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Research Article: *Chlamydia trachomatis* IgM and gG antibodies and associated risk factors of among positive and negative HIV women attending consultation at the Mbouo-Banjoun Presbyterian Hospital West Region of Cameroon

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Author Name:

Leonard Fonkeng Sama^{1*}, Michel Noubom², Romeo Joël Nguekam¹, Solange Dabou³, Thibau Flaurant Tchouangueu¹ and Christopher Bonglavnyuy Tume^{1,5}

1 Unit of Microbiology and Antimicrobial Substances, Department of Biochemistry, University of Dschang
2 Faculty of Medical and pharmaceutical Sciences, Department of Microbiology and Immunology, University of Dschang, Cameroon, University of Dschang

3 Laboratory of Biochemistry, Medicinal Plants, Food Security and Nutritional Sciences (LABPMAN), University of Dschang, Dschang, Cameroon, P.O. Box 67, Dschang, Cameroon

5 University of Bamenda, Faculty of Science, Department of Biochemistry, University of Bamenda, Cameroon

Corresponding Author:

Leonard Fonkeng Sama

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Leonard Fonkeng Sama
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Abstract

Background

Chlamydia trachomatis (CT) infection in the genitourinary tract is the most prevalent bacterial sexually transmitted disease (STD) worldwide. Genital chlamydial infection has a huge impact on sexual and reproductive health, and it is very common in developed and developing countries. This study aimed to determine the seroprevalence and risk factors for *C. trachomatis* infection in women seeking medical care in the locality of Mbouo-Banjoun West Region of Cameroon.

Methods: We conducted a cross-sectional hospital based study from November 2016 to June 2017 in which we recruited 204 consenting women aged 18 to 55 years. A questionnaire was administered to study participants and potential risk factors for *Chlamydia* exposure sought. Venous blood was collected and serum from each participant analysed for *C. trachomatis* infection as evidenced by positive *anti-C. trachomatis* IgG and IgM antibodies detected using the Sandwich Enzyme-Linked ImmunoSorbent Assay (ELISA) technique. The proportion of *anti-C. trachomatis* antibody was calculated and predictors of *C. trachomatis* infection analysed by univariate and multivariate regression. Epi-Info 7 was used for statistical analyses. A $p < 0.05$ was considered significant in all analyses.

Results: The seroprevalence of *anti-C. trachomatis* antibodies (IgM or IgG) was found to be 62.25% [127/204]. Among seropositive women, 37.15% [77/204] were seropositive for IgG antibodies while 47.54% [97/204] were seropositive for IgM antibodies and 23.04% [47/204] were seropositive for both IgM and IgG antibodies. Among the risk factors evaluated, marital status ($P = 0.03$) and knowledge of *Chlamydia* ($P = 0.001$) were observed to be an independent risk factor of *C. trachomatis* infection.

Conclusions: Our findings suggest recent *C. trachomatis* exposure is high in our study population, and may constitute a significant risk factor for, ocular and pulmonary infection in new born child, infertility to women. Education and screening of HIV-positive individuals and pregnant women for *C. trachomatis* infection may be important primary prevention strategies in this population.

Keywords: Chlamydia, Seroprevalence, IgG, IgM, Antibodies, women, Cameroon

Introduction:

Chlamydia trachomatis (CT) infection in the genitourinary tract is the most prevalent bacterial sexually transmitted disease (STD) worldwide [1]. Genital chlamydial infection has a huge impact on sexual and reproductive health, and it is very common in developed and developing countries [2–4]. Chlamydia is also known as the silent STI due to its lack of symptoms in infected individuals particularly females. Chlamydia infection can lead to serious sequelae such as pelvic inflammatory diseases (PID), tubal factor infertility, ectopic pregnancy and chronic pelvis pain in women [5]. In men Chlamydia leads to non-gonococcal urethritis (NGU), epididymitis and proctitis among others [5]. Understanding the epidemiology of chlamydia is therefore the first step towards its control. According to the WHO estimates, globally 92 million new cases of *C. trachomatis* infection occur each year and about two-thirds of these cases occur in the developing world, where diagnostic and treatment services are scarce [6]. Most epidemiological data on *Chlamydia trachomatis*

