



## **Seroprevalence and Factors Associated with Herpes Simplex Virus Type 2 and *Chlamydia trachomatis* among Women Attending AIDS Care Unit at the Bonassama District Hospital**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author KMML designed the study, wrote the protocol, coordinated the study and wrote the first draft of the manuscript. Author DNRR handled experimental process, data collection and analyses. Author NNRA participate in study design and to draft and review the manuscript. Author AAJ managed coordinated the experimental process and review manuscript. Author WM managed recruitment and study site coordination. Author EE managed the literature searches and manuscript review. Author AZPH review the manuscript. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Background:** Infections with Herpes Simplex Virus Type-2 (HSV-2) and *Chlamydia trachomatis* is the most common cause of genital ulcer disease and cervicitis worldwide. There is little information on the prevalence of these sexually transmitted infections (STIs) in Cameroon. The aim of this

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study was to evaluate the seroprevalence and behavior factors associated with *Chlamydia trachomatis* and HSV-2 seropositivity among women attending AIDS care unit in the district hospital in Douala.

**Methodology:** One hundred and fifty seven consenting women (80 HIV-1 positive and 77 HIV-1 negative) were invited to participate in this study. A structured questionnaire was used for each woman to obtain informations about socio-demographic characteristics and sexual risk behavior. Peripheral venous blood sample were collected, labeled and sent to the laboratory, where the presence of IgG antibody against HSV-2 and *Chlamydia trachomatis* was detected, using ELISA test. Data management was carried out using the SPSS 16.0 statistical software.

**Results:** The mean age of women was 37.22±9.13 years (range, 20 to 74 years). Of the 157 women tested, 122 (77.7%) were HSV-2 positive and 60 (38.2%) were positive for chlamydial infection. The prevalence of HSV-2 and chlamydial infections in HIV positive patients were 73.7% and 43.7% respectively versus 81.8% and 32.5% in HIV negative individuals. Women with multiple infections were those belonging to the group aged 30-39 years old, single and having had more than three sexual partners during the six months preceding the study.

**Conclusion:** HIV status appears to be a factor in the acquisition of chlamydial infection in our population. These results suggest the need for extension and expansion of the current study in Cameroon.

**Keywords:** HSV2; *Chlamydia trachomatis*; AIDS; Cameroon.

## 1. INTRODUCTION

Sexually transmitted infections (STIs), including HIV/AIDS, constitute a public health problem worldwide especially in sub-Saharan Africa where 70% of HIV/AIDS infected persons live [1]. The burden of the disease is most felt in developing countries due to inadequate finance and poor logistics, to curtail the spread of the infection. In Africa, where sexual transmission is the predominant mode of spread for HIV, one of the most important risk factors for seropositivity is the history or the clinical evidence of STIs, particularly genital ulcerations. Many cofactors for HIV genital shedding has been suggested, including systemic factors, such as immunosuppression, hormonal contraceptive use, or micronutrient deficiencies, and local factors, such as the presence of other sexually transmitted infections and STIs [1,2].

Among STIs, *Herpes simplex virus type 2* (HSV-2) represent the leading cause of genital ulcer disease worldwide, and the main etiological agent of genital herpes [3,4]. HSV-2 infection represents a risk factor for the acquisition and transmission of other sexually transmitted infections (STIs) [5,6]. Epidemiological studies have shown that HSV-2 facilitates the acquisition and transmission of human immunodeficiency virus (HIV), indicates the relevance of the pathogenesis and epidemiology of HSV-2 [7-10]. Rotermann et al. in 2013 found from the 2009 to 2011 Canadian Health Measures Survey 13.6%

of Canadians (2.9 million) were tested positive for HSV-2, and another 0.7% (158,000), for chlamydia. HSV-2 affects higher percentages of women than men, and individuals aged 35 to 59 versus 15 to 34 [11]. On the same way, a household-based survey of the general population in Peru where 15 261 individuals from the general population and 4485 females sex workers (FSWs) agreed to participate in the survey. The overall prevalence of infection with HSV2, weighted for city size, was 13.5% in men, 13.6% in women, and 60.6% in FSWs. The prevalence of *C. trachomatis* infection was 4.2% in men, 6.5% in women, and 16.4% in FSWs [12]. In addition, infection with *Chlamydia trachomatis* is the most prevalent bacterial STI causing symptomatic and, more commonly, asymptomatic genital infection. In women, *Chlamydia trachomatis* is an important cause of cervicitis and salpingitis as well as pelvic inflammatory disease [13].

