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Sero-diagnosis of *Chlamydia trachomatis* and Molecular Detection of Genital Oncogenic *Human Papilloma Virus* Among Cameroonian Women

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Abstract: Cervical cancer is a preventable public health concern ranking second among women's cancer in Cameroon. *Human papilloma virus* (HPV) is the main causative agent with *Chlamydia trachomatis* being suggested as the co-factor. Our objective was to characterize high risk (hr) HPV types and to detect *Chlamydia trachomatis* antibodies among Cameroonian women with and without cervical cancer. *Methods*: This unmatched case-control study enrolled 100 cases with cervical cancer and 200 controls with normal cytology aged 25- 65 years in four reference hospitals in Douala and Yaoundé (Cameroon). Consented participants filled a structured questionnaire and data on socio-demographic characteristics collected. *Chlamydia trachomatis* antibodies were detected by the Enzyme Linked Immuno-Sorbent Assay technique (ELISA) and hr HPV- DNA by PCR technique. Descriptive statistics was conducted to provide frequencies and percentages and the logistic regression analysis to assess the association between categorical data. $p < 0.05$ was considered significant. *Results*: Our data showed 39 (39.0%) cases aged 39-52 years compared to 96 (48.0%) controls aged 25-38 years (p=0.001). We found 82 (82.0%) cases compared to 131 (65.5%) controls with hr HPV infections. HPV 16 was most prevalent being found in 29 (29.0%) cases compared to 69 (34.5%) controls. *Chlamydia trachomatis* IgG / hr HPV co-infections were detected in 20 (20.0%) cases compared to 33 (16.5%) controls but with no significant association with cervical cancer (aOR=1.87; 95%CI: 0.58-5.97; p=0.293). *Chlamydia trachomatis* IgM (aOR=3.50; 95%CI: 1.16-10.49; p=0.025) was significantly associated with cervical cancer. *Conclusion*: Hr HPV- DNA was high in cases than in controls. *Chlamydia trachomatis* single infection and *Chlamydia trachomatis*/hr HPV co-infections were not significantly associated to precancerous lesions thus, necessitating further investigations.

Keywords: Precancerous Lesions, *Human papilloma virus*, *Chlamydia trachomatis*, Co-infection, Case-control Study

1. Introduction

The *Human papilloma virus* (HPV) family comprises more than 200 different types [1]. Almost all cervical cancers are caused by persistent infection with certain types of HPV [2]. Infections caused by high risk (hr) HPV are responsible for 7.7% cases of cervical cancer mainly in developing countries [3, 4]. Most HPV infections are transient and only a small minority of women with long term, persistent infections are at an increased risk of progression to precancerous cervical lesions and cervical cancer [5].

A number of factors have been found to contribute to cervical cancer including: high parity, tobacco smoking, long-term hormonal contraceptive use, co-infection with *C. trachomatis, Herpes simplex virus* type 2, HIV, immunosuppression, certain dietary deficiencies, cancer,

imbalanced vaginal flora, having an uncircumcised male partner and low socio-economic status [1, 6]. The role of sexually transmitted infections (STIs) other than HPV as co-factors in cervical carcinogenesis due to HPV is a matter of debate. Most STIs are suggested to be associated with an inflammatory response/process which facilitate HPV entry into target cells, as well as with the persistence of infection [7].

C. trachomatis is an intracellular bacterium with spherical to ovoid obligate shape that is found worldwide [8]. There are 17 different serotypes of *C. trachomatis*: E, F, G, D, K, J, H, B, Ia with the serovars D-K causing the urogenital tract infection [9]. Most *C. trachomatis* infections in women may result in pelvic inflammatory diseases, preterm delivery, and premature rupture of membranes, low birth weight and still birth. There are also an increased risk of post abortion, post caesarean section and post-partum maternal infections [10].

HPV and *C. trachomatis* are the cause of most common STIs worldwide; they share similar behavioural risk factors, such as younger age and higher numbers of sexual partners [11]. As a result, the two infections could occur concurrently [11, 12]. This association has also been suspected in the aetiology of cervical cancer independently of HPV status [13]. Given that the endocervix is the major site of *C. trachomatis* infection in the lower genital tract, it is hypothesized that *C. trachomatis* infection may play a role in the initiation of malignant transformation of the glandular endocervical epithelium via the mechanisms related to HPV co-infection. In addition, *C. trachomatis* may play a role in the progression of adenocarcinoma by evoking the inflammatory response that damages the mucosal barrier including the basement membrane [11, 12, 14].

