

Tubal Infertility and Chlamydia Trachomatis in a Congolese Infertile Population

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Abstract

Infertility of tubal origin is the most frequent in sub-Saharan area. It is due to tuboperitoneal lesions mainly because of infection; especially sexually transmitted infection. Worldwide, Chlamydia trachomatis is the main pathogen. In our setting, some studies failed to establish the link between tubal infertility and chlamydia trachomatis. The current study aimed to determine the local data related to chlamydia trachomatis role in tubal infertility and the usefulness of Chlamydia trachomatis antibody titer test (CAT) in discrimination of the patients with and without tuboperitoneal lesions. Patients' average age was 33.9 ± 4.8 years, average coitarche 19.4 ± 4.4 years and average number of partners: 3.1 ± 1.6 . The level of CAT is correlated to the tuboperitoneal severity. CAT was more specific (93.3%; CI 95%: 81.7 - 98.6) than sensitive (72.7% CI 95%: 49.8 - 89.3) and discriminated correctly 89% (AUC = 0.89) of the patients with or without tuboperitoneal lesions. In conclusion, as it is stated worldwide, Chlamydia trachomatis is the most frequent sexually transmitted pathogen associated with tubal infertility. CAT has to be used as a tool to select patients to be submitted to invasive investigation, like laparoscopy.

Keywords

Tubal Infertility, Chlamydia Trachomatis, Chlamydia Trachomatis Antibody Titer Test, Sub-Saharan Area

1. Introduction

Infertility, as an inability for a couple to achieve pregnancy after a year of adequate sexual exposure, is consi-

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dered by the WHO as an important health problem [1]. Frequently, it is of tubal origin, especially in sub-Saharan area, and mainly due to tuboperitoneal lesions because of chlamydia trachomatis infection [2] [3]. Therefore Chlamydia trachomatis antibody titer test (CAT) had become one of the well-known tests in infertility work-up [4] [5].

Tuboperitoneal lesions (*i.e.* tubal occlusion; peritoneal and peritubo-ovarian adhesions...) responsible for tubal infertility can be caused by surgical trauma, endometriosis or more frequently by infectious conditions (the upper genital tract infections) [6]. In our setting, apart from infection of post abortion and post partum origin, some are secondary to medical procedures (HSG, hysteroscopy, endometrial biopsy...); lots are due to sexually transmitted pathogen with chlamydia trachomatis as the most commonly causative organism [7] [8].

Chlamydia trachomatis has a direct cytotoxic infect on tubal epithelial mucous and indirect effect through individual immune response (humoral or cell mediated response) [3]. Immunological response is directed to chlamydia trachomatis component (the Major Outer Membrane protein (MOMP), Heat shock protein 60 (Hsp 60), lipopolysaccharides). The Immunoglobulin G (IgG) against Hsp 60 is associated with chronic infection or the presence of chlamydia trachomatis sequelae: the base of its usefulness in infertility work-up [8]. The chlamydial infection consequences in short term are tubal epithelial cell and microvillousities and ciliae destruction; and in a long term: ectopic pregnancy, chronic pelvic pain and tubal infertility. The chlamydia trachomatis prevalence continues to grow up steadily year by year [9]. The WHO estimated at 100,000, the number of new cases, around the world. Women are two times more infected than men; and teenagers, twelve times more than those above 25 [10].

In sub-Saharan area, tubal assessment is therefore, one of the most important steps for couple's infertility work-up. The traditional tests for tubal patency and adhesions assessment include mainly: hysterosalpingography, hysterocontrastsonography (HyCosy) and laparoscopy with tubal dye as gold standard test. Those tests are not only demanding and but also not available sometimes in our area. Sometimes, they can be associated with severe drawbacks such as allergic reaction, pelvic infection and sepsis for HSG and hysteroscopy; more skilled personnel and special supplies requirements for Laparoscopy. Furthermore, some severe events, namely death, might occur when performing. Then, after Punumenn paper (1999) related to the usefulness of CAT test in infertility work-up [4], some protocols included this test, as a non-invasive investigation capable to discriminate patients with high certainty of having tuboperitoneal lesions from the ones without [11]-[14]. It was so important that, the Dutch Society of Obstetrics and Gynaecology adopted the CAT as the first line test in couple infertility management with the cut-off-level (IgG MIF > 1:32 or Elisa > 1.1), above which, invasive investigations can be performed [5].

