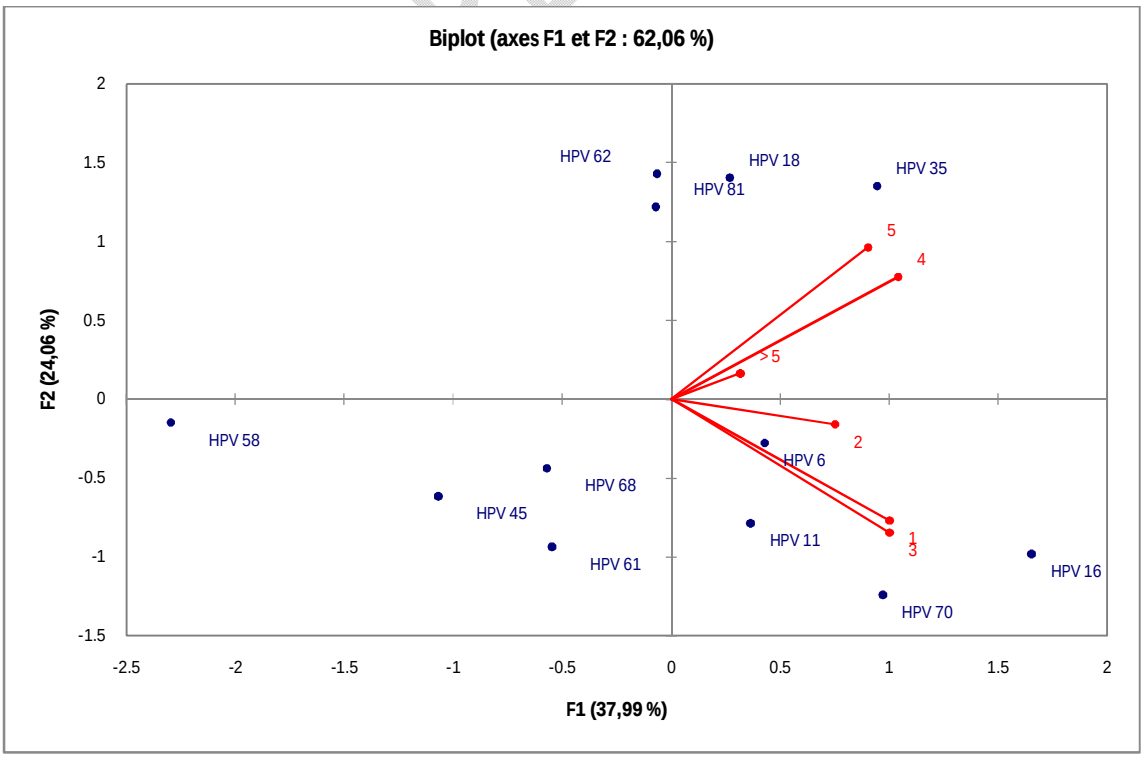

Figure 3: HPV-Pregnancy

III-1-5- HPV infection and contraceptive methods

The figure 4 below presents, contraception action and HPV infection. Indeed, we observed that women who used intrauterine devices were more likely to be infected with HPV genotypes 18 and 35. HPV genotype 16 was discovered more frequently in women who used oral contraceptives. Genotype 45 was the most prevalent in this study and was most common in women who had used methods such as implants and injections