Issues related to HPV/ *C. trachomatis* single or co-infections remain a matter of debates. In Cameroon, there is paucity in the epidemiological data on HPV/ *C. trachomatis* co-infections and their role in the aetiology of cervical cancer. The objective of this study was to detect genital HPV- DNA types and *C. trachomatis* antibodies in women with and without cervical precancerous lesions in two main cities of Cameroon

2. Methods

2.1. Study Design

This unmatched case-control study enrolled women screened for cervical cancer between June to November 2018 at the Gynaecology units of four reference hospitals in Douala [the Douala General Hospital (DGH) and the Douala Gynaeco-Obstetric and Paediatric Hospital (DGOPH)] and in Yaoundé [the Yaoundé University Teaching Hospital (YUTH) and the Yaoundé Gynaeco-Obstetrics and Paediatrics Hospital (YGOPH)] respectively the economic and political capitals of Cameroon.

Inclusion criteria for cases: Cases were 100 cervical cancer patients randomly selected among those diagnosed with abnormal cytology as atypical squamous cells of undetermined significance (ASCUS), low grade squamous intraepithelial lesions (LSIL), high low grade squamous intraepithelial lesions (HISIL). Only cases who participated in the free cervical cancer screening campaign organized for the purpose of this study were included.

Exclusion criteria for cases: Those cases who had initiated cervical cancer therapy and those with prolong cervico-vaginal bleeding were excluded from the study.

Inclusion criteria for controls: We included 200 non-cervical cancer patients randomly selected among those diagnosed with normal cytology by the Pap test. Only controls who participated in the free cervical cancer screening campaign organized for the purpose of this study were included.

Exclusion criteria for controls: We excluded controls with the following characteristics: HPV vaccination, pregnancy, radical hysterectomy, virgins and those with prolong cervico-vaginal bleeding.

2.2. Sample size Calculation

The sample size for a case - controls was obtained using the Kelsey method [15]. Where: N=sample size for cases=100, 80% the power, Z_β=0.84, at a 0.05 significance level Z_α=1.96, OR=odds ratio of 2.0. The proportion exposed in the control group was 36.3% [16] (Pcontrol exp), P_1=proportion of exposed in the control group, P_2=proportion of exposed in the case group. Using the ratio (r) of one case to two controls, we included 100 cases and 200 controls in the study.

2.3. Sampling Technique

Sampling method for cases: A systematic random sampling technique using the calculated skip interval of 1.13 (skip intervals=K=Total population/sample size) was conducted to select 100 cases for this study: thus starting from the first case, any other case was chosen at an interval of two until we got 100 cases.

Sampling method for controls: A systematic random sampling technique using the calculated skip interval of 4.06 (skip intervals=K=Total population/sample size) was conducted to select 200 controls for this study: thus starting from the first case, any other case was chosen at an interval of five until we got 200 controls.

2.4. Ethical Clearance

The study was approved by the Faculty of Health Science Institutional Review Board of the University of Buea, Cameroon (N°: 2018/0254/UB/SG/IRB/FHS). The aim of the study, benefits and potential risk were clearly explained to each participant. Verbal and written informed consent to attest adherence in the study was provided by each of them. Anonymity and confidentially were respected.

2.5. Sample Collection

2.5.1. Administration of Questionnaire

The ward of the gynaecology or family planning unit of each selected health facility was used to recruit participants

who filled a structured questionnaire consisting of questions on socio-demographic and reproductive histories. Following verification of the questionnaire for its completeness, an identification code was attributed to each of them. They were later conveyed for the blood and the endocervical sample collection in a private gynaecology consultation room following an attributed identification code.

2.5.2. Collection and Handling of Venous Blood Samples

Each participant was comfortably seated and reassured of the little pain they might feel when the sterile needle will be introduced into the cubital vein. Following sterilisation with 70% alcohol, 4 millilitres (ml) of venous blood sample was aseptically obtained by venepuncture using sterile vaccutainer needle and gently introduced into a plain dried 5 ml vaccutainer tube. The blood was then allowed to clot. At the end of the day, all blood samples were centrifuged at 12 000 g for 10 minutes and 500 µl of serum extracted avoiding mixing with blood cells and aliquoted into a 1.5 ml eppendorf tube clearly labelled with the participants' identification number. The serum sample obtained was stored at -20°C freezer until analysis [17]. Blood samples from different study sites were transported in the cool environment (+4- 8°C) to the Applied Biochemistry Laboratory of the Faculty of Health Sciences- University of Buea for analysis.