infection (CTI) are from industrialised nations and reliable data from the developing nation's area where the disease burden is concentrated are rare. However, it is important to document laboratory-confirmed incidence and prevalence of CTI from the developing world as well. The available African data obtained from the studies conducted in Kenya, Zimbabwe, Nigeria and South Africa, have shown a wide variation in CT prevalence with infection rates ranging from 6% to 56% depending on the population sampled [7–10]. Few data are available regarding CTI epidemiology in Cameroon. Furthermore, chlamydial infection have been shown to be associated with 3-6-fold increase in the transmission of HIV infection and are attributed to be a risk factor for the development of cervical carcinoma. Also, infected patients, unaware of their infection, may serve as a reservoir of infection to their partners [11,12]. The control of these infections represents a unique opportunity to improve reproductive health of women living with HIV [13]. HIV-infected individuals affected by an STI have increased viral load in genital secretions, [14, 15] thereby increasing considerably their potential of infectiousness and transmission. The aim of the current study was to determine the prevalence and risk factors associated with CT among HIV-infected and HIV non-infected women attending consultation at the Mbouo-Banjoun Presbyterian Hospital.

Material and methods

Study design

A cross-sectional study was carried out from November 2016 to June 2017 in Mbouo-Banjoun Presbyterian Hospital, West Region of Cameroon.

Study site and population

The Mbouo-Banjoun Presbyterian Hospital, is a private health care institution run by the Sisters of the Presbyterian Congregation whose convent is located near the hospital. The hospital was created in 1928. This hospital has an HIV treatment centre, and majority of services rendered are in the domains of gynaecology, general and orthopaedics surgery. The hospital is situated at about 2 miles away from Bafoussam the regional capital of the West Region of Cameroon. About 75 % of the women in this locality depend on agriculture and trading for their livelihoods. Participants in this study were volunteer sexually active women (HIV-infected or uninfected) attending antenatal or medical check-ups at the hospital and who duly provided consent by signing or placing their thumbprint on the consent form and those who were diagnosed as being infected received standard treatment in accordance with the Sexually Transmitted Diseases Control Guidelines of the Ministry of Health.

Questionnaire administration and sample collection

After agreeing to participate, the women were interviewed by the research team. Each participant to this study was provided with a structured questionnaire. Those who could not read were assisted by the laboratory technician or antenatal care nurse. The questionnaires contained simple closed ended questions regarding socio-demographic characteristics (age, schooling, marital status, and social activity), sexual characteristics (protected or no, number of partner).

Five millilitres of venous blood were collected into two tubes pre-labelled with an anonymised patient codes. The blood from the EDTA containing tubes was immediately tested for the presence of HIV antibodies using Determine® (*Alere Determine™ HIV-1/2*) test strips according to manufacturer's instructions. The second blood sample was allowed to clot

completely before centrifugation at 3000 rpm for 15 min at 4°C to obtain serum. Aliquoted serum samples were then stored at –25°C. These frozen sera were later tested for the presence of *Chlamydia trachomatis* (CT) antibodies.

Serological test for *Chlamydia trachomatis* antibodies

The presence of *Chlamydia trachomatis* antibodies in the participants' sera was determined using an indirect Enzyme-Linked Immunosorbent Assay (ELISA). This was done using the NovaLisa *Chlamydia trachomatis* IgM and IgG ELISA Kits CHLG0070 (NovaTec Immunodiagnostica, GmbH, Dietzenbach, Germany). The *Chlamydia trachomatis* IgM and IgG ELISA Kits had a specificity of 100 % and a sensitivity of 100 %. Calibrator and controls were run with each test assay. The procedure outlined in the kit's brochure was strictly adhered to. The micro titre wells were pre-coated with *C. trachomatis* antigen. About 10µl of participant's serum diluted at 1:100 with IgM and IgG sample diluent was added to each well, incubated for one hour at 37°C and later washed with a washing solution. After the addition of conjugate, further incubation for 30 minutes was done at room temperature. At the end of incubation, plates were again washed, TMB substrate was added and incubated for 15 mins at room temperature in the dark. Finally, stop solution was added to all the wells and Optical Density (OD) readings were taken with the wavelength of 450 nm using iMark Microplate Reader, Bio-Rad, Germany.