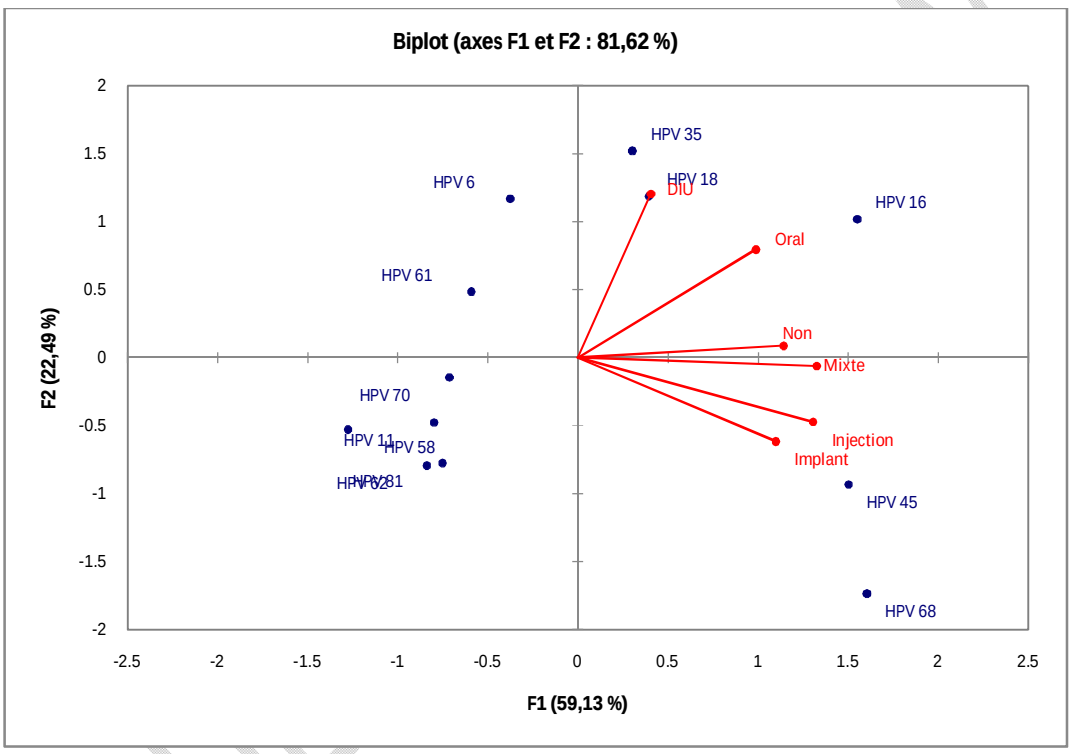

Figure 4: Observations Diagram (HPV-MC)

III-7- Human papilloma virus and other infections

The figure 5 below, presents co-infections between HPV viruses and other microorganisms, such as parasites, bacteria and fungi. In this investigation, *Candida albicans* (CA) was the most prevalent microorganism. HPV 18, 35, 45, and 68 were found to be co-infected with *Trichomonas vaginalis* (TV) in up to 2% of cases. *Candida albicans* was found in all of the high-risk genotype infections, with a 30 percent incidence frequency. The presence of Aspergillus in the case of HPV 45 infection is not noteworthy. However, the Pearson correlation matrix reveals a substantial association between the existence of CA and the presence of TV, as well as numerous other positive correlations.

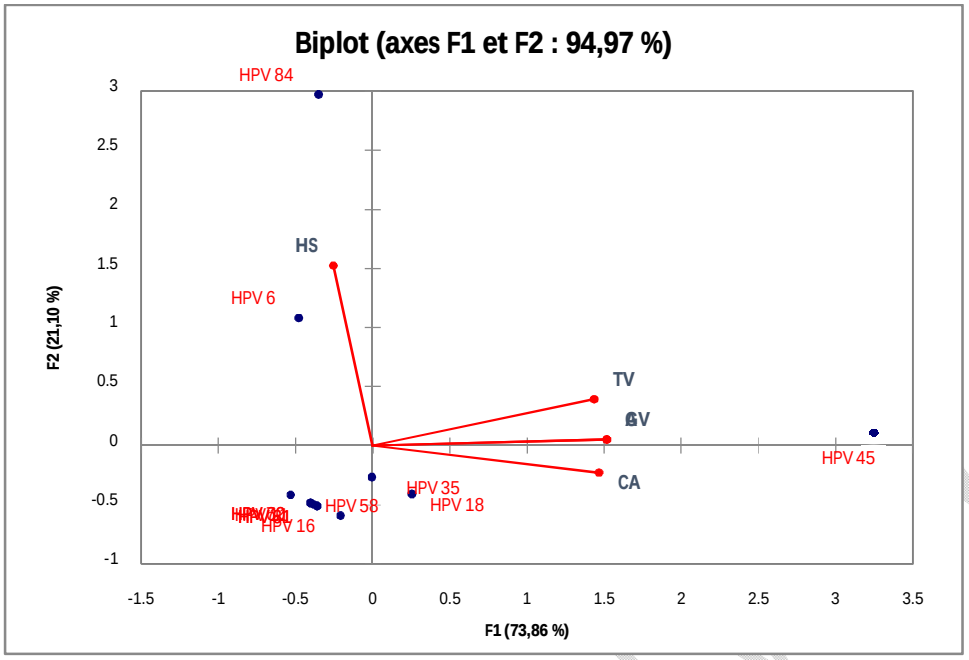

Figure 5: HPV and other infections
TV:*Trichomonasvaginalis*
CA:*Candidaalbicans*
HS: *Herpès simplex*
GV:*Gardnellavaginalis*
A :*Aspergillus*

Discussion

Human papillomavirus infection is still a major problem around the world, particularly in developing countries. The goal of this study was to find out how often HPV is in malignant lesions while also defining these lesions. HPV appears to be present in the majority of lesions, and its prevalence appears to be favored by specific socio-demographic factors.During this study, HPV 58 was absent in the whole study sample.Many HPV genotypes were identified. We found in this study that the Cameroonian population has a wide range of HPV genotypes, which could be a burden; this opinion is shared by many Latin American studies as shownby Schicero*et al.*[9]. The HPV 6 virus was most commonly found in women over the age of 40, and the 18 and 35 genotypes, which appear to be predominant in adults, were also found in