2.5.3. Collection and Handling of Cervical Smear

Following physical examination by a gynaecologist, a sterile disposable speculum was aseptically inserted into the illuminated vagina to observe the external os [18]. A sterile cytobrush was then aseptically inserted into the endocervical canal (about 1.0-1.5 centimeters) until the largest bristles touched the ectocervix through a disposable speculum. The brush was rotated in an anti-clockwise direction (180°C) and slowly removed from the cervical canal avoiding vaginal secretions and mucus. The secretions of the endocervix obtained with the cytobrush were suspended in 500 µl of Sacace transport medium plus solution (Sacace Biotechnologies Srl, Italy) contained in a 2.0 ml disposable polypropylene Eppendorf tubes, stored at -20°C and transported sealed in cooled environment (+4- 8°C) to the "Yaoundé Military Health Research Centre" for HPV- DNA testing.

2.5.4. Laboratory Procedures

i) Venous Blood Sample Analysis

Data on *C. trachomatis* (IgG, IgM, IgA) antibodies were indirectly obtained in the serum by Enzyme linked immune-Sorbent assay (ELISA) technique using Vircell diagnostic kits according to the manufacturer's instructions (Vircell Microbiologists, Spain). The validity of ELISA test results was considered at Optical Density > 0.9 for positive control, < 0.5 for negative control and > 0.5 and < 1.5 for the cut-off control. A participant was classified as negative if the antibody index was ≤ 9 and positive if > 9 according to the formula: Antibody index=(sample OD/cut-off OD) X 10.

ii) Detection of High Risk Human Papilloma Virus by Polymerase Chain Reaction

The PCR technique was used to detect 12 hr HPV- DNA in the endocervical secretions with strict adherence to the manufacturer's instruction. The procedure involved:

An extraction of the HPV- DNA in the endocervical samples and controls using DNA-Sorb-A extraction kit according to the manufacturer's instructions (Sacace Biotechnologies Srl, Italy). The purity of the extracted DNA samples was confirmed using Nanodrop® 2000 spectrophotometer (Fisher thermoscientific, USA).

Multiplex amplification of the isolated DNA was conducted on Biorad T100 thermocycler (Biorad) using HPV 12 high risk typing kit (Sacace Biotechnologies Srl, Italy). Each PCR-mix-1 tube contained primers directed against regions of four HPV types and β-globine gene used as Internal Control and the TaqFpol enzyme. The amplification procedure had three cycles with different reaction temperatures: 1 cycle of 15 minutes at 95°C, 42 cycles (30 seconds at 95°C, 40 seconds at 63°C, and 50 seconds at 72°C), and 1 cycle of 1 minute at 72°C. Storage was done at 10°C for 1 minute.

The amplified products were detected by gel electrophoresis and the bands revealed using a white 12 UV transilluminator (Gel logistic 112 manufactured by Kodak). The length of the amplified samples was determined using a 100 bp ladder.

2.6. Statistical Analysis

Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS) v 20 and the results presented on tables. Continuous variables were computed as mean and standard deviation (SD) and categorical variables as frequencies and percentages. The association between categorical data and the infections was obtained using the Chi square (X^2) and the logistic regression analysis (odd ratio at 95% confidence interval). The level of significance was considered at $p< 0.05$.

3. Results

3.1. Socio-demographic, Behavioural and Reproductive Characteristics of Cases and Controls

Table 1 presents 29 (29.0%) cases and 96 (48.0%) controls aged 25-38 years. Their mean ages were 44.7 +/-11.1 SD years for the cases and 39.3+/-9.7 SD years for the controls. Of the 180 married participants, 61 (61.0%) were cases and 119 (59.5%) were controls. Likewise, 34 (34.0%) cases had salary jobs compared to 81 (40.5%) controls. Furthermore, 46 (46.0%) cases compared to 94 (47.0%) controls had secondary school education respectively.

Of the 282 participants with no history of smoking, 95 (95.0%) were cases compared to 187 (93.5%) controls. Similarly, 81 (81.0%) cases compared to 135 (67.5%) controls were casual alcohol consumers (Table 1).

