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Original Article

Studies on *Chlamydia Trachomatis* Infection among Infertile Males in Benin City, Nigeria

Helen Oroboghae Ogefere[1], Osaigbovo Edogun[1,2], James Agbonwanre Osaikhuwuomwan[3], Richard Omoregie[4]

[1]*Department of Medical Laboratory Science, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria*
[2]*Department of Medical Microbiology, School of Medicine, University of Benin, Benin City, Nigeria*
[3]*Human Reproduction Research Program Unit, Department of Obstetrics and Gynaecology, University of Benin Teaching Hospital, Benin City, Nigeria*
[4]*Medical Microbiology Division, Medical Laboratory Services, University of Benin Teaching Hospital, Benin City, Nigeria*

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Corresponding author's email:
richard.omoregie@ubth.org

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ABSTRACT

Introduction: *Chlamydia trachomatis* is a major cause of sexually transmitted infection. This study aimed to ascertain the association of *Chlamydia trachomatis* with semen health in males seeking fertility assistance and to compare two diagnostic tests for current infection.

Methods: Blood and urine samples were collected from 197 male spouses in childless unions and screened for *Chlamydia trachomatis* IgG antibodies and antigens respectively. Semen was also collected from the participants to determine their semen health.

Results: Chlamydial antigens were detected in urine samples from 7 (3.55%) participants. Chlamydial antibodies were detected in blood samples in 16 (8.12%) of patients. The presence of *Chlamydia trachomatis* antigens and antibodies were not affected by age of the participants as well as their duration of marriage ($p>0.05$). Current *Chlamydia trachomatis* infection was associated with volume of seminal fluid (OR=34.88, 95%CI=5.79, 210.00; $p<0.0001$), abnormal morphology (OR=235.00, 95%CI=27.31, 2022.00; $p<0.0001$), reduced motility (OR=5715.00, 95%CI=105.90, 308400.00; $p<0.0001$) and reduced sperm count (OR=1134.00, 95%CI=63.07, 20390.00; $p<0.0001$).

Conclusion: The prevalence of past and current *Chlamydia trachomatis* infection in this study was 5.58%and 3.55% respectively. Current C. *trachomatis* infection resulted in abnormal seminal fluid analysis parameters, albeit, this should be interpreted with caution due to the small number of positive cases. Measures to reduce or eliminate chlamydial infections are advocated.

Keywords: *Chlamydia trachomatis;* Infertility; Antibodies; Antigens; Prevalence

Introduction

Infertility affecting couples all over the world today constitutes both a medical as well as social problem, particularly in Nigeria. In Nigeria, as well as in several other African countries, marriages are conducted almost for the sole purpose of child bearing and hence when there is infertility the psychological problems are highly distressful and pervasive. Not until recently, female partners of infertile couples were thought to be source of problem but studies have shown that male partners (factors) are not exempt and account for 40-50% of infertility, though this figure differs from one region to another for the obvious reasons of different cultural believe and poor knowledge (1,2).

According to the WHO's 2022 infertility report, the lifetime prevalence and point prevalence of infertility worldwide were 17.5% and 12.6%, respectively, and they are the same in high-income and low-income countries. As shown in that report, the WHO Western Pacific Region had the greatest lifetime prevalence (23.2%) and the WHO African Region had the highest point prevalence (16.4%) (3). Although the incidence of infertility is on the increase in this part of the world (Africa), no cogent effort or program is being put in place to deal with the problem, in Nigeria in particular, thus making the effect of infertility to be on the increase (4). Infertility due to male partners can only be established when recognizable causes of infertility in the female partner are excluded, alongside the failure of semen quantity and quality to meet the WHO standard criteria.

The most prevalent causes of infertility in the male reproductive system are issues with semen ejection, low or non-existent sperm counts, or aberrant sperm morphology and motility, as well as infection (3, 5). Genital *Chlamydia trachomatis* infections are classified as sexually transmitted diseases (STDs) and have been severally implicated in infertility cases. In most cases, infected patients are asymptomatic. Thus, the infection persists undiagnosed and patient's failure to seek proper treatment on time may lead to decreased fertility (6).