this age group. A physiopathological dynamic in aging could explain this occurrence. Indeed, research conducted elsewhere confirms that,among the population which both received HPV and pathological examination, the peak age of onset of precancerous lesion was between 35 and 49 years, while the previous study[10].The age of first sexual exposure was a key variable that allowed us to see that certain genotypes were more prevalent in women under the age of 20, particularly genotypes 18, 11, 61, and 68. Certain genotypes, such as genotypes 70, 62, and 16, were not associated with the age of first sexual exposure. Only genotype 6 and, in a few rare cases, genotype 45 were detected in women who had sexual activities at a young age.For most genital infections, the issue of early sexual activity remains a critical issue. The presence of more than concomitant HPV infections, a phenomenon very common in younger populations, is reported to not influence the duration of infection [11]. Epidemiology portrays the young woman as immature for specific genital system-harming behaviours. There have been studies that show the fragility of the female genital system especially for the cervix, because the cervix plays a fascinating gatekeeping role in first, preventing the ascent of pathogens from the vagina into the uterus[12].The high rate of factors supporting the development of HPV and related disorders are more common in region of this study, where the average financial income per capita was low (Far-North region, and south region), as they are for other sexually transmissible infectious pathogens. This observation can be explain by the fact that, the majority of these district have a low quality of life, with inadequate access to basic necessities including healthy food, clean water, acceptable housing, proper hygiene, and medical treatment. The study leads by Arnaud*et al.*,~~showed~~demonstratedthat, Due to extreme poverty, even the young women engage in unsafe sexual practices such as prostitution to meet their basic needs, consequently increasing the risk of infection. Low education and/or schooling levels and the cultural model that requires women to marry early are other factors associated with a high spread of HPV and a high disease rate in young women [13]. The

number of sexual partners has been shown to be one of the most important risk factors for HPV transmission by Chelimo and collaborators [14].In Cameroon, high-risk and low-risk HPV, including HPV 45 and HPV 6, are found in almost all women who have had at least one sexual exposure and more than one pregnancy, demonstrating the importance of sexual intercourse in the emergence of lesions and the development of infections. According to different Africa studies, the number of pregnancies appears to be a determinant in the development of HPV infection[15–17].In this study, we observed that women who had 0, 1, 2, or 3 pregnancy episodes had a statistically higher infection rate than the other groups.Infections with the HPV 6 genotype were common in women who use intrauterine devices, accounting for more than 3% of all cases. Oral contraceptives appear to be a significant influence in the development of high-risk infections.The combination of oral contraceptives and injections appear as dangerous combination, for patients and favor the development of HPV virus infections. Fulya and collaboratorsconsidershowed that,the most important risk factor in HrHPV positivity was the use of contraceptive methods other than LNG-IUS and copper IUD. Although the relationship between IUD usage and cervical cancer has been extensively reported, there are conflictingresults and Hormonal contraceptive methods are associated with CIN3 and cervical cancer[18]. Further studies with a larger sample size may provide clearer information.

Conclusion

This workstudy consisted in establishing a relationship between HPV distribution and factors able to promote his development.Statistical analyses were possible usingXLSTAT 2015 software to perform PCAtest. It has been possible to observe that, the mean age of the sampleswas significant, withthe most representative individuals being the age range between [20-25[. Results allowed us to confirm that, HPV infectionalone may not be sufficient to cause cervical cancer and other exogenous or endogenous factors might contribute.It therefore appears that, the presence of HPV genotypes depends on many socio-demographic factors that may favor infections with the virus. In this study, it has been possible to classify into two groups:Environmental factors, including use of oral, contraceptives, tobacco smoking, parity,

number of pregnancies, early sexual ~~sexual~~exposure , number of sexual partners and the social factors which include genital microbial and fungal infections as *Trichomonasvaginalis*, *Candida albicans*, *Herpes simplex*, *Gardnerellavaginalis* and *Aspergillus.*

What is already know on this topic

1- Human papillomavirus (HPV) infection is the most common sexually transmitted infection worldwide;
2- Almost all cervical cancer cases (99%) are linked to infection with high-risk human papillomaviruses (HPV);
3- Although most infections with HPV resolve spontaneously and cause no symptoms, persistent infection can cause cervical cancer in women, with the help of external factors that aren't well-known.

What this study adds

This research provides a wealth of information.

1- On the distribution of virus genotypes based on socio-demographic information
2- Concerning factors that influence the progression of precancerous lesions into cancer;
3- Forbetter effective strategies for combating cervix cancer in Cameroon, which is the second leading cause of death after breast cancer;

Declarations

Ethics approval and consent to participate

The study protocol was written based on the Helsinki ethical principles for medical researches and approved by the National Ethics Committee for Human Health Research **(n° 2014/08/485/CE/CNERSH/SP).**

Consent for publication

Informed consent was obtained from all individual participants included in the study

Availability of data and materials

The data will be available upon reasonable request to the corresponding author

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STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1-2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2-3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4

Data sources/measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4
Bias	9	Describe any efforts to address potential sources of bias	4
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4-5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5
		(*b*) Describe any methods used to examine subgroups and interactions	5
		(*c*) Explain how missing data were addressed	5
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	5
		(*e*) Describe any sensitivity analyses	5
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	NA
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6
		(b) Indicate number of participants with missing data for each variable of interest	NA
Outcome data	15*	Report numbers of outcome events or summary measures	7
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	NA
		(*b*) Report category boundaries when continuous variables were categorized	7
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	8

Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	9
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9
Generalisability	21	Discuss the generalisability (external validity) of the study results	9
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	NA

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.