Contribution of the ELISA and PCR tests in the diagnosis of *Chlamydia trachomatis* infection in fertile and infertile women in resource-limited settings: a case-control study

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ABSTRACT

Background and Purpose: *Chlamydia trachomatis* (CT) is the most common infectious cause of infertility. It can be diagnosed using several techniques. In resource-limited settings, most tests are performed using ELISA and this leads to an excessive intake of antibiotics. The aim was to compare the frequency of CT infection in infertile and fertile women submitted to ELISA and PCR tests, and to determine the sensitivity and specificity of ELISA for current infection.

Methods: We carried out a case-control study over a period of seven months. Patients with infertility (cases) and with confirmed pregnancy (controls) were included in the study after giving their informed consent. Data collected included sociodemographic characteristics, past medical history, clinical examination, ELISA test (IgG, and IgM) and PCR test for *Chlamydia*. Chi-square, Fisher's and Student's tests were used to explore associations between variables.

Results: Two hundred patients, 100 fertile and 100 infertile, were enrolled. The ELISA test was positive in 34.3% of the infertile women as compared with 12.5% of the fertile women (OR:3,652 [1,135-11,749]). The PCR test was positive in 8% of the cases and 8% of the controls. On multivariate analysis, low monthly income, cervical motion tenderness and adnexal mass were found to be independent predictors of infertility. Considering PCR as the reference test, ELISA showed lower sensitivity in the diagnosis of CT. However, IgM had better specificity. The association of a positive IgM test with clinical signs predicted the diagnosis of current CT infection in 81.3% of cases.

Conclusion: The proportion of CT seropositivity (IgG and IgM) was higher in infertile than fertile women. No difference in PCR positivity was found between the two groups. It is important to raise awareness, among women, of the influence of this infection on their fertility and to carry out PCR tests to identify patients to whom treatment should be given.

KEYWORDS

Infertility, Chlamydia trachomatis, PCR, ELISA.

Introduction

Infertility is a public health problem with a two to three times higher frequency in developing countries [1]. In Cameroon, about 20 to 30% of couples suffer infertility and the frequency varies from region to region [2]. In our setting, infections, including sexually transmitted infections, are the most common causes of infertility.

Chlamydia trachomatis (CT) is the most common bacterial cause of sexually transmitted infections [3]. Most affected persons are asymptomatic, and thus provide an ongoing reservoir for infection.

According to the WHO, there are 101 million *Chlamydia* infections worldwide each year [4]. *Chlamydia trachomatis* is a highly contagious bacterium that is responsible for 60 to 70% of cases of pelvic inflammatory disease [5]. This infection can lead to tubal causes of infertility in women. Kamel *et al*. and Casari *et al*. found the rate of CT infection to be three times higher in infertile than in fertile women [6,7].

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1. Many diagnostic methods for *Chlamydia* infection exist, but currently the most effective is based on the identification of DNA of CT by polymerase chain reaction (PCR). This method is not readily available in developing countries, hence the use of other methods (anti-*chlamydia* antibody test or ELISA method, direct or indirect immunofluorescence), each of which has certain limits. Thus, the diagnosis of CT infection in infertile women varies according to the efficiency of the method used. Mahmoud *et al*. found that direct and indirect immunofluorescence test showed *Chlamydia* infection to be four to five times higher in infertile women than in fertile ones, but with PCR, it was three times higher [8]. Another study, done in Iran in 2017, found no significant difference between fertile and infertile groups for CT infection using PCR and IgM antibody tests [9]. A study done in Rwanda revealed that the PCR prevalence of CT infection was relatively low and did not differ significantly between sub-fertile and fertile women (3.3 and 3.8% respectively). Similarly, no significant differences in overall prevalence rates of anti-*chlamydia* IgG and IgA were observed between these groups [10].

In Cameroon, a few studies have examined the association between CT and infertility. In 1986, Sende *et al*., using the ELISA method, found prevalence rates of 8.6 and 11.4% respectively among fertile and infertile Cameroonian women [11]. A retrospective study performed in 2003 using ELISA found that 66.2% of infertile women had anti-*chlamydia* antibodies [12]. The ELISA test is the most frequently used in Cameroon due to its low cost. After a positive test followed by treatment, patients like to undergo follow-up tests, which are very often positive as antibodies persist after treatment. Furthermore, some patients consult different medical doctors or nurses, and when each medical practitioner needlessly prescribes antibiotics, over time this may negatively impact on patients' health.