Of the 200 participants who had never used condom, 64 (64.0%) were cases compared to 136 (68.0%) controls. On the other hand, 28 (28.0%) cases compared to 50 (25.0%)

controls used oral contraceptive occasionally. We also found that 44 (44.0%) cases compared 82 (41.0%) controls with more than five parities (Table 1).

Table 1. *Socio-demographic and reproductive characteristics of cases and controls.*

Characteristics	Total (N=300) No (%)	Cases (N=100) No (%)	Controls (N=200) No (%)	X2	p-value
Age range (years)					
25-38	125 (41.7)	29 (29.0)	96 (48.0)	21.1	0.001
39-52	120 (40.0)	39 (39.0)	81 (40.5)		
53-65	55 (18.3)	32 (32.0)	23 (11.5)		
Marital status				5.50	0.141
Divorced	4 (1.3)	2 (2.0)	2 (1.0)		
Married	180 (60.0)	61 (61.0)	119 (59.5)		
Single	95 (31.7)	26 (26.0)	69 (34.5)		
Widowed	21 (7.0)	11 (11.0)	10 (5.0)		
Profession				7.20	0.066
Traders	47 (15.7)	12 (12.0)	35 (17.5)		
Salary jobs	115 (38.3)	34 (34.0)	81 (40.5)		
Housewives	85 (28.3)	38 (38.0)	47 (23.5)		
Unemployed	53 (17.7)	16 (16.0)	37 (18.5)		
Level of education				3.60	0.310
Illiterates	7 (2.3)	1 (1.0)	6 (3.0)		
Primary	46 (15.3)	20 (20.0)	26 (13.0)		
Secondary	140 (46.7)	46 (46.0)	94 (47.0)		
Tertiary	107 (35.7)	33 (33.0)	74 (37.0)		
Smoking				0.31	0.854
Former	8 (2.7)	2 (2.0)	6 (3.0)		
Occasional	10 (3.3)	3 (3.0)	7 (3.5)		
Never	282 (94.0)	95 (95.0)	187 (93.5)		
Alcohol consumption				6.96	0.073
Permanent	19 (6.3)	4 (4.0)	15 (7.5)		
Former	10 (3.3)	1 (1.0)	9 (4.5)		
Never	55 (18.3)	14 (14.0)	41 (20.5)		
Occasional	216 (72.0)	81 (81.0)	135 (67.5)		
Use of oral contraception				0.77	0.858
Former	4 (1.3)	1 (1.0)	3 (1.5)		
Permanent	18 (6.0)	7 (7.0)	11 (5.5)		
Occasional	78 (26.0)	28 (28.0)	50 (25.0)		
Never	200 (66.7)	64 (64.0)	136 (68.0)		
Use of condom				2.50	0.290
Always	21 (7.0)	6 (6.0)	15 (7.5)		
Occasional	180 (60.0)	55 (55.0)	125 (62.5)		
Never	99 (33.0)	39 (39.0)	60 (30.0)		
Parity				3.91	0.141
<3	111 (37.0)	30 (30.0)	81 (40.5)		
3-5	63 (21.0)	26 (26.0)	37 (18.5)		
>5	126 (42.0)	44 (44.0)	82 (41.0)		

3.2. Molecular Detection of hr HPV- DNA Types Among Cases and Controls

Of the 300 participants, 213 (71.0%) [95%CI: 65.7-76.0] were hr HPV-DNA positive among whom, 82 (82.0%) were cases compared to 131 (65.5%) controls. HPV infections were statistically different among cases and controls (p=0.003). HPV multiple infections were found in 112 (37.3%) [95%CI: 31.7-43.0] participants among whom 38 (38.0%) were cases and 74 (37.0%) were controls (Table 2).