Cut-off value calculation:

- Cut-off value was considered as the mean absorbance value of cut-off controls kept in duplicate wells.
 - Samples were considered positive if O.D readings were 10% higher than the cut-off values and those with 10% less than cut-off negative. Those in the middle Grey zone were repeated.
- Calculation in Nova Tec Units Antibody index = (Sample O.D/ Cut-off O.D) x 10.

The samples with O.D values above 11 NTU (NovaTec units) were considered as positive and those below 9 NTU were taken as negative. Borderline samples with 9-11 NTU were repeated in triplicate.

Data management and statistical analysis

Data were entered into Microsoft Excel program and then transferred to Epi Info 7 statistical program. The *Chlamydia trachomatis* prevalence was calculated as the proportion of serologically positive *anti-C.trachomatis* samples among all samples tested at 95 % confidence interval (CI). A multivariate logistic regression model was used to investigate the association between the potential risk factors for *C.trachomatis* as defined by seropositivity for *C.trachomatis* antibodies of any kind. Variables used in the multiple regression were selected through step-wise backward elimination using a 20 % cut-off. The strength of associations was measured using the odds ratio (OR) at 95 % CI. The Chi-square test was used for group comparisons. Statistical significance was set at 5% and all the associations that showed a $p < 0.05$ were considered significant.

Results

A total of 357 eligible women that were approached, 204 women aged 18 to 55 years provided consent for this study. The mean age of participating women was 30 ± 8.12 years. For the women that provide consent to participate to the study, 46.08% were aged between 26 and 35 [94/204], 32.84% [67/204] between 18 to 25 and 21.08% [43/204] above 36 years. A total of 45.59%

(93/204) of the women were single, 14.22% [29/204], 64.71% [132/204] and 21.08% [43/204] had primary, secondary and higher level of education whereas 59.31% [121/204] has no Knowledge of Chlamydia infection.

Amongst all the participants, 32.84% [67/204] had more than one sex partner, 26.96% [55/204] has protected and unprotected sexual intercourse, 67.16% [137/204] are using public toilet and 44.12% [90/204] where HIV positive (table 1).

Prevalence of anti-*C. Trachomatis* antibodies

The overall seroprevalance of *anti-C.trachomatis* antibodies among the 204 women in this study was 62.25% [127/204]. Among this seropositive women, 37.15% [77/204] were seropositive for IgG antibodies while 47.54% [97/204] were seropositive for IgM antibodies and 23.04% [47/204] where seropositive for both IgM and IgG antibodies. From the overall seropositive patients to *anti-C.trachomatis* antibodies, significant difference ($P=0.001$) were observe between HIV positive and HIV negative with 63.33% [57/90] HIV positive women been positive for *anti-C.trachomatis* antibodies and 67.31% [70/104] HIV-negative women been positive for *anti-C.trachomatis* antibodies. Significant difference ($P=0.0000$) was observe between HIV-positive and HIV-negative women seropositive for *C.trachomatis* IgG antibodies with 36.67% [33/90] HIV-positive women been seropositive for *C.trachomatis* IgG antibodies and 42.31% [44/104] HIV-negative women been positive for IgG antibodies. However, between HIV-positive and HIV-negative significant different ($P=0.0025$) was observe for *C.trachomatis* IgM antibodies with 55.56% [50/90] of HIV-positive women been positive for *C.trachomatis* IgM antibodies and 45.19% [47/104] been positive for *C.trachomatis* IgM antibodies. Significance difference ($P=0.0070$) was also observe between HIV positive been positive to both *C.trachomatis* IgM and IgG antibodies with 28.89% [26/90] HIV-positive women been positive to both *C.trachomatis* IgM and IgG antibodies and 20.19% [21/104] HIV-negative been positive to both *C.trachomatis* IgM and IgG antibodies (table 2).

Table 3 shows the prevalence of *C. trachomatis* in relation to socio-demographic characteristic. From this table, we observe according to age category, HIV status, IgM and IgG antibodies that, the highest prevalence of 30.00% IgM occurred for patient age between 25 – 35 years ($X^2=0.9195$; $P=0.6314$) whereas for IgG, the highest of 27.19% occurred for HIV negative age between 18-25 years 26 – 35 years ($X^2=3.3465$; $2P=0.1794$). The highest prevalence of 44.44% IgM occurred for single HIV positive women ($X^2=3.0606$; $P=0.3824$) whereas for IgG, the higher prevalence's of 38.60% single occurred for HIV negative ($X^2=6.0913$; $P=0.1073$).