Despite its importance, data on the seroprevalence of HSV-2 and *Chlamydia trachomatis* infections in several populations are still limited, especially in some African countries. In Cameroon, the prevalence of sexually transmitted infections except HIV is poorly documented. This cross-sectional pilot study aims to evaluate the seroprevalence and behavior factors associated with *Chlamydia trachomatis* and HSV-2 seropositivity among women attending AIDS care unit in Douala, Cameroon.

## 2. MATERIALS AND METHODS

### 2.1 Study Population and Data Collection

The present pilot study was performed in Bonassama District Hospital of Douala (Cameroon). Two populations were tested for HSV-2 and *Chlamydia trachomatis* infections. Group I consisted of 80 HIV seropositive non pregnant women randomly selected in the HIV unit. Group II consisted of 77 HIV seronegative non pregnant women apparently healthy. Participants, staff conducted face-to-face interviewed directly according to a structured questionnaire. Information on the socio-demographic characteristics and sexual risk behaviors was obtained. Socio-demographic variables included age, education level, marital status and contraceptive use. Sexual risk behaviors history included age of first sexual intercourse with a male, number of sexual contacts in the past 6 months and condom use. Informed consent was obtained from all women and the study was approved by National Ethic Committee.

### 2.2 Sample Collection and Laboratory Procedures

For each patient, approximately 5 ml of blood was collected and transported to the laboratory. The blood was then centrifuged at 4000 rpm for 10 minutes, and the sera obtained were collected in ependorf tubes and stored at - 30°C for later analysis. Specific IgG antibodies present in the sera were detected and measured by ELISA according to the protocols of Puiria et al. for *Chlamydia trachomatis*; and Hampar et al. for HSV-2 [14,15]. All tests were performed according to manufacturer's instructions.

### 2.3 Statistical Analysis

The data collected were digitized using Microsoft Office Excel 2007 software, coded and exported to the Statistical Package for the Social Sciences software version 16.0 (SPSS Inc., Chicago, IL, USA), where appropriate statistical calculation were performed. Results are presented as frequency and means with standard errors. Chi square test was carried out to compare variables. Statistical significance was taken at  $P < 0.05$ .

## 3. RESULTS

One hundred and fifty seven women with baseline data, HSV-2 and *Chlamydia Trachomatis* serology were included in this study.

The mean±SD age of women was 37.22±9.13 years (range, 20 to 74 years). Of the 157 participants, 80/157 (50.9%) and 77/157 (49.1%) were HIV-1 positive and negative respectively. Table 1 summarizes the socio-demographic information as well as information on reproductive and sexual risk behavior of the study groups. Most of the participants were aged between 30-39 years and among them 40% were HIV -1 positive and 40.3% were HIV-1 negative. From participants with first sexual intercourse at an age greater than 16 years 65.0% were HIV-1 positive and 66.2% were HIV-1 negative. Regarding the number of sexual partners during the last 6 months preceding the study greater than or equal to four, we obtained 68.7% HIV-1 positive versus 54.5% HIV-1 negative. According to marital status most of the participants were single and among them 45.0% were HIV-1 positive and 50.6% were HIV-1 negative. In addition most of the participants stopped their studies in secondary school and among them 60.0% HIV-1 positive versus 54.5% HIV-1 negative. Regarding the use of contraceptive methods, many did not use contraception at the time of the study. Significant differences were observed between marital and HIV status ( $P = 0.001$ ); and between education level and HIV status ( $P = 0.002$ ). Of the 157 participants 122 (77.7%) and 60 (38.2%) were infected with HSV-2 and *Chlamydia trachomatis* respectively. The prevalence of *Chlamydia trachomatis* and HSV-2 infections among HIV-1 positive patients were 43.7% (35/80) and 73.7% (80/59) respectively.

Multiple infections with *Chlamydia trachomatis*, HSV-2 and HIV-1 was found in 22 (27.5%) participants (Table 2). Mean age±SD of women with coinfection was 38.60±8.79; 39.00±8.50 and 38.14±9.38 for HIV-1/*Chlamydia trachomatis*, HIV-1/HSV-2 and HIV/*Chlamydia trachomatis*/ HSV-2 respectively.