Table 2. *Molecular detection of hr HPV among cases and controls.*

Hr HPV infection	Total No (%)	Cases No (%)	Controls No (%)	OR (95% CI)	p-value
HPV-DNA				2.40 (1.33-4.31)	0.004
Positive	213 (71.0)	82 (82.0)	131 (65.5)		
Negative	87 (29.0)	18 (18.0)	69 (34.5)		
Total	300 (100)	100 (33.3)	200 (66.6)		
Multiple infections				1.04 (0.64-1.71)	0.866
Positive	112 (37.3)	38 (38.0)	74 (37.0)		
Negative	188 (62.7)	62 (62.0)	126 (63.0)		
Total	300 (100)	100 (33.3)	200 (66.6)		

3.3. Molecular Diversity of High Risk HPV- DNA Types Among Cases and Controls

The overall hr HPV- DNA detection was: 98 (32.7%) for HPV16, 19 (6.3%) for HPV 31, 36 (12.0%) for HPV33, 29 (9.7%) for HPV35, 39 (13.0%) for HPV18, 44 (14.7%) for HPV39, 25 (8.3%) for HPV45, 16 (5.3%) for HPV59, 14 (4.7%) for HPV52, 11 (3.7%) for HPV56, 13 (4.3%) for HPV58 and 54 (18.0%) for HPV66 (Table 3). Among cases hr HPV- DNA was detected in 29 (29.0%) for HPV16, 12 (12.0%) for HPV33, 13 (13.0%) for HPV35, 14 (14.0%) for HPV18, 17 (17.0%) for HPV39, 14 (14.0%) for HPV 45 and 20 (20.0%) for HPV66. In controls, hr HPV- DNA detection was as followed: 69 (34.5%) for HPV16, 24 (12.0%) for HPV33, 25 (12.5%) for HPV18 and 27 (13.5%) for HPV39. HPV16 was involved in most multiple infections. The most prevalent co-infections were observed in 27 (9.0%) for HPV16/66 and in 22 (7.3%) for HPV16/18 (Table 3).

Table 3. *Molecular diversity of hr HPV-DNA types among cases and controls.*

Hr HPV genotypes	Total (N=300) No (%)	Cases (N=100) No (%)	Controls (N=200) No (%)	X^2	p-value
HPV16	98 (32.7)	29 (29.0)	69 (34.5)	0.92	0.338
HPV31	19 (6.3)	5 (5.0)	14 (7.0)	0.45	0.503
HPV33	36 (12.0)	12 (12.0)	24 (12.0)	0.00	1.000
HPV35	29 (9.7)	13 (13.0)	16 (8.0)	1.90	0.167
HPV18	39 (13.0)	14 (14.0)	25 (12.5)	0.13	0.716
HPV39	44 (14.7)	17 (17.0)	27 (13.5)	0.65	0.419
HPV45	25 (8.3)	14 (14.0)	11 (5.5)	6.03	0.012
HPV59	16 (5.3)	6 (6.0)	10 (5.0)	0.13	0.716
HPV52	14 (4.7)	8 (8.0)	6 (3.0)	3.74	0.053
HPV56	11 (3.7)	6 (6.0)	5 (2.5)	2.31	0.128
HPV58	13 (4.3)	3 (3.0)	10 (5.0)	0.64	0.423
HPV66	54 (18.0)	20 (20.0)	34 (17.0)	0.13	0.716
Multiple hr HPV infections					
HPV16/66	27 (9.0)	3 (11.1)	24 (88.9)	7.11	0.008
HPV16/18	22 (7.3)	8 (36.4)	14 (63.6)	0.99	0.754
HPV33/39	16 (5.5)	8 (50.0)	8 (50.0)	2.11	0.146

3.4. Sero-diagnosis of Chlamydia Trachomatis Infections Among Cases and Controls

C. trachomatis antibodies were detected in 30 [10.0%, 95% CI: 6.3-13.3] cases for IgM, 70 [23.3%, 95% CI: 18.3-28.3] cases for IgA and 77 [25.7%, 95%CI: 21.0-30.3] cases for IgG. Among cases, 4 (4.0%), 17 (17.0%) and 22 (22.0%) participants were positive for IgM, IgA and IgG respectively compared to 26 (13.0%), 53 (36.5%) and 55 (27.5%) for controls. IgM participants had higher odds of cervical cancer than those negative (aOR=3.50; 95%CI: 1.16-10.49; p=0.025) (Table 4).