Many studies in western and developed countries confirmed the role of chlamydia trachomatis in couple infertility and the accuracy of CAT in the infertility work-up. On the contrary, some studies in sub-Saharan did not confirm the preponderant role of this pathogen in tubal pathology [15] [16]. Because of this discordant findings and the lack of local data, the current study aims to assess the role of Chlamydia trachomatis in couple infertility and the importance of CAT test for couple infertility work-up in our setting.

2. Material and Methods

The current descriptive study included patients seeking infertility care at the University clinics of Kinshasa (Infertility unit) and at two private clinics (Centre Medical Edith de Lemba and Polyclinique Assossa Medical), from January to September 2012. The three Centers were elected because they, especially, take care of infertile patients. After getting her consent, 149 patients who underwent a HSG and/or a laparoscopy were enrolled in the present study. Among them, patients with a history of abdominal or pelvic surgery, abdominal and pelvic infection related to pregnancy (post abortion and post partum) and those with endometriosis at laparoscopy were excluded. Finally, a consecutive series of 67 cases out of the 149 patients were included.

The materials for sampling blood were got ready (syringes, tourniquet, tubes, Pasteur pipettes for ELISA (kit cHSP60-Ig-ELISA medac; spectrophotometer)).

The variables of interest were: age, parity, gravidity, abortion, marital status, profession, education level, number of partners, coitarche, history of: sexually transmitted infection (STI), illegal induced abortion, previous investigations and treatments. Para clinical findings at HSG, laparoscopy and CAT were also considered.

Procedures: first, medical history was taken through an interview, and Para clinical findings from medical files and operation protocol were registered. For HSG, we checked tubal patency; for laparoscopy, we checked

tubal patency at dye test, the quality of the tube and peritubal and pelvic adhesions. The Hull and Rutherford classification [17] was calculated in order to appreciate the severity of the tuboperitoneal lesions at laparoscopy and the chance of achieving a pregnancy after tubal surgery [13]. After that, a 5 ml of blood sample was taken from the forearm venous in a non-citrate tube and centrifuged to separate serum from figurative blood elements. Sera were conserved in a cryotube, at -20° and -80° Celsius. Finally, the sera were tested for chlamydial trachomatis antibodies IgG titer using cHSP60 IgG-Elisa kit (Medac Diagnostika, Hamburg, Germany). According to the manufacturer instructions, the sera were diluted at 1/50 and incubated at 37°C for an hour, than washed; after that, the substrate was added before the optic density (OD) could be read by the photometer. The interpretation respected the producer instructions: the cut-off equals average DO value of the negative control plus 0.350. The grey zone equals the cut-off value plus minus 10%. Than samples with OD below grey zone were considered negative; samples with OD inside the grey zone were doubtful and the ones above the grey zone were positive. Positive sample means it contains chlamydia trachomatis HSP60 antibodies. Final titer equals 55.5 multiplied by OD value divided by cut-off (final titer = $55.5 \times (\text{OD}/\text{cut-off})$).

We defined number of partners as the number of sexual partners a patient has ever had in her live. Coitarche was the age at the first intercourse; Infertility duration was the duration between the beginning of the maternity desire and the interview day. The Hull and Rutherford classification: grade I for minimal lesions (peritubal adhesions), grade II for moderate lesions (unilateral tubal occlusion, peritubo-ovarian limited adhesions) and grade III for severe lesions (bilateral tubal occlusion, hydrosalpinges, tubal mucosa alteration and extended adhesions). History of STI was defined as the existence of an episode of abdominal pain with or without leucorrhoea related to intercourse and not associated with illegal abortion, intrauterine device insertion, neither any intrauterine manoeuvre.