In view of all these considerations, we felt it important first to update the data on the frequency of CT infection in fertile and infertile women, and second to compare the contribution of ELISA and PCR tests in the diagnosis of current infection.

Methods

This was a case-control study carried out over a period of 7 months at the Douala General Hospital, Douala Gyneco-Obstetrics and Pediatric Hospital, Bel Air Plus Clinic and Louis Pasteur Labo. The study population consisted of infertile women, defined as women unable to conceive despite regular unprotected sexual intercourse over one year (cases), and fertile women, that is women with pregnancy by confirmed ultrasound (controls). All patients were recruited during outpatient consultations. The cases and controls were matched for age ±2 years. None of the patients had received antibiotic treatment during the 4 weeks prior to the visit.

Data collection

All participants were administered a structured questionnaire which gathered information on sociodemographic characteristics, previous gynecological problems, and past medical and surgical history.

The questionnaire also included detailed questions on genital tract symptoms such as vaginal discharge, pruritus and lower abdominal pain. Thereafter, the women were examined and variables from the physical examination were noted (vaginal discharge, state of the cervix, cervical motion tenderness or Chandelier sign, and presence of any adnexal mass). A single venous blood sample for ELISA test and a swab of upper vaginal secretions for CT PCR testing were collected from all participants.

Laboratory procedures

ELISA

After collection of 4 ml of venous blood in a dry tube, the samples were centrifuged (3000 revolutions for 5 min using a Hanshin Medical Co. LTD centrifuge). The serum obtained was aliquoted and stored at -20°C.

When carrying out the test, these samples and the reagents (DRG® *Chlamydia trachomatis* IgM and IgG, EIA-3463) were brought back to room temperature for 15 to 20 minutes, then we proceeded with the dilution phase. Then we started the enzymatic reaction. After stopping the enzymatic reaction, we read the optical density using the ELISA reader (Stat Fax AW) at 450 ± 10 nm.

The cutoff values established by the manufacturer were used to interpret the anti-*Chlamydia* IgM and IgG antibody serum levels. An Ig titer < 9DU/ml was considered a negative result, 9-10 DU/ml a borderline range result (equivocal result), and ≥ 11DU/ml a positive result.

PCR

We proceeded with manual extraction of CT DNA. Then, the amplification phase was carried out in a Hain Lifescience FluoroCycler 96R Real Time PCR System®, and the results were read using FluoroSoftware. DNA was detected when at least one CT-specific valid peak was observed.

Statistical analysis

The data collected were analyzed using SPSS (Statistical Package for Social Sciences) software version 23.0. After some descriptive statistics, we ran Chi-square and Fisher's exact tests to assess associations between the qualitative variables and Student's test for quantitative variables. A p-value < 0.05 was considered significant. For comparison of ELISA and PCR test, two-by-two tables were used to calculate sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

Ethical approval

Ethical clearance (ref. number 1687 CEI-UDo/05/2019/T) was obtained from the institutional (University of Douala) ethics committee for research on human health. Permission was granted by the directors of the Douala General Hospital, Douala Gyneco-Obstetrics and Pediatric Hospital, Bel Air Plus Clinic and Louis Pasteur Labo. Informed consent was obtained from the participants.

Results

We enrolled a total of 200 women (100 fertile and 100 infertile). Their mean age was 33.68 ± 5.35 years, with a median age of 34 years. The most represented age group was 35–40 years. The majority of the women were married. A monthly income below 50,000 Central African currency XAF was associated with a threefold increased risk of infertility (Table I). Mean parity was significantly lower in the infertile patients (0.67 ± 0.990 vs 1.70 ± 1.425, p<0.05).

A history of *Chlamydia* infection, ectopic pregnancy and myomectomy increased the risk of infertility. Furthermore, the presence of cervical motion tenderness and adnexal mass multiplied, by 9 and 3 respectively, the risk of infertility (Table II).

A positive ELISA test (presence of IgG and IgM antibodies) carried a threefold increased risk of infertility, while the PCR test was positive in 8.0% fertile women and 8.0% infertile women (Table III).