Table 4. *Sero-diagnosis of C. trachomatis infections among cases and controls.*

Ab Types	Total No (%)	Cases No (%)	Controls No (%)	OR 95% CI	p-value	aOR 95% CI	p-value
IgM							
Positive	30 (10.0)	4 (4.0)	26 (13.0)	3.60 (1.2-10.6)	0.014	3.50 (1.2-10.5)	0.025
Negative	270 (90.0)	96 (96.0)	174 (87.0)				
Total	300 (100)	100 (33.3)	200 (66.7)				
IgA							
Positive	70 (23.3)	17 (17.0)	53 (26.5)	1.80 (0.9-3.3)	0.067	1.60 (0.8-3.0)	0.156
Negative	230 (76.7)	83 (83.0)	147 (73.5)				
Total	300 (100)	100 (33.3)	200 (66.7)				
IgG							
Positive	77 (25.7)	22 (22.0)	55 (27.5)	1.35 (0.8-2.4)	0.304	1.12 (0.6-2.0)	0.720
Negative	223 (74.3)	78 (78.0)	145 (72.5)				
Total	300 (100)	100 (33.3)	200 (66.7)				
IgG/IgM					0.045	-	-
Positive	13 (4.3)	1 (1.0)	12 (6.0)			-	-
Negative	287 (95.7)	99 (99.0)	188 (34.5)			-	-
Total	300 (100)	100 (33.3)	200 (66.7)			-	-

3.5. High Risk HPV- DNA/ C. trachomatis Co- infections Among Cases and Controls

Hr HPV- DNA/C. *trachomatis* IgM was detected in 4 (4.0%) cases compared to 16 (8.0%) controls, hr HPV- DNA/ *C. trachomatis* IgA in 13 (13.0%) cases compared to 36 (18.0%) controls and hr HPV- DNA/ *C. trachomatis* IgG in 20 (20.0%) cases compared to 33 (16.5%) controls (Table 5).

Table 5. *Comparison of hrHPV-DNA/C. trachomatis co-infections among cases and controls.*

Co-infections	Total No (%)	Cases No (%)	Controls No (%)	OR (95% CI)	p-value	aOR (95% CI)	p-value
HPV/IgM							
Positive	20 (6.7)	4 (4.0)	16 (8.0)	2.08 (0.7-6.4)	0.190	1.87 (0.6-5.9)	0.293
Negative	280 (93.3)	96 (96.0)	184 (92.0)				
Total	300 (100)	100 (33.3)	200 (66.7)				
HPV/IgA							
Positive	49 (16.3)	13 (13.0)	36 (18.0)	1.46 (0.8-2.9)	0.269	1.42 (0.7-2.9)	0.340
Negative	251 (83.7)	87 (87.0)	164 (82.0)				
Total	300 (100)	100 (33.3)	200 (66.7)				
HPV/IgG							
Positive	53 (17.7)	20 (20.0)	33 (16.5)	0.79 (0.4-1.5)	0.454	0.74 (0.4-1.4)	0.741
Negative	247 (82.3)	80 (80.0)	167 (83.5)				
Total	300 (100)	100 (33.3)	200 (66.7)				
HPV/IgM+IgG				-	0.111	-	-
Positive	10 (3.3)	1 (1.0)	9 (4.5)				
Negative	290 (96.7)	99 (99.0)	191 (95.5)				
Total	300 (100)	100 (33.3)	200 (66.7)				

4. Discussion

4.1. Characteristics of Cases and Controls

Participants in this study were aged between 25- 65 (mean=41.1+/- 10.5) years quite different from that of another study which included participants aged between 35 - 78 (mean=53.2 +/-9.8 years) [19]. The mean ages of our cases (mean=44.7 years) and controls (mean=39.3 years) were lower than that reported in India (cases mean=50.6 years and controls mean=45.5 years) [20] but greater than 36.89 years recorded in women with cervical cancer in the Northern Region of Cameroon [21]. The characteristics of cases and controls were similar except for their age. A case-control study in Egypt reported no significant differences between patient and control groups as regarding risk factors of cervical cancer as age, educational level, parity, sexarche, total life time sex partner, smoking and contraceptive history [22].

4.2. Prevalence of hr HPV Among Cases and Controls

The overall prevalence of hr HPV genotypes was 71.0% for single and 37.3% for multiple types, similar to 71.9% in Angola [23] but higher than 35.9% and 41.4% for multiple hr HPV types in USA [24]. Compared to Cameroon, a well implemented HPV vaccine programme might have contributed to a reduced risk of HPV infection in the USA. We found high prevalence of hr HPV infections among cases compared to controls which was similar to the findings of another study in Brazil (2016) which reported high prevalence of HPV in women with LSIL than in women with negative cytology [25]. The disparities observed in the prevalence of HPV infections between studies may be justified by the epidemiologic diversity of the virus types, factors favouring the persistence or clearance of the virus, the diagnostic range of the HPV kits and the selection criteria of the study population. Findings of high prevalence of hr HPV-DNA types in the current study might be due to the persistence of the infection while lower prevalence in controls might presumably be attributed to the immunological clearance of the virus [26].