The antibody response to *Chlamydia trachomatis* infections involves the production of both IgG and IgA antibodies by invoking a humoral cell response that produces circulatory immunoglobulin M (IgM), immunoglobulin G (IgG), and secretory immunoglobulin A (IgA) antibodies as well as a cellular immune response, which can persist after the infection is cleared (7). Immunopathologic reaction has been linked to a 40-kd major outer membrane protein (MOMP) and 10- and 60-kd chlamydial heat-shock proteins (cHSPs) (8). However, further research is required to fully comprehend these cell-mediated immune responses. The serum antibody response to sexually transmitted chlamydial infection involves the production of specific antibodies, primarily IgG, IgM, and IgA, which indicate a current or past exposure to the bacterium Chlamydia trachomatis. These antibodies are useful as biomarkers for an infection and its potential complications, but generally offer only partial and short-lived protective immunity against future infections. Serology may not be able to differentiate between a chronic ongoing infection and a previous infection since anti-chlamydial antibodies seem to linger for extended periods of time after the infection has cleared. Chlamydial IgG antibodies have been shown to survive at a steady level for up to eight years, despite the fact that they may go away in a few months(9). However, an immunological response to a chlamydial infection might be either pathogenic or protective. Chlamydia has minimal inherent toxicity because it is an intracellular infection. The main cause of illness seems to be immune recognition of certain antigens expressed either directly by the organism or indirectly on the surface of infected host cells. Inflammatory injury inside the upper reproductive tract could result from either an exaggerated or overstimulated Th-1 response or from a failed or feeble Th-1 action that results in chronic infection, given the cell-mediated Th-1 response's primary protective role in resolving infection (9).

Infections of urinary and genital tract origins (testes and prostrate) can affect sperm cells at different developmental stages such as formation, maturation and transportation (10). When this happens, the sperm deteriorates leading to decreased sperm motility, viscosity, viability, count and a high proportion of abnormal forms and leucocytospermia (11). Ejaculatory ducts have been reportedly damaged by *Chlamydia trachomatis* leading to reduction in semen volume, which in some cases may lead to azoospermia in the infected men (12). It is believed and well documented that male fertility is directly affected when sperm is damaged by *Chlamydia trachomatis* infections, resulting in impairments in the semen and semen quality, which is regarded as an alternative indicator of male fertility (13).

It is extremely concerning since 50% of men and 75% of women who contract *Chlamydia trachomatis* through sexual contact do not show any symptoms. Unfortunately, the difficulty of cell culture and non-inclusion of a screening test like ELISA in routine diagnostic methods makes early detection of chlamydial infection impossible and many of the chlamydial infections escape undiagnosed. This makes

both asymptomatic infected male and female partners undetected reservoirs of infection.

At the moment, the use of urine samples is becoming widely accepted for detecting *Chlamydia trachomatis* infection (14). This is due to the fact that detecting serum antibodies to *Chlamydia trachomatis* in men without symptoms is not very beneficial as such IgG titre values do not usually give any clear indication whether it is an active or a previous infection that elicited such immune response. Molecular techniques like nucleic acid amplification tests (NAATs), which are regarded as the gold standard for diagnosis, are not commonly used in resource-poor environments like Nigeria. Thus, this study's objective was to assess the effectiveness of an antigen detection assay using first catch urine samples and comparing it with IgG measurement in venous blood samples of male partners seeking fertility assistance.

Materials and Methods

Study Area

This investigation was conducted at Human Reproduction Research Program (HRRP) (in vitro fertilization) Clinic in University of Benin Teaching Hospital (UBTH), Benin City, Edo State, Nigeria. The hospital has a geo-spatial location of latitude 6.3903oN and longitude 5.6118oE, and it's a tertiary hospital with referral status and over 900 bed space.

Study Population

For this study, 197 male partners of infertile couples (MPIC) who had been married for at least a year but were childless and seeking fertility treatment from the HRRP Clinic between April 2019 and February 2021 were enrolled. The ages of the participants ranged from 30 – 65 years and the duration of marriage of the participants were documented as well.

Sample Collection

Blood, urine and semen samples were collected from each participant. Whole blood, 5ml was collected through venepuncture into ethylene diamine tetraacetetic acid (EDTA) bottle and mixed properly. Two sterile universal containers were also given to the patient; one was to collect 20ml of first void early morning urine and the second for semen, which was to be collected through masturbation after 3-5 days period of abstinence. Within one hour of collection, the semen samples were sent to the laboratory. Semen analysis was done using WHO criteria (15).

Sample Processing

Detection of C. *trachomatis* IgG antibodies using blood samples: IgG antibodies to *Chlamydia trachomatis* in plasma were detected using an indirect solid-phase enzyme immunoassay utilizing the ImmunoComb *Chlamydia trachomatis* IgG rapid test kit (Orgenics Limited, Israel) following the manufacturer's instruction.

Detection of C. *trachomatis* antigens using urine samples: The test kit, Solid Chlamydia Rapid Test Device (Rapid Labs Limited, United Kingdom) was used. Using an antibody-specific test kit, the Chlamydia Rapid Test Device, a rapid chromatographic immunoassay was performed in accordance with the manufacturer's instructions to qualitatively identify C. trachomatis antigens (elementary bodies) in urine specimens.