Coinfections with multiple infectious agents according to socio-demographic characteristic are presented in Table 3. The results show that most of coinfecting participants were aged between 30-39 years. Women who had their first sexual intercourse at an age greater than 16 years were likely coinfecting. Data indicated that 40/59 (67.8%); 22/35 (62.9%) and 15/22 (68.2%) were coinfecting with HSV-2/HIV-1; *Chlamydia trachomatis*/ HIV-1 and HSV-2/*Chlamydia trachomatis*/ HIV-1 respectively. Similarly, the majority of coinfections were detected in women who had a number of sexual contact  $\geq 4$ , during the past 6 months preceding the study with the

rate of 41/59 (69.5%), 27/35 (77.1%) and 17/22 (77.3%) respectively for HSV-2/HIV-1; *Chlamydia trachomatis*/ HIV-1 and HSV-2/ *Chlamydia trachomatis*/HIV-1. When we considered marital status, single women were more likely to be coinfecting than others socio demographic characteristic. We recorded results of 28/59 (47.5%) with HSV-2/HIV-1, 16/35 (45.7%) with *Chlamydia trachomatis*/HIV-1 and 10/22 (45.4%) with HSV-2/*Chlamydia trachomatis*/HIV-1. Low prevalence was observed in women with university level. Only 5 (8.5%) were coinfecting with HSV-2/HIV-1, 2 (5.7%) with *Chlamydia trachomatis*/HIV-1 and 2 (9.1%) with HSV-2/*Chlamydia trachomatis*/HIV-1 (Table 3).

#### 4. DISCUSSION

Infections with *Chlamydia trachomatis* and *herpes simplex virus type 2* are recognized as the major cause of cervicitis and genital ulcers in women. Their prevalence depending upon the population studied and the sensitivity of the laboratory methods used. The present study provides data on the seroprevalence and behavior factors associated with *Chlamydia trachomatis* and HSV-2 seropositivity. The results indicate that the IgG (HSV-2) was detected. This high HSV-2 seroprevalence is consistent with prevalences recorded in others studie [16,17]. In Nigeria, Agabi et al. reported a HSV-2 seroprevalence of 87.0% among patients attending the sexually transmitted infections

clinic in Jos [16]. In a community-based trial of mass STIs treatment in the Rakai district, Uganda, 43% of subjects with genital ulcers were identified with genital herpes by PCR analysis [18]. Similarly, we found a high prevalence of *Chlamydia trachomatis* infection in this study (38.2%) which makes it comparable with those obtained by Siemer et al. in Ghana and Joyee et al. in India. These studies obtained the prevalence of 39.3% and 30.8% respectively [19,20]. However the studies conducted by Ngandjio et al. in Yaounde and by Aseffa et al. in Ethiopia who had revealed a prevalence of 3.78% and 5.9% respectively [21,22]. This difference can be explained in terms of a difference not only between the populations studied but also between the detection techniques used in each study. In our population, HIV status did not appear to be a factor in acquiring HSV-2, since the prevalence of infection was higher among HIV-negative in HIV-positive participants. This result is inconsistent with that obtained by Mbopi-Kéou et al. and Weiss et al. who observed a significant difference in the prevalence of HSV-2 in HIV positive individuals compared to HIV negative [23-25]. Unlike the prevalence of HSV-2, the prevalence of chlamydial infection seems to be associated with the HIV status. It is recorded at higher levels in the group of positive HIV which is in keeping with results obtained by Mbu et al. among pregnant women [26].