HPV 16 and HPV 18 were identified in most studies as the most prevalent oncogenic HPV types worldwide [16, 23, 24, 27]. Our results showed HPV 16 as the most prevalent hr HPV type followed in decreasing order of frequency by HPV 66, HPV 39, HPV18 and HPV 33 which was different from another study in Cameroon which reported HPV16 and HPV18 to account for 36.3% of HPV infection [28]. In another part of Africa, HPV 52 followed by HPV 35, HPV 16, HPV 53, HPV58, HPV 6 and HPV 51 were most prevalent in Mozambique thus, showing some difference with our findings presumably resulting from the range of HPV genotyped [29]. Still in this study, HPV 39 and HPV45 were most frequent in cases compared to controls. Findings of HPV 39 (17%) in high prevalence than that of a worldwide (3%) report further supported its role in cervical cancer as suggested by Wohlmeister *et al*. (2016) [25]. Our results suggest HPV 39, HPV 45 and HPV 66 as the emerging oncogenic HPV genotypes associated to increased cervical cancer incidence in Cameroon; as such we suggest their consideration during epidemiological studies and future vaccine development as effective preventive measures. Unfortunately, facilities for HPV genotyping and effective HPV vaccination programme are lacking in Cameroon [16].

The most prevalent multiple-infections observed in our population were HPV 16/66, HPV 16/18, and HPV 33/39 with HPV 16/18 and HPV 33/39 being associated with the risk of progression to precancerous lesions. There are disparities in the distribution of HPV multiple-infections according to studies with HPV 66/53, HPV 16/53 and HPV 16/58), HPV 6/35, HPV 6/66 [23] being reported in Angola and HPV 31/35 in Nigeria (2017) [30].

4.3. Prevalence of C. trachomatis Among Cases and Controls

C. trachomatis is one of the most prevalent sexually transmitted pathogens in the world. Most urogenital *C. trachomatis* infections are initially asymptomatic and may subsequently cause considerable long-term morbidity. In

most cases, *C. trachomatis* antibodies may be the only indication of active/past *C. trachomatis* involvement [31].

The prevalence of *C. trachomatis* varies greatly in different studies. The global prevalence for *C. trachomatis* infection was estimated to 3.8% [32]. Higher prevalence such as 26% was found in Nigeria, [33] and 26.4% in Argentina [34]. Our results showed the overall sero-prevalence of *C. trachomatis* antibodies in decreasing order to be 25.7% for IgG, 23.3% for IgA and 10.0% for IgM. In Rwanda, the IgG prevalence of *C. trachomatis* antibodies was 18.8% in sub-fertile and 18.3% fertile and IgA prevalence rates of 7.4% in sub-fertile and 4.1% in the fertile women lower than in our report [31]. The disproportionate burden of C. trachomatis infection in most studies might be attributed to unsafe socio-behavioural characteristics of the study population in addition to the choice of the diagnostic technique with the sero-diagnosis yielding higher prevalence due to cross-reactivity than the molecular technique which more accurate [33, 35].

4.4. C. trachomatis as an Independent Predictor of Cervical Cancer

We investigated and found high prevalence of *C. trachomatis* in controls than in cases which were in disagreement with Adegbesan-Omilabu *et al*. (2014) in Nigeria who reported higher prevalence of *C. trachomatis* infection in cases than in controls [36]. Equally, Kwaśniewska *et al*. (2007) reported a significantly higher frequency of *C. trachomatis* occurrence in tissue sections from patients with invasive cervical cancer compared to control without neoplastic lesions [37].

C. trachomatis infection as predictor of cervical cancer has also been subject of controversies. There are suggestions that *C. trachomatis* independently initiate cervical carcinogenesis through cellular transformation and tumour development thus suggesting that its detection in serum might be a possible method to predict the risk of cervical cancer in clinic [12]. In the contrary, another author reported that *C. trachomatis* was not significantly associated with cervical cancer in prevalent cases [38]. By sero-diagnosis we found that, *C. trachomatis* (IgA, IgG) was not significantly associated with the increased risk of cervical cancer among cases compared to controls. In the contrary, *C. trachomatis* IgM a marker of primo-infection was significantly associated with cervical cancer but its implementation as predictor of cervical cancer might not be practically feasible considering its short half-life time in the circulation. As such, the role of *C. trachomatis* as independent predictor of cervical cancer can accurately be evaluated using cell culture which requires further investigations.