Data were recorded in a Microsoft Excel office 2007 software and analyzed with Stata. IC 12.1 for statistics analysis. Continuous quantitative variables were summarized as means and standard deviation; and the categorical variables as proportions. The average values were compared by Kruskal Wallis test, and the proportions by Chi2 test. The logistic regression was used to appreciate the association between CAT and other predictors, and the linear regression, the relation between, in one hand the CAT, and in the other, the infertility duration and the lesions severity; and the diagt command, for appreciation of CAT results validity. The p-value of <0.05 was considered for statistical significance.

To check the usefulness of CAT, for infertility work up, we calculated its diagnostic and discriminative capacity, in comparison with HSG, and Laparoscopic findings as gold standard test. In one hand, the CAT was taken as dichotomic test according to the manufacturer cuff-of value; allowed the calculation of the sensitivity, the specificity, the positive predictive value (PPV), the negative predictive value (NPV). The sensitivity was defined as the proportion of patients with tuboperitoneal lesions diagnosed correctly; and the specificity, the proportion of patients without tuboperitoneal lesions diagnosed correctly. The PPV was defined as the proportion of patients with tuboperitoneal lesions among the ones with positive test and the NPV as the proportion of patients without tuboperitoneal lesions among the ones with negative test. In the other hand, to quantify its discriminative capacity, the CAT was considered as a numeric test, evaluated by the Receiver operating Characteristics curve (ROC) analysis. The Area Under Curve (AUC) was calculated to express the performance of CAT (the probability of positive test for a patient with tuboperitoneal lesions) and to determine the accurate cut-off for tuboperitoneal lesions diagnosis in the current study.

The current research received the approval of the Department of Obstetrics and Gynecology ethical board. The patient informed consent was required before her enrollment.

2.1. Socio-Demographic Characteristics

The patients age was ranged from 23 to 43 years, with an average of 33.9 ± 4.8 years; most of them (78.9%) were above 30 and nulliparous (70%). The majority (62.9%) achieved at least one pregnancy previously and many (40.3%) at least one illegal abortion. Nearly all (92.5%) were married and many (59.7%) had got a university degree and worked as civil servant (41.7%). The coitarche varied from 13 to 37 years old with an average of 19.8 ± 4.4 years, many (59.6%) have had coitarche before 20; most of them had more than one sexual partners (79.1%). The average number of partner was 3.1 ± 1.6 raging from 1 to 11 (Table 1).

Many patients (43.2%) had a history of STI and almost two third (62.6%) suffered from secondary infertility. Before they met our team, 16.6% had seen a traditional practitioner and 31.8% a specialist. For previous treatments, a tenth (10.5%) had herbal treatment and about one-fifth (19.3%) modern drogues (ovulation inducers,

Table 1. Patients' socio-demographic characteristics.

Variables	% (N = 67)	Min	Mean \pm SD	Maximum
Age (years)		23	33.9 \pm 4.8	43
≤ 25	5.9			
26 - 30	14.9			
31 - 35	41.7			
36 - 40	28.3			
> 40	8.9			
Parity				
Nulliparous	77.6			
Pauciparous	5.9			
Multiparous	2.9			
Type of infertility				
Primary	37.3			
secondary	62.6			
Illegal abortion				
0	59.7			
1	13.4			
≥ 2	26.8			
Coitarche (years)		13	19.8 \pm 4.4	37
≤ 15	8.9			
16 - 19	50.7			
20 - 25	29.8			
> 25	10.4			
Number of partner		1	3.1 \pm 1.6	7
1	20.9			
2	17.9			
3 - 7	61.1			
Professions				
Housewife	32.0			
unemployed	8.9			
civil servant	41.7			
trader/accountant	16.7			
Education				
Elementary	7.4			
High school	32.8			
University	59.7			

antibiotics...). The average duration of infertility was 5.1 ± 3.5 years; many patients (79%) had infertility duration of more than 2 years.

At HSG, tuboperitoneal abnormalities were noticed in 64.0% of patients. Among all the patients, 54.7% had bilateral tubal occlusion and 7.5% unilateral. The laparoscopic abnormalities summarized according to H & R classification, showed that 5.97% had a grade II lesions and 25.37% a grade III. The CAT test, expressed by index, ranged from 10 to 350, noticed that 48 patients (71.64%) were seropositive (**Table 2**).