According to the Knowledge on *Chlamydia*, the higher prevalence of 34.44% IgM occurred for HIV positive women whom have no knowledge on *Chlamydia* infection ($X^2=7.5824$; $P=0.0058$) whereas the higher prevalence of 21.05% IgG occurred for HIV negative whom known about *Chlamydia* infection ($X^2=4.3380$; $P=0.0037$). The higher prevalence of 41.23% IgM occurred for HIV negative women using common toilet and higher prevalence of 38.60% IgG occurred in HIV negative women using common toilet ($X^2=1.5830$, $P=0.4532$). The higher prevalence of 43.33% IgM occurred for HIV negative women having more than one sexual partner ($X^2=0.3862$; $P=0.5343$) whereas the higher prevalence of 38.60% IgG also occurred among HIV negative patient but having one sexual partner ($X^2=0.0808$; $P=0.7762$).

Looking for the Type of sexual intercourse, the higher prevalence

of 33.33% IgM occurred for HIV positive women having mixed sexual intercourse (protected and non-protected) ($X^2=1.9229$; $P=0.3823$) whereas for IgG, the higher prevalence of 20.00% occurred for HIV positive women also having mixed sexual intercourse ($X^2=0.1905$; 0.6625). According to social activity, the higher prevalence of 27.19% IgM occurred among HIV negative student ($X^2=8.1787$; $P=0.0425$), also the higher prevalence of 27.19% IgG occurred among HIV negative student ($X^2=8.8541$; $P=0.0313$).

Risk factors for *C.trachomatis* seropositivity

A simple logistic regression analysis (table 4) with suspected variables indicated that no suspected risk factor was significantly associated with *C.trachomatis* IgG seropositivity antibodies type whereas Marital status ($P=0.03$), Knowledge of Chlamydia ($P=0.001$) and HIV status ($P=0.04$) were predictors of *C. trachomatis* seropositivity IgM antibody type. However, results of multiple logistic regression analysis of selected variables as shown in Table 5 showed that only the Knowledge of Chlamydia was an independent predictor of IgM ($P=0.001$) and IgG ($P=0.04$) *C. trachomatis* seropositivity whereas Marital status ($P=0.03$) was independent predictor for IgM *C. trachomatis* seropositivity antibodies. No significant association was found between *C. trachomatis* seropositivity and HIV status [IgM ($P=0.2$), IgG ($P=0.8$)].

Discussion

It has been prove that Chlamydia is an accelerator of HIV transmission and acquisition [16]. In most women and about half of men chlamydia do not experience symptoms [17]. Since symptoms may not be present, the only way to know if a person who may be at risk, is infected with chlamydia is to be tested. In the present study, we sought to investigate Chlamydia infection, as evidenced by *anti-Chlamydia* antibodies in the serum of HIV positive and HIV negative women in a rural locality in Cameroon. The overall prevalence of 62.25% of *anti-C. Trachomatis* found in this study is higher compared to those described in those found among sex workers or among prostitutes in Cameroon [18-19] but also higher than prevalence of chlamydia in Cameroonian University Context: case of the University of Dschang, Western Region of Cameroon where the prevalence of 2% was obtain [20], to 32.1 % obtained among HIV positive and HIV negative patients in the Vhembe District of South Africa [21] and to the 1.8% prevalence obtain in São Paulo, Brasilia in the study on Prevalence and factors associated with Chlamydia trachomatis infection among women with HIV in São Paulo [22]. This high prevalence could be attributed to chlamydia being asymptomatic in most cases particularly in females whom constitute our study population. The prevalence of *C. trachomatis* was significantly different ($P=0.001$) between HIV positive (63.33%) and HIV negative (67.31%). Similar results were obtained in a study carry in the Mopani District South Africa on prevalence of chlamydia and gonorrhoea among HIV infected women in rural Mopani District [23], but also in the study carry in Vhembe District of South Africa [21] where the prevalence of *C.trachomatis* was found to be high among HIV-negative compare to HIV-positive. This could mean that the prevalence of *chlamydia* is not always associated with the reported HIV status. The use of ARV also appeared to reduce the risks and sequelae of *chlamydia* infection [21].