Considering PCR as the reference test, our results revealed that IgG and IgM showed low sensitivity. However, IgM had better specificity and a higher NPV (94.9%) (Table IV).

Curves were plotted, comparing the results of ELISA and PCR in the diagnosis of CT infection. The first ROC curve comparing IgG with PCR found that IgG are not useful for diagnosis (p=0.211). By contrast, a significant difference was found between IgM and PCR; this means that IgM can be used in the diagnosis although their sensitivity is low (sensitivity: 62.5%, specificity: 60.99%, p=0.019) (Figures 1 and 2). Given the low economic level of the majority of our population (Cameroon is a developing country), we decided to investigate whether certain clinical variables associated with IgM antibodies can increase the sensitivity of this test. A third ROC curve was drawn comparing abnormal vaginal secretion, Chandelier's sign, inflammatory cervix and positive IgM antibodies to PCR. A significant difference (p = 0.002) with a sensitivity of 81.3% and a specificity of 60.03% was found. It appears that the presence of these four variables results in a model of prediction superior to the presence of IgM alone. Hence if a patient presents these four variables, then she is likely to have a current CT infection in 81.3% of cases (Figure 3).

Discussion

During the study, 100 infertile women and 100 pregnant women were recruited. Positive IgG and M anti-*Chlamydia* antibodies were associated with a three-fold increased risk of infertility. However, when the diagnosis was made by PCR, the frequency of CT infection was the same in both groups.

Sociodemographic characteristics

The mean age of the study population (33.68 years) was in line with the findings reported by Abdella *et al*. and Becker *et al*., who reported a mean age of 30.78 and 33 years, respectively [13]. Abida Malik *et al*. and Mallika Gosh *et al*. found different results, reporting a mean age of 26.4 and 24.85, respectively [14,15]. The difference versus the Malika study could be due to sample size: in fact, we had 200 patients as compared to their 80. In addition, we recruited infertile patients (who often consult later in our community) before matching them with fertile women.

A low economic level led to a three-fold increase in the risk of infertility (OR = 3.640, p = 0.031). This may be due to poor treatment of sexually transmitted infections in this group (because of poverty), hence the increased risk of infertility. In addition, Dyer and colleagues found that the treatment of infertility is very expensive, even for basic or ineffective inter-

Table 1 Sociodemographic characteristics of the study population.

Variables	Total N (%)	Fertile N (%)	Infertile N (%)	OR (95%CI)	*p*-value
Age group					
[20 – 25]	10 (5.0)	5 (5.0)	5 (5.0)	0.833 (0.202 – 3.435)	1.000
[25 - 30]	38 (19.0)	20 (20.0)	18 (18.0)	0.750 (0.294 – 1.911)	0.546
[30 - 35]	59 (29.5)	30 (30.0)	29 (29.0)	0.805 (0.342 – 1.893)	0.619
[35 – 40]	60 (30.0)	30 (30.0)	30 (30.0)	0.833 (0.355 – 1.953)	0.674
≥ 40 years	33 (16.5)	15 (15.0)	18 (18.0)	----------	Reference
Marital status					
Married	145(72.5)	76 (76.0)	69 (69.0)	----------	Reference
Single	55 (27.5)	24 (24.0)	31 (31.0)	1.422 (0.7671 –2.657)	0.267
Monthly income					
0 – 50,000	18 (12.8)	5 (7.1)	13 (18.3)	3.640 (1.150 –11.519)	**0.031**
50 – 100,000	24 (17.0)	10 (14.3)	14 (19.7)	1.960 (0.705 – 5.119)	0.166
100 – 200,000	39 (27.7)	20 (28.6)	19 (26.8)	1.330 (0.591 – 2.992)	0.490
>200,000	60 (42.6)	35 (50.0)	25 (35.2)	----------	Ref
Level of education					
Primary	7 (3.5)	2 (2.0)	5 (5.1)	----------	Reference
Secondary	54 (27.1)	18 (18.0)	36 (36.4)	0.800 (0.141 – 4.533)	1.000
University	138(69.3)	80 (80.0)	58 (58.6)	0.290 (0.054 – 1 .547)	0.239

Table 2 Clinical characteristics.