Semen Analysis: The time of collection was noted alongside the time of examination. The semen was analysed by determining semen volume, pH, viscosity, sperm cell progressive motility and morphology, as well as total sperm cell count using WHO guidelines (15).

Statistical Analysis: The data were analysed with Chi square (X2) test using the statistical software GraphPad Prism, version 5.01 (GraphPad Software Inc., San Diego, CA, USA). The level of statistical significance was set at $p < 0.05$.

Ethical clearance: The University of Benin Teaching Hospital's (UBTH) Ethics and Research Committee in Benin City granted approval in a letter with reference number ADME/E 22/A/VOL. VII/1244.

Results

A total of 197 male spouses in childless unions seeking fertility assistance from the Human Reproduction Research Program (HRRP) Clinic in University of Benin Teaching Hospital, Benin City, were recruited for this study. The ages of the participants ranged from 30 – 65 years. Table 1 shows the number of urine samples positive for *Chlamydia trachomatis* antigens and the number of serum samples positive for *Chlamydia trachomatis* IgG antibodies according to age. Positive urine samples ranged from 2.27% to 6.06% while positive serum samples ranged from 5.26% to 11.36% of the total tested. Their respective p values were p= 0.5883 in urine and p= 0.3231 in serum, indicating that there was no significant relationship between age and test result. Similarly, the duration of marriage of the participants was not significantly associated with *Chlamydia trachomatis* infection in either urine (p= 0.6984) or serum (p= 0.4157) samples (Table 2).

Table 1: Prevalence of Chlamydia trachomatis antigens and antibody in relation to patient age

Age (years)	No. tested	Antigen test positive (%)*	Antibody test positive (%)†
30 – 41	76	3 (3.95)	4 (5.26)
42 – 53	88	2 (2.27)	10 (11.36)
54 – 65	33	2 (6.06)	2 (6.06)
Total	197	7 (3.55)	16 (8.12)

**p=0.5883; †p=0.3231*

Table 2: Prevalence of Chlamydia trachomatis antigens and antibody in relation to duration of marriage

Duration of marriage (years)	No. tested	Urine positive (%)*	Blood positive (%)†
≤5	46	1 (2.17)	2 (4.35)
6 – 10	83	4 (4.82)	9 (10.84)
≥11	68	2 (2.94)	5 (7.35)
Total	197	7 (3.55)	16 (8.12)

**p=0.6984; †p=0.4157*

Chlamydia trachomatis antibodies were detected in 11 (5.58%) blood specimens and *Chlamydia trachomatis* antigens were detected in 2 (1.02%) urine specimens while 5 (2.54%) cases were detected by both antibody and antigen diagnostic techniques (Table 3). There was a statistical significant difference between antigen and antibody detection (p= 0.0271), which is indicative that more *Chlamydia trachomatis* antibodies were detected than its antigens.

Table 3: Comparison of Chlamydia trachomatis antigen and antibody detection

Methods	No. tested	No. positive (%)
Antibody detection (blood)	197	11 (5.58)
Antigen detection (urine)	197	2 (1.02)
Antibody + Antigen	197	5 (2.54)

p=0.0271

There was a significant association between *Chlamydia trachomatis* infection and some abnormal seminal fluid analysis parameters – semen volume (OR=34.88, 95%CI=5.791, 210; p<0.0001), sperm cell morphology (OR=235.0, 95%CI=27.31, 2022; p<0.0001), motility (OR=5715, 95%CI=105.9, 308400; p<0.0001) and total sperm count (OR=1134, 95%CI=63.07, 20390; p<0.0001) (Table 4).

Table 4: Association between Chlamydia trachomatis urine test result and semen parameters

Abnormal Semen parameters	*Chlamydia trachomatis* infection	No. tested	No.in each category (%)	OR	95%CI	P values
Volume (<1.5ml)	Present	7	3 (42.86)	34.88	5.791, 210.0	<0.0001
	Absent	190	4 (2.11)			
Morphology (<4% normal form)	Present	7	5 (71.43)	235.0	27.31, 2022	<0.0001
	Absent	190	2 (1.05)			
Motility (<32% motility)	Present	7	7 (100.00)	5715	105.9, 308400	<0.0001
	Absent	190	0 (0.00)			
Count (<15 million/ml)	Present	7	6 (85.71)	1134	63.07, 20390	<0.0001
	Absent	190	1 (0.53)			

Discussion

Due to the pathogen's extreme lack of symptoms, the prevalence rates of genital chlamydial infections in men are probably underreported. The age range of the male spouses of childless unions considered in this work ranged from 30-65 years. Although the study found that the infection rate was 8.12% with antibody testing and 3.55% with antigen testing, there was no discernible variation in *Chlamydia trachomatis* infection between the study population's age groups (p=0.3231 and p=0.5883, respectively). These numbers are lower than the 20.6% positive instances reported by Keikha et al. (16). A prevalence of 3.33% was recently reported from Cotonou (17), and is comparable to our current finding. The Cotonou study used urine and molecular techniques to detect Chlamydia trachomatis. However, higher percentages of 33% and 34%, respectively, were observed in South Africa and India (18). The different prevalence published from different studies can be attributed to the use of different samples, different testing methods and difference in population studied. Due to its often asymptomatic nature, the actual prevalence of chlamydial infection is believed to be underrated (19).