The presence of IgG antibodies to trachomatis in the sera of the HIV positive and HIV-negative women as observed in this may be indicative of past *chlamydia* infection. The detection rate in

HIV positive women (36.67%) is lower than 54.2% in India [24,25], and the 42.31% detection rate in HIV negative women was higher than 15 - 40% reported in Nigeria[26].

The lower prevalence (36.67%) of *Chlamydia trachomatis* IgG antibodies among HIV positive women is significantly lower ($P=0.000$) than that of the HIV negative women (42.31%). Which suggests that it is unlikely that previous exposure to HIV induce a higher prevalence of Chlamydia trachomatis infection, but rather *Chlamydia trachomatis* infection facilitate the transmission of HIV which is in support with [27,28,29] who reported that *Chlamydia trachomatis* infection increases the risk of HIV transmission and acquisition.

The presence of IgM antibodies to trachomatis in the sera of the HIV-positive and HIV-negative women as observed in this may be indicative of new chlamydia infection. The detection rate in HIV positive women (55.56%) is higher than 3% obtain by Padmavathy Kesavaram et al. [30] and the 45.19% detection rate in HIV negative women was higher than 1.5% reported among pregnant women in Makkah [31]. The higher prevalence (55.56%) of Chlamydia trachomatis IgM antibodies among HIV positive women is significantly higher ($P=0.0025$) than that of the HIV negative women (45.19%). Which suggests that it is likely that previous exposure to HIV induce a higher prevalence of Chlamydia trachomatis infection.

In the present study, the prevalence was found to be high among people aged between 25–35 years (35.29 %). Similar results have been reported by previous studies in the African continent. For example, a study carried in Vhembe District of South Africa found a prevalence of 35.6% reported in people of between 26–45 years in a study on the prevalence of chlamydia among HIV positive and HIV negative patients in the Vhembe District as detected by real time PCR from urine samples [21]. A previous study carry in South Eastern Nigeria found a high prevalence of 43.9 % was reported in people of between 26–30 years in a study on the prevalence of chlamydia in patients attending gynaecological clinics in South Eastern Nigeria [32]. A high prevalence of 52.8 % was also reported in female miners particularly those who were aged between 46–50 years old [33]. In general no significant difference was observe between socio-demographical characteristics and the prevalence of *C.trachomatis* except with level of education where significant different was observe regard to IgG antibody ($X^2 = 8.6474$, $P = 0.0133$) with high prevalence found among patient of secondary level of education. Similar results have been reported in New Caledonia [34], in Vhembe District of South Africa[21] although the difference was not significant with other educational levels. Significant difference was also reported with knowledge of Chlamydia with both IgM($X^2 = 7.5824$, $P = 0.0058$) and IgG($X^2 = 4.3380$, $P = 0.0037$) antibodies. Similar results have been reported in Scotland, UK in the study of Knowledge of Chlamydia trachomatis among men and women approached to participate in community-based screening, Scotland, UK. Significant difference was reported in type of toilet regard to IgM antibody ($X^2 = 6.4594$, $P = 0.0396$) with high prevalence among patients using common toilet. Which suggests that it is unlikely that chlamydia could not be catch from toilet seats.

Socio-economic status of study participants has often been cited as risk factor for the occurrence of several diseases. In our study, we found that the prevalence of *C.trachomatis* was higher among student compare to house wife and civil servant with significant difference. This correlates with a study that

was conducted in Vhembe District of South Africa[21]. Similar results have been reported by previous studies in the United States by Klovstad et al. [27], where the percentage in people who were unemployed was higher compared to those who were employed also the difference was not significant.

The marital status and Knowledge of Chlamydia were significantly a risk factor associated with *C.trachomatis* in the univariate and multivariate analysis analyses (tables 4 and 5). In addition, HIV status was observed to be significantly associated with *C.trachomatis* in the univariate analysis, although it was not significant in the multivariable analysis Table 4. Similar results have been observed in a review article publish by Kucinskiene et al. [41] showed a significant association between *C.trachomatis* infection and Knowledge of Chlamydia.

Our study however should be interpreted with some caution. Our sample size was small and conclusions from the present study must be measured. It is likely that we may obtain different results with increased sample size.