2.2. Chlamydia Trachomatis and Patients' Characteristics

By linear regression, the coitarche and the number of partners were negatively, not significantly ($p = 0.567$), correlated to the CAT titers: the earlier the patient began sexual activities, the higher was the CAT value (**Figure 1**) and higher was the number of the sexual partner the lesser was the CAT (**Figure 2**). The duration of infertility and the severity of tuboperitoneal lesions were positively correlated to CAT: the higher was the duration of infertility, the higher ($p = 0.86$) was, not significantly, the CAT titer (**Figure 3**); and the more severe were the tuboperitoneal lesions, the significantly higher ($p = 0.002$) was the CAT (**Figure 4**). Most of the patients (74.06%) with tuboperitoneal were above 30 years old (**Table 3**).

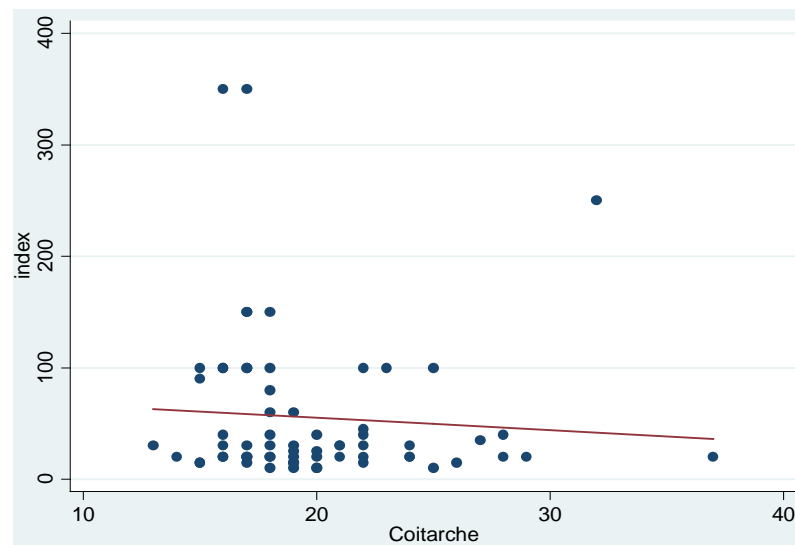
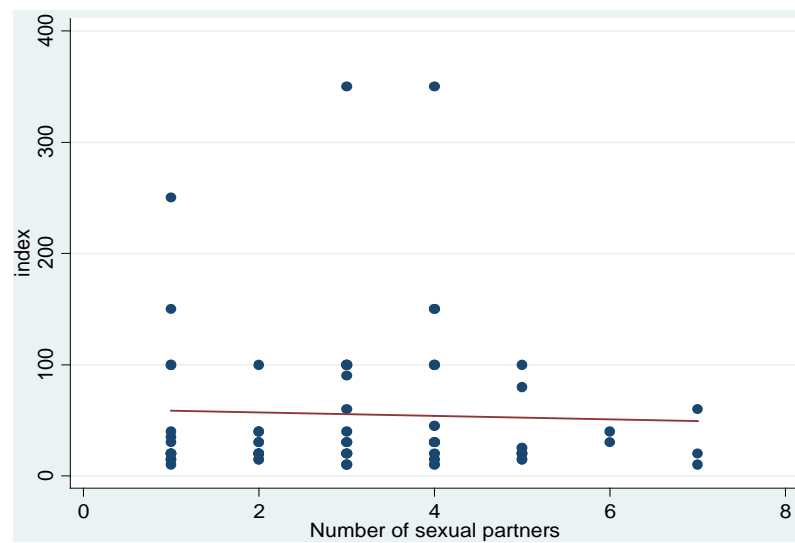
2.3. Discriminative Power of CAT

In comparison with laparoscopic findings (**Table 4**), HSG had a sensitivity of 94.7% (95% IC: 74 - 99.9) speci-

Table 2. Tuboperitoneal lesions at para clinic investigations.

Variables	% (N = 67)	Min	Mean \pm SD	Maximum
HSG				
Normal	35.8			
Bilateral occlusion	54.7			
Unilateral occlusion	7.5			
Adhesions	1.8			
Laparoscopy				
Normal	59.7			
Grade I	1.4			
Grade II	5.9			
Grade III	25.3			
CAT				
Normal	71.6			
Pathologic	28.3			

Legends: figure represents percentage.