Variables		Total N (%)	Fertile N (%)	Infertile N (%)	OR (95%CI)	*p*-value
Past history of Chlamydia	Yes	57 (28.5)	22 (22.0)	35 (35.0)	1.909 (1.020 –3.573)	0.042
	No	143 (71.5)	78 (78.0)	65 (65.0)		
Myomectomy	Yes	17 (8.5)	3 (3.0)	14 (14.0)	5.264 (1.463-18.94)	0.009
	No	183 (91.5)	97 (97.0)	86 (86.0)		
Ectopic pregnancy	Yes	7 (3.5)	0 (0.0)	7 (7.0)	----------	0.014
	No	193 (96.5)	100(100.0)	93 (93.0)		
Anormal examination	Yes	104 (52.8)	62 (63.9)	42 (42.0)	2.446 (1.378-4.342)	0.002
	No	93 (47.2)	35 (36.1)	58 (58.0)		
Pelvic pain	Yes	75 (37.5)	31 (31.0)	44 (44.0)	1.749 (0.980-3.121)	0.058
	No	125 (62.5)	69 (69.0)	56 (56.0)		
Fever	Yes	13 (6.5)	7 (7.0)	6 (6.0)	0.848 (0.275-2.619)	1.000
	No	187 (93.5)	93 (93.0)	94 (94.0)		
Abnormal vaginal discharge	Yes	65 (32.5)	27 (27.0)	38 (38.0)	1.657(0.911-3.014)	0.097
	No	137 (67.5)	73 (73.0)	62 (62.0)		
Chandelier sign[a]	Yes	37 (19.0)	5 (5.0)	32 (33.7)	9.651 (3.569-26.09)	0.001
	No	158 (81.0)	95 (95.0)	63 (66.3)		
Adnexal mass	Yes	54 (27.6)	15 (15.0)	39 (40.6)	3.877(1.957-7.680)	0.001
	No	142 (72.4)	85 (85.0)	57 (59.4)		
Post-coital bleeding	Yes	12 (6.0)	4 (4.0)	8 (8.0)	2.087(0.608-7.167)	0.373
	No	188 (94.0)	96 (96.0)	92 (92.0)		
Cervix	Inflamm[b]	37 (18.5)	17 (17.0)	20 (20.0)	----------	Reference
	Normal	163 (81.5)	83 (83.0)	80 (80.0)	0.819 (0.400-1.676)	0.585

[a] Chandelier sign: cervical motion tenderness; [b] inflammatory

Table 3 ELISA and PCR results according to groups.

Variables	Total N (%)	Fertile N (%)	Infertile N (%)	OR (95%CI)	*p*-value
Age group					
Positive IgG	78 (44.1)	33 (37.9)	45 (50.0)	0.611 (0.336 – 1.112)	0.106
Positive IgM	45 (27.8)	27 (33.8)	27 (33.8)	0.552 (0.275 – 1.110)	0.094
Equivocal (IgG)	23 (11.5)	13 (13.0)	10 (10.0)	0.744 (0.310 – 1.785)	0.508
Equivocal (IgM)	38 (19.0)	18 (18.0)	20 (20.0)	1.139 (0.561 – 2.310)	0.718
Negative (IgG and M)	58 (77.3)	35 (87.5)	23 (65.7)		
Positive (IgG and M)	17 (22.7)	5 (12.5)	12 (34.3)	**3.652 (1.135 – 11.749)**	**0.030**
IgG+ and IgM-a	46 (31.5	20 (28.2)	26 (34.7)	1.353 (0.670 – 2.732)	0.398
IgG- and IgM+a	25 (17.1)	11 (15.5)	14 (18.7)	1.252 (0.526 – 2.997)	0.611
Chlamydia PCR					
Negative	184(92.0)	92 (92.0)	92 (92.0)	3.640 (1.150 –11.519)	0.031
Positive	16 (8.0)	8 (8.0)	8 (8.0)	1.960 (0.705 – 5.119)	0.166

[a] Equivocal modality has been excluded here

Table 4 Sensitivity, Specificity, PPV and NPV of ELISA compared with PCR test.