Conclusions

C.trachomatis infection appears to be a public health concern in Mbouo-Banjoun with a global antibody sero-prevalence of 62.25% among HIV infected and HIV non-infected women attending consultation in our study area. In this study, recent infections were found in 47.54% of the study population while up to 37.15% of patients were reactivating and significant difference ($P = 0.001$) was observe between HIV positive and HIV negative. Marital status, Knowledge of Chlamydia and HIV status were observed to be risk factors for *C.trachomatis* infection among HIV-positive and HIV-negative women attending consultation at the Mbouo-Bandjoun Presbyterian Hospital West Region, Cameroon

Data Availability

Data used to support the findings of this study are included within the article.

Competing interests

The authors declare that they have no competing interests.

Ethical consideration

Ethical clearance for this study was obtained from the Ethics Review and Consultancy Committee of the Cameroon Bioethics Initiative (CAMBIN) under the reference number CBI/370/ERCC/CAMBIN of August 18th, 2015.

Authors' contributions

LFS, MN and CBT conceived the study. RJN, LFS and TFT carried out sample and data collection. EJN, LFS and SD participated in analysis of the samples, data management and statistics. SD and LFS drafted the manuscript. All authors reviewed the manuscript the final version prior to submission. All authors read and approved the final manuscript.

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Table 1: General characteristics of study participants

Characteristic	Number (%)
Age category	
18-25	67 (32.84%)
26-35	94 (46.08%)
>36	43 (21.08%)
Marital status	
Married	88 (43.14%)
Divorced	14 (6.86%)
Single	93 (45.59%)
Widow	0 (0, 00%)
Cohabiting	9 (4.41%)
Level of education	
None	0 (0.00%)
Primary	29 (14.22%)
Secondary	132 (64.71%)
Higher	43 (21.08%)
Knowledge of Chlamydia	
Yes	83 (40.69%)
No	121 (59.31%)
Type of toilet	
Personal	55 (26.96%)
Public	137 (67.16%)
Commune	12 (5.88%)
Number of sexual partner	
1	137 (67.16%)
More than 1	67 (32.84%)
Type of sexual intercourse	
Protected	42 (20.59%)
Non protected	55 (26.96%)
mixed	107 (52.45%)
Social activity	
Trader	50 (24.51%)
Civil servant	23 (11.27%)
House wife	66 (32.35%)
Student	65 (31.86%)
HIV positive	
Yes	90 (44.12%)
NO	114 (55.88%)

Table 2 : HIV status and anti-Chlamydia trachomatis IgM and IgG as a function of sociodemographic data.

Characteristic	HIV positive	HIV negative	Total	P-Value
IgG+	33(36.67%)	44(42.31%)	77(37.15%)	0.0000
IgM+	50(55.56%)	47(45.19%)	97(47.54%)	0.0025
IgG+ and IgM+	26(28.89%)	21(20.19%)	47(23.04%)	0.0070
IgG+ or IgM+	57(63.33%)	70(67.31%)	127(62.25%)	0.6750

Table 3 : Seroprevalence of antibodies to *C. trachomatis* according to HIV status