**Figure 1.** CAT evolution according to the coitarche.**Figure 2.** CAT evolution according to the number of sexual partners.

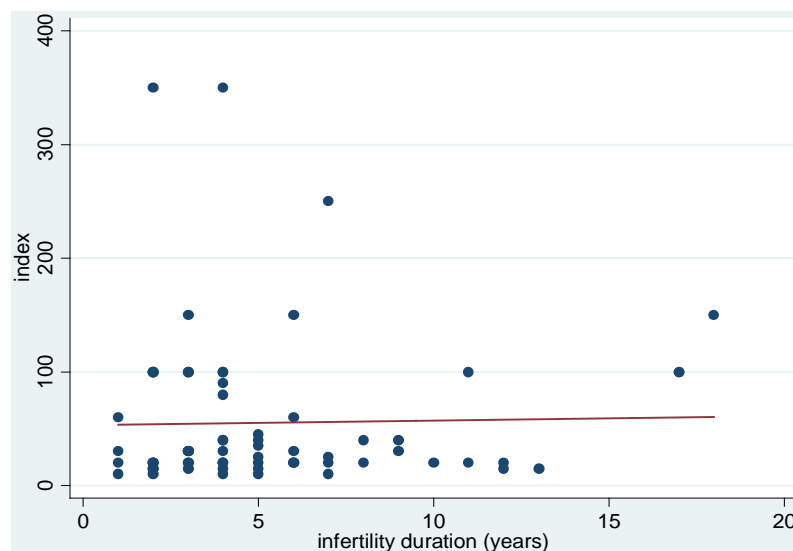


Figure 3. CAT titers evolution according to the duration of infertility.

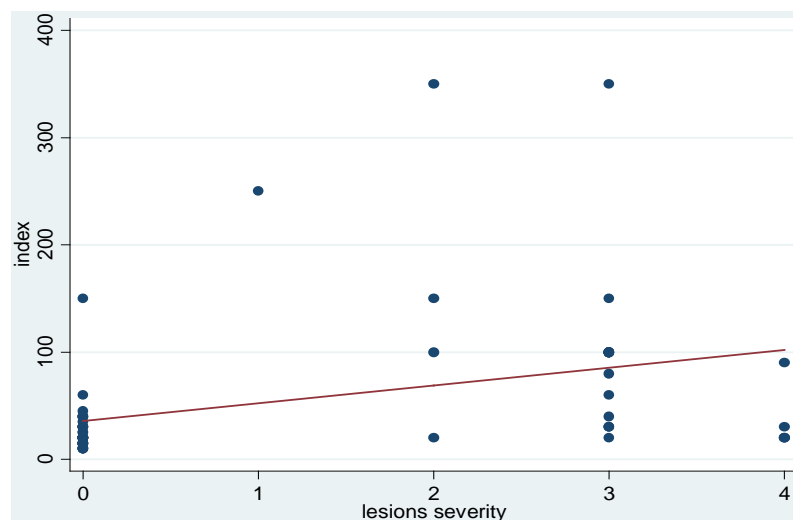


Figure 4. CAT titers evolution according to the severity of the lesions.

Table 3. The relations between CAT titers and the patients' characteristics.

Characteristics	Coefficient	St Err	p	IC 95%	r
Coitarche	-1.10	1.91	0.564	-4.9 - 2.7	0.07
Partner numbers	-1.58	5.0	0.75	-11.7 - 8.58	0.03
Infertility duration	0.40	2.37	0.86	-4.33 - 5.13	0.02
Lesions severity	16.5	5.17	0.002	6.2 - 26.8	0.36

St Err = standard error; IC 95% = interval of confidence at 95%.

Table 4. Validity of HSG and CAT results in comparison of laparoscopic findings.