**Table 1. Socio-demographic characteristics according to HIV status**

Socio-demographic factors	HIV-1 positive (n = 80)		HIV-1 negative (n = 77)		P-value
	N	(%)	N	(%)	
Age					0.113
20 - 29	12	(15.0)	22	(28.6)	
30 - 39	32	(40.0)	31	(40.3)	
40 - 49	25	(31.2)	19	(24.7)	
≥ 50	11	(13.5)	5	(6.5)	
Age at the first vaginal sex					0.871
≤ 16	28	(35.0)	26	(33.8)	
> 16	52	(65.0)	51	(66.2)	
Number of sexual contact in past 6 months					0.067
< 4	25	(31.3)	35	(45.5)	
≥ 4	55	(68.7)	42	(54.5)	
Marital status					0.001
Single	36	(45.0)	39	(50.6)	
Married	21	(26.3)	33	(42.8)	
Widow	23	(28.7)	5	(6.5)	
Education					0.002
Primary	26	(32.5)	14	(18.2)	
Secondary	48	(60.0)	42	(54.5)	
University	6	(7.5)	21	(27.3)	
Contraceptive use					0.198
Yes	18	(22.5)	11	(14.3)	
No	62	(77.5)	65	(84.4)	

**Table 2. Seroprevalence of infections according to HIV-1 status**

Infections	HIV-1 positive (N = 80)		HIV-1 negative (N = 77)	
	N	(%)	N	(%)
<b><i>Chlamydia trachomatis</i></b>				
Positive	35	(43.7)	25	(32.5)
Negative	45	(56.3)	52	(67.5)
<b>HSV-2</b>				
Positive	59	(73.7)	63	(81.8)
Negative	21	(26.3)	14	(18.2)
<b><i>Chlamydia trachomatis</i>-HSV-2</b>				
Positive	22	(27.5)	21	(27.3)
Negative	58	(72.5)	56	(72.7)

**Table 3. Socio-demographic characteristics among coinfecting women**

Socio-demographic factors	HSV-2/HIV-1 positive (N = 59)		<i>Chlamydia trachomatis</i> /HIV-1 positive (N = 35)		<i>Chlamydia trachomatis</i> /HSV-2/HIV-1 positive (N=22)	
	N	(%)	N	(%)	N	(%)
<b>Age</b>						
20 - 29	9	(15.2)	5	(14.3)	4	(18.2)
30 - 39	24	(40.7)	14	(40.0)	9	(40.9)
40 - 49	17	(28.8)	12	(34.3)	6	(27.3)
≥ 50	9	(15.2)	4	(11.4)	3	(13.6)
<b>Age at the first vaginal sex</b>						
≤ 16	19	(32.2)	13	(37.1)	7	(31.8)
> 16	40	(67.8)	22	(62.9)	15	(68.2)
<b>Number of sexual contact in past 6 months</b>						
< 4	18	(30.5)	8	(22.9)	5	(22.7)
≥ 4	41	(69.5)	27	(77.1)	17	(77.3)
<b>Marital status</b>						
Single	28	(47.5)	16	(45.7)	10	(45.4)
Married	16	(27.1)	8	(22.9)	6	(27.3)
Widow	15	(25.4)	11	(31.4)	6	(27.3)
<b>Education</b>						
Primary	20	(33.9)	11	(31.4)	8	(36.4)
Secondary	34	(57.6)	22	(62.9)	12	(54.5)
University	5	(8.5)	2	(5.7)	2	(9.1)
<b>Contraceptive use</b>						
Yes	14	(23.7)	10	(28.6)	7	(31.8)
No	45	(76.3)	25	(71.4)	15	(68.2)

A potential limitation of our study is that the positive cases was not detected using molecular techniques such as PCR. However, the sensitivity and specificity of detection methods that we used in this study has been already demonstrated [14,15]. In this study, the highest rate of coinfections was found in the 30-39 years age group. It is certainly the most sexually active group, thereby increasing their risk of contracting infections. These groups of individual often have many sexual partners than younger and older age group [16]. Also, it was observed a higher prevalence of coinfections among singles compared to non single participants. This may be attributed to the impact of alternative

determinants; such as frequenting several sexual partners [27,28]. This study has equally indicated that there was a higher rate of coinfection among women with more than three sex partners during the past six months preceding the study. Our results are in agreement with previous reports carried out in others sites indicating that people who have multiple sexual contacts are more risk of contracting several infections [11,12,16,29].

## 5. CONCLUSION

In this study, it was observed that HIV status appears to be a factor in the acquisition of Chlamydial infection. Multiple infections seem to

be more frequent among patients aged 30–39 years old, singles and having several sexual contacts. These results suggest the need for extension and expansion of the current study in order to evaluate the prevalence Chlamydial infection among HIV-positive and negative women in Cameroon.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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