4.5. C. trachomatis as Co-factor for Hr HPV Infection in Developing Cervical Cancer

There are suggestions that co-infections with HPV/*C. trachomatis*, HPV/gonorrhoea followed by HPV/syphilis and HPV/HIV [37] may increase a woman's risk of developing cervical neoplasia [11] since cellular damage caused by *C. trachomatis* infection promotes HPV acquisition or persistence. Such observations were supported by the findings of a significantly higher prevalence of *C. trachomatis* infection in women with positive HPV- DNA than controls as well as in infections with one or multiple viral types [34].

From our results, the overall prevalence of *C. trachomatis*/HPV co-infections was 17.7%, 16.3% and 6.7% for IgG, IgA and IgM respectively which was higher than 8.6% found in Uganda (2019) [39] and 5.6% in Iran (2018) [40]. The prevalence of HPV/*C. trachomatis* co-infections is low in most studies when assessed by DNA using PCR technique than by antibodies testing presumably because of the high specificity of DNA testing. The association of HPV/*C. trachomatis* and its role in developing cervical cancer remains a matter of debates. Some authors suggested that reactivation or persistence of HPV following the co-infection with *C. trachomatis* might support such association [13]. There are two suggestions supporting the association of HPV/*C. trachomatis* in developing cervical cancer. The first hypothesis was that *C. trachomatis* acts as a co-factor trough immune system modulation to favour HPV acquisition/persistence [41]. The second hypothesis being that, the association between these two agents is more related to a mutual potentiation than to the fact that they share a common route of transmission [34]. Adding to the findings of high prevalence of HPV in *C. trachomatis* infected women [42], another author in Egypt documented high prevalence of HPV/*C. trachomatis* co-infections in HPV-infected cancer patients than in HPV- infected control cases [22]. In the current study, the prevalence of *C. trachomatis* /HPV co-infection was not significantly high in cases than in controls for IgG presumably as the result of persisting IgG of past infection. These findings were in line with a similar case-control study where Smelove *et al*. (2016) found *C. trachomatis* not associated with increased risks of subsequent invasive adenocarcinoma and adenocarcinoma in situ [42]. Another study in Greece (2018) also reported that *C. trachomatis* detection was associated with the presence of lr-HPV and mixed lr- and hr-HPV but not with hr HPV alone [7].

Despite high prevalence of *C. trachomatis* IgG in cases than in controls which may be attributed to past infection, our data showed no significant association between *C. trachomatis* (IgG/IgM/IgA)/HPV co-infection and cervical cancer thus suggesting that *C. trachomatis* might not be a co-factor of hr HPV in initiating cervical cancer.

5. Conclusion

Cervical cancer is a chronic disease of women with high morbidity and mortality in Cameroon. Our study suggested a high prevalence hr HPV- DNA among cases than controls. HPV 16 and HPV 66 were the most frequent genotypes found among cases and controls. *C. trachomatis* antibodies were prevalent among cases and controls with the controls bearing the highest burden of the infection. Serologically, *C. trachomatis* infection was not significantly associated with

the risk of precancerous lesions among Cameroonian women. Assessment of the hr HPV- DNA/*C. trachomatis* co-infections revealed high prevalence of the co-infections among controls than cases but with no statistical significance in this study. From our data, we came to the conclusion that *C. trachomatis* infection assessed by serology was neither a predictor nor a co-factor for hr HPV in causing cervical cancer among Cameroonian women. However, further studies become necessary to establish their mutual actions in the development of cervical cancer using accurate diagnostic techniques like Chlamydia cell culture and PCR. Meanwhile, co-morbidity of *C. trachomatis* and hr HPV can be prevented by a sustainable national information/education programme on safe sex behaviour and practice.

Authors' Contributions

WB: The main investigator contributed in study design, sample collection, data management and statistical analysis and drafted the manuscript. DSN: Contributed in study design, suggested appropriate statistical analysis and revised the manuscript. ANJC: Contributed in study design and revision of the manuscript. TNN: Provided the logistic for patient recruitment, contributed in sample collection and revised the manuscript.

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

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