Variable	PCR -	PCR +	Sensitivity	Specificity	PPV[a]	NPV[b]
IgG						
Negative	92 (56.8)	7 (47.6)	53.33	56.79	10.26	92.93
Positive	70 (43.2)	8 (53.3)				
IgM	13 (13.0)	10 (10.0)				0.508
Negative	111 (74.5)	6 (46.2)				0.718
Positive	38 (25.5)	7 (53.8)				

[a] positive predictive value; [b] negative predictive value

Figure 1 ROC curve of IgG compared to PCR.

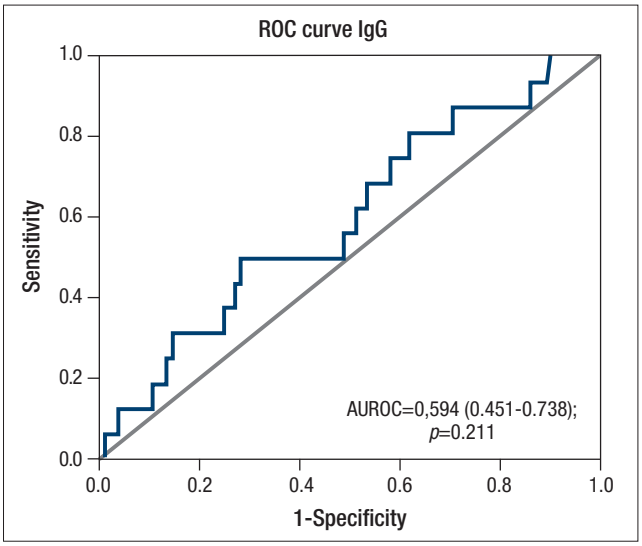


Figure 2 ROC curve of IgM compared to PCR.

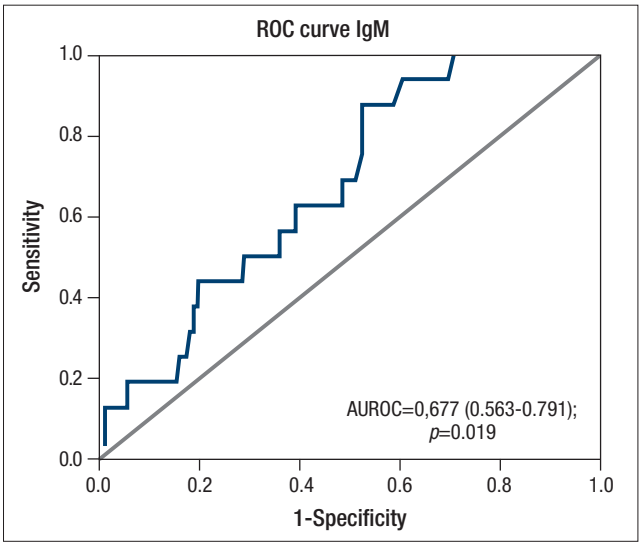
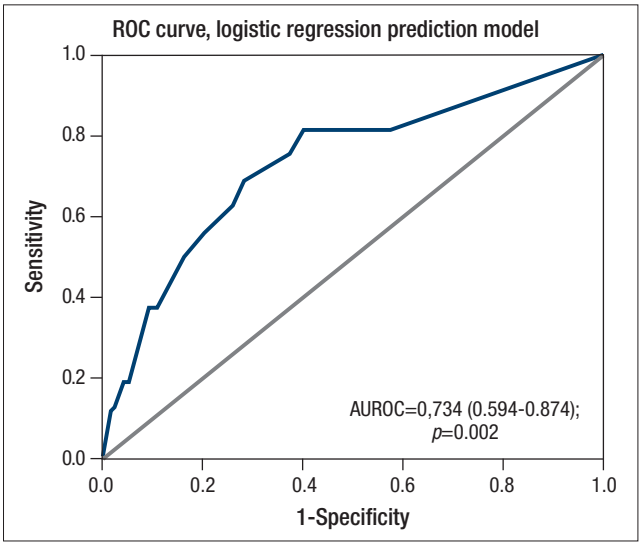


Figure 3 Logistic regression model of some variables (abnormal vaginal secretion, Chandelier's sign, positive IgM and inflammatory cervix) with the PCR reference technique.



ventions [16]. Mallika Gosh *et al*. in 2019 found different results from ours: according to their study, there was no association between low economic status and infertility (p = 0.054). This difference could be explained by their small sample size [15].