Tests	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	LR+	LR-	OR (p) (95% CI)
HSG	94.7 (74 - 99.9)	55.9 (37.9 - 72.8)	54.5 (36.4 - 71.9)	95 (75.1 - 99.9)	2.1 (1.4 - 3.1)	0.1 (0.0 - 0.6)	22.8 (0.004) (2.72 - 190)
CAT	72.7 (49.8 - 89.3)	93.3 (81.7 - 98.6)	84.2 (60.4 - 96.6)	87.5 (74.8 - 95.3)	10.9 (3.5 - 33.5)	0.3 (0.1 - 0.5)	37.3 (0.000) (8.69 - 158)

ficity of 54.5% (95% IC: 37.9 - 72.8), a negative predictive value of 95% (95% IC: 75.1 - 99.9). Tuboperitoneal lesions were two times ($LR+ = 2.1$) more likely to be noticed at HSG when they were present. Taking as dichotomic test, according to manufacturer instructions, the CAT was fairly less sensitive: 72.7% (95% IC: 49.8 - 89.3) than specific: 93.3% (95% IC: 81.7 - 98.6). A positive test was eleven times ($LR+ : 10.9$) more likely to be found when tuboperitoneal lesions were present.

Taking as a numeric test (**Table 5**), the CAT was capable to discriminate correctly 89% ($AUC = 0.8924$) of patients with and without tuboperitoneal lesions at laparoscopy. According to the ROC of the current study (**Figure 5**), the accurate cut-off was the titer of ≥ 60 (**Table 5**). Because at this value the CAT had the highest sensitivity (72.73%) and specificity (93.33%).

Table 5. Discriminative capacity of CAT as numeric test.

Cut point \geq	Sensitivity %	Specificity %	Correctly classified	LR+	LR-
≥ 10	100.00	0.00	32.84	1.00	
≥ 15	100.00	11.11	40.30	1.13	0.00
≥ 20	100.00	26.67	50.75	1.36	0.00
≥ 25	90.91	62.22	71.64	2.41	0.15
≥ 30	90.91	66.67	74.63	2.73	0.14
≥ 35	77.27	80.00	79.10	3.86	0.28
≥ 40	77.27	82.22	80.60	4.35	0.28
≥ 45	72.73	91.11	85.07	8.18	0.29
≥ 60	72.73	93.33	86.57	10.91	0.29
≥ 80	68.18	95.56	86.57	15.34	0.33
≥ 90	63.64	95.56	85.07	14.31	0.38
≥ 100	63.64	97.78	86.57	28.63	0.37
≥ 150	22.73	97.78	73.13	10.23	0.79
≥ 250	13.64	100.00	71.64		0.86
≥ 350	9.09	100.00	70.15		0.91
> 350	0.00	100.00	67.16		1.00

Observations: 67; ROC $AUC \pm \text{Std Err} = 0.89 \pm 0.04$; IC 95%: 0.81 - 0.97.

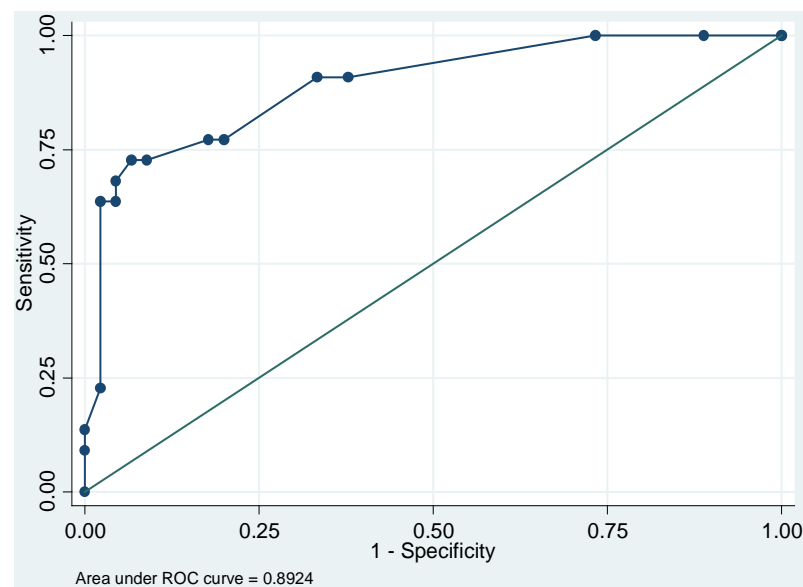


Figure 5. The ROC of CAT in a Congolese infertile women.