Characteristic	Actual positive IgM (Frequency%) in HIV⁻ women	Actual positive IgM (Frequency%) among HIV⁺ women	Actual positive IgM (Frequency %)	Actual positive IgG (Frequency%) in women HIV⁻	Actual positive IgG (Frequency%) in women HIV⁺	Actual positive IgG (Frequency%)
<i>Age category</i>			(X² =0.9195, P =0.6314)			(X² = 3.3465, P = 0.1794)
[18-25]	32 (28.07%)	0 (0.00%)		31 (27.19%)	0 (0.00%)	
[26-35]	15 (13.16)	27 (30.00%)		13 (11.40%)	17 (18.89%)	
>36	0 (0.00%)	23 (25.56%)		0 (0.00%)	16 (17.78%)	
<i>Marital status</i>			(X² =3.0606, P =0.3824)			(X² =6.0913, P = 0,1073)
Married	8 (7.02%)	40 (44.44%)		4 (3.51%)	26 (28.89%)	
Divorced	0 (0.00%)	6 (6.67%)		0 (0.00%)	2 (2.22%)	
Single	39 (34.21%)	0 (0.00%)		44 (38.60%)	0 (0.00%)	
Widow	0 (0.00%)	0 (0.00%)		0 (0.00%)	0 (0.00%)	
Cohabiting	0 (0.00%)	9 (10.00%)		0 (0.00%)	9 (10.00%)	
<i>Level of education</i>			(X² =2.2712 , P =0.3212)			(X² =8.6474, P =0.0133)
None	0 (0.00%)	0 (0.00%)		0 (0.00%)	0 (0.00%)	
Primary	11 (9.65%)	0 (0.00%)		17 (14.91%)	0 (0.00%)	
Secondary	16 (14.04%)	46 (51.11%)		13 (11.40%)	28 (31.11%)	
Higher	20 (17.54%)	4 (4.44%)		14 (12.28%)	5 (5.56%)	
<i>Knowledge of Chlamydia</i>			(X² =7.5824 P =0.0058)			(X² =4.3380 , P =0.0037)
Yes	29 (25.44%)	19 (21.11%)		24 (21.05%)	15 (16.67%)	
No	18 (15.79%)	31 (34.44%)		20 (17.54%)	18 (20.00%)	
<i>Type of toilet</i>			(X² =6.4594 , P = 0.0396)			(X² =1.5830 , P =0.4532)
Personal	0 (0.00%)	34 (37.78%)		0 (0.00%)	23 (25.56%)	
Common	47 (41.23%)	10 (11.11%)		44 (38.60%)	4 (4.44%)	
Public	0 (0.00%)	6 (6.67%)		0 (0.00%)	6 (6.67%)	
<i>Number of sexual partner</i>			(X² =0.3862 , P =0.5343)			(X² =0.0808 , P =0.7762)
1	47 (41.23%)	11 (12.22%)		44 (38.60%)	9 (10.00%)	
More than 1	0 (0.00%)	39 (43.33%)		0 (0.00%)	24 (26.67%)	
<i>Type of sexual intercourse</i>			(X² =1.9229, P = 0.3823)			(X² =0.1905, P =0.6625)
Protected	10 (8.77%)	6 (6.67%)		8 (7.02%)	4 (4.44%)	
Non-protected	13 (11.40%)	14 (15.56%)		15 (13.16%)	11 (12.22%)	
mixed	24 (21.05%)	30 (33.33%)		21 (18.42%)	18 (20.00%)	
<i>Social activity</i>			(X² =8.1787 , P =0.0425)			(X² = 8.8541, P =0.0313)
Trader	0 (0.00%)	26 (28.89%)		0 (0.00%)	19 (21.11%)	
Civil servant	0 (0.00%)	16 (17.78%)		0 (0.00%)	11 (12.22%)	
House wife	16 (14.04%)	8 (8.89%)		13 (11.40%)	3 (3.33%)	
Student	31 (27.19%)	0 (0.00%)		31 (27.19%)	0 (0.00%)	

Table 4: Simple logistic regression with suspected variables

Variable	No of subjects tested N(%)	IgM		IgG	
		Odd ratio (95 % CI)	p value	Odd ratio (95 % CI)	p value
Age category					
18-35	161	1.122 (0.46-2.71)	0.7	0.783 (0.33-1.85)	0.5
>36	43				
Marital status					
Married	116	0.448 (0.20-1.38)	0.03	1.365 (0.63-2.95)	0.4
Unmarried	88				
Knowledge of Chlamydia					
Yes	121	0.295 (0.14-0.59)	0.001	0.544 (0.28-1.04)	0.06
No	83				
Type of toilet					
Personal	55	0.553 (0.22-1.38)	0.2	0.572 (0.23-1.04)	0.2
Public	149				
Type of sexual intercourse					
Protected	42	1.385 (0.66-2.88)	0.3	1.654 (0.77-3.57)	0.1
Non-protected	162				
HIV status					
Negative		1.78 (1.01-3.15)	0.04	0.92 (0.52-1.63)	0.7
Positive					

Table 5: Multiple logistic regression of selected variables (to be selected by the statistitian)

Variable	No of subjects tested N(%)	IgM		IgG	
		Odd ratio (95 % CI)	p value	Odd ratio (95 % CI)	p value
Marital status					
Married	116	0.441 (0.20-0.96)	0.03	1.03 (0.52-2.10)	0.9
Unmarried	88				
Knowledge of Chlamydia					
Yes	121	0.3 (0.15-0.60)	0.001	0.518 (0.27-0.98)	0.04
No	83				
HIV status					
Negative		1.48 (0.74-2.96)	0.2	1.06 (0.52-2.16)	0.8
Positive					

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