Clinical characteristics associated with infertility

A history of *Chlamydia* infection increased the risk of infertility almost two times (OR = 1.909 p = 0.042). This was found in most of the previous studies [17, 18, 19, 20, 21]. In fact, CT infection can cause adnexal adhesions and tubal obstruction, both responsible for infertility.

With regard to physical examination, we found that an abnormal examination, i.e., the presence of cervical motion tenderness (OR = 9.651) or an adnexal mass (OR = 3.877), was associated with infertility. This result is similar to that reported by Tao Xin *et al*. in 2018 [22]. According to their study, pelvic inflammatory disease (PID) is associated with infertility (p <0.05). In fact, the causative agents of PID cause inflammation of the tubes and the pelvis, leading to obstruction of tubes or formation of adhesions responsible for tubal infertility.

ELISA and PCR for CT diagnosis.

CT ELISA test was positive (IgG and IgM antibodies) in 34.3% of the infertile and 12.5% of the fertile women (OR 3,652 [1,135–11,749]) in our sample. Studies published by Hoenderboom *et al*. and Tukur *et al*. showed similar results [23, 24]. By contrast, Munvunyi *et al*. in 2011, Joolayi *et al*. in 2017, found no significant difference between these two groups using the ELISA test [10,9]. These discrepancies may be due to: the method used for the diagnosis of *Chlamydia* infection, the sensitivity of the tests, and the sample sizes. In the study published by Munvunyi, IgG and IgA were used with a sample size of 303 infertile women compared to 312 fertile women [10], whereas in the study of Joolayi *et al*., IgG and IgM antibodies were used and the sample was smaller [9].

The PCR test was systematically performed in all our patients and was positive in 8.0% of fertile and 8.0% of infertile women. This is consistent with the result of many studies [10,15,19,25]. We can therefore conclude that infertile patients have been in contact with *Chlamydia* which has caused damage to their tubes resulting in infertility. They do not have more current infections than pregnant women. A high anti-*chlamydia*l IgG titer is often detected in upper genital tract infections and cannot be taken as proof of a current infection. There is therefore no need to treat women with isolate positive IgG test, especially if they have recently received antibiotics. *Chlamydia*-induced antibodies may persist for a long time after an infection that was resolved by cellular immune response.

Sensitivity and specificity of IgG and IgM antibodies compared with PCR for current infection

Our results revealed low sensitivity of the IgG anti-*Chlamydia* antibodies test (53.33%) and low specificity (57.5%), with a NPV of 92.9%. We concluded that CT infection cannot be diagnosed by IgG antibody testing alone. Since the NPV is high, we can use it to rule out the infection. Muvunyi *et al*. and Joyee *et al*. obtained similar results with a NPV > 90% [10,26]. The sensitivity of the IgM assay was also low (53.8%) but the specificity

was higher (75.5%), with a NPV of 94.9%. In addition, a significant difference was found between IgM and PCR ROC curve (p = 0.019). This means that IgM can be used in the diagnosis of current CT infection, although its sensitivity is low. This is in agreement with the results of other studies [9,26].

Conclusion

Some factors, like a history of CT infection and cervical motion tenderness on physical examination, are associated with infertility. The proportion of CT seropositivity (IgG and IgM) was higher in infertile women than in pregnant women, although there was no significant difference in PCR positivity between the two groups. Thus, CT infection must be recognized as a risk factor of infertility. The low PCR positivity further leads us to conclude that not many patients seen in consultation settings have a current infection. It is therefore important to raise awareness among women of the influence of this infection on fertility and to carry out PCR tests to identify those needing treatment. If a patient cannot afford the PCR test, clinical manifestations of infection associated with positive IgM antibodies may be an alternative. Large studies should be carried out to validate this hypothesis and the government should make the PCR testing method more widely available and less expensive.

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Limitation: *The main limitations of our study are: first, we carried out the study with hospital data instead of community data; second, participants with equivocal results (ELISA) should have been re-tested two weeks later, to allow more precision concerning their results.*

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Conflict of interest: *None Declared*