3. Discussion

3.1. The Role of Chlamydia Trachomatis in Infertility

The patients' average age was 33.9 ± 4.8 years, with 78.9% above 30. Many of them (40.3%) had a history of illegal abortion. That means pregnancies achieved after unprotected and non-desired intercourse, which is risky for STI [18]. The average coitarche was 19.8 ± 4.4 years and the majority (59.7%) had got a university degree. In that current study, population was fairly older, according to the reproductive women-age span (16 - 40 years old). The impairment of fertility related to ovarian ageing begins by the age of 35 [19] [20]. In our setting, patients seeking care for infertility see the appropriate medical practitioner more and more late, at advanced age: they begin by consulting the generalist, traditional care provider (herbalist), pasta and the priest, before meeting the specialist. Because, for some of them, infertility is due to bad luck from any family member or sorcerer [21]. Not only, it is a waste of time, but also patients are in touch with some bad practices and drugs that can worsen their fertility. Obviously, it increases the risk of STD by chlamydia trachomatis through multiple partners as we noticed in the present study (average number of partners: 3). Furthermore, many of them had conceived previously but underwent illegal abortion (40%), another risk factor of chlamydia trachomatis infection [22].

Most of patients (59.6%) began their sexual activities earlier, before 20. This period is known as more risky for chlamydia trachomatis infection, with its consequences becoming visible by their thirties [23] [24]. In current study, we noticed any relation, not statistically significant, between the duration of sexual activity and the number of sexual partners in one hand and CAT in the other hand. In fact, the earlier patient had had coitarche, the higher the level of CAT titer and the higher the number of partner, the lesser the CAT titer. With some reserve, in respect of the smallness of our sample, those findings are consistent with the closely relation between the sexual activity and the chlamydia trachomatis infection. Indeed, Rahm *et al.*, 1991 [23] stated that sexually active teenage girls are more at risk of chlamydia trachomatis infection and Hansfield *et al.*, 1986 [24] noticed that for sexual partners and chlamydia trachomatis, the recent partner is by more a risk factor than the number of partner.

At laparoscopy, the tuboperitoneal lesions were noticed preferentially (74.06%) in patients above 30 years old. Furthermore, the majority of them (62.6%) had secondary infertility, the most prevalent in sub-Saharan area and associated with chlamydia trachomatis infection [7]. Chlamydia trachomatis consequences (tuboperitoneal lesions and secondary infertility) become visible in patients' thirties. Patients with history of previous abdominal and pelvic surgery and infection; the same with pelvic endometriosis were excluded to prevent from de confusion with others tuboperitoneal etiologies.

3.2. The Discriminative Capacity of CAT

In the current study, the CAT titer was positive for 71.6% of patients, with a sensitivity of 72.7% and a high specificity (93.3%). The higher positive likelihood ratio (LR+: 10.9) of CAT compared to HSG [2] [1], are consistent with others authors, among them: Akande *et al.*, 2003 [25]; Hartog *et al.*, 2005 [18] and Tiitinen *et al.*, 2006 [26] who affirmed the strong association of tuboperitoneal lesions and CAT titers. Even if Muvunyi [15], and Nzintcheu [16], who performed their research in the central Africa area, close to ours, failed to establish that link. Our findings reinforce the usefulness of CAT. Taking in account its higher specificity, the CAT can be used as a non invasive test capable to determine the ones, who can be submitted to invasive investigation (laparoscopy) because of high probability of the presence of tuboperitoneal lesions. Furthermore, in his late paper in the subject; Muvunyi *et al.* [27] stressed the usefulness of CAT in sub fertile women workup. Apart from the sample smallness, the current study highlights the preponderant role of chlamydia trachomatis in our setting and the CAT usefulness in infertility work-up.

4. Conclusion

In conclusion, the Chlamydia trachomatis is the most frequent microorganism associated with tubal infertility in the current study. The CAT level rose steadily with the severity of the tuboperitoneal lesions. With its higher specificity, the CAT has to be used to select patients for the more invasive investigations, like laparoscopy, also in our setting.

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