Though the duration of marriage did not show any significant statistical difference with C. trachomatis infection in both antigen testing (p=0.6984) and antibody testing (p= 0.4157), but it is worth noting that the highest prevalence of C. trachomatis was 4.82% in antigen testing and 10.84% in antibody testing were among those who have been married for 6-10 years. This could be explained by the fact that in Nigeria as well as in several other African countries, marriages are conducted almost for the sole purpose of child bearing. About 30% to 40% of couples become pregnant within the first three months of having sufficient, unprotected sex, and about 85% of couples conceive within the first twelve months (20). Thereafter, family and societal pressures causes infertile couples to become more agitated and to desperately to seek medical assistance and help from in vitro fertilization (IVF) clinics.

Although blood screening detected more positive cases than using early morning urine, it has been reported that the use of *Chlamydia trachomatis* antibody detection to establish current infection is not very reliable; as it does not differentiate between past infection and current infection (21). Also, in most *Chlamydia trachomatis* antibody screening, there appears to be cross-reactivity among species and this sometimes make significant interpretation of result clinically difficult (22). Most individuals with urogenital chlamydial infection develop serum antibodies (7).The serum IgG antibodies produced in response to urogenital infections by Chlamydia do remain in most individuals for years and hence useful as an indicator of past infection (23). Consequently, serological methods for screening individuals for *Chlamydia trachomatis* antibodies in serum or seminal plasma have little diagnostic use in male infertility studies (24). For this reason, we considered rather to study the association of *Chlamydia trachomatis* infection and male factor infertility using urine sample.

Several male fertility studies have used different methods as well as different samples (urethral swab, semen, urine and serum) but the use of urine sample is becoming widely accepted for detecting *Chlamydia trachomatis* infection (14). However, infection rates from male partners of childless couples urine samples have been reported to range from 0.3% to 7.1% (25, 26), which agrees with the results of our study, which found a 3.55% infection rate in urine. Studies on male factor fertility have shown notable variations in the incidence of *Chlamydia trachomatis* infections and these variations may be attributed to *Chlamydia trachomatis* detection techniques and the study area (14).

Three (42.86%) of the *Chlamydia trachomatis* infected male partners had semen volume <1.5ml. WHO semen analysis criteria recommends that a semen volume of <1.5ml per ejaculate is not adequate for fertilization (15). The urine positive *Chlamydia trachomatis* was significantly (p<0.0001) associated with semen volume. *Chlamydia trachomatis* have been reported to damage ejaculatory ducts leading to reduction in semen volume (12). The finding that *Chlamydia trachomatis* infection was significantly (p<0.0001) associated with abnormal sperm cell morphology agrees with a previous report (27).

The WHO states that within the first hour of testing, a viable sperm's total progressive motile spermatozoa should surpass 32% (15). In this study, all the infected semen presented with progressive motility of <32%, and this agrees with a previous report (28). The reduction in sperm motility by *Chlamydia trachomatis* is probably due to its attachment to spermatozoa. This attachment can be both on the surface and in the nucleus, thereby immobilizing them; causing the sperm to show a dramatic decrease in motility (29, 30).

According to earlier research, a low total sperm count is linked to *Chlamydia trachomatis* infection (29, 31, 32). This was observed in this study. Thus, a current *Chlamydia trachomatis* infection appears to be associated with male infertility. It is noteworthy that the 95% Confidence Intervals observed are quite far apart, which may be because there weren't many positive cases. Because of their poor accuracy, these estimations should be looked at with caution.

A weakness of the study was that it was a single-center cross-sectional study with few positive cases utilizing antigen. Therefore, care should be taken when extrapolating the results of this study, particularly the impact on semen quality. Also, fertile men were not used as control making the generalization of the effect of *Chlamydia trachomatis* on semen quality difficult even though previous authors appears to have established the link.

Conclusion

The prevalence of past and current *Chlamydia trachomatis* infection in this study was 5.58%and 3.55% respectively. Age and duration of marriage did not affect this prevalence. Current *Chlamydia trachomatis* infection resulted in abnormal seminal fluid analysis parameters. Measures to reduce or eliminate chlamydial infections as well as the use of first morning urine sample to detect current *Chlamydia trachomatis* infection are advocated.

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