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SHORT COMMUNICATION

The prevalence of *Chlamydia trachomatis* infection among infertile males and its association with abnormal semen characteristics in Delta State, Nigeria

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Abstract: *Chlamydia trachomatis* is the most common cause of sexually transmitted diseases that is not of viral origin and there is accumulating evidence of a significant role played by this pathogen in causing male factor infertility. This study thus aimed to determine the prevalence of *C. trachomatis* among infertile males and to evaluate their association with fertility status and abnormal semen characteristics. This study included 215 infertile male subjects who visited a major fertility clinic in Warri, Delta state, Nigeria. Forty apparently healthy males without complaints of infertility were enrolled as controls. Blood samples were collected from patients aseptically using venous puncture and semen samples were obtained after masturbation. *C. trachomatis* IgG antibodies were assayed for in blood specimens using the Dot rapid Assay Kit flow through Ct cassette and positive samples were further screened with an enzyme immunoassay technique. Semen samples were analyzed following World Health Organization guidelines. Forty-two (19.5%) out of 215 infertile male subjects were found sero-positive for *C. trachomatis*. *C. trachomatis* was significantly associated with male infertility when compared to controls (*P<0.001*). Age groups 20-29 years (43.3%) and 30-39 years (22.1%) significantly had higher prevalence of *C. trachomatis* (*P<0.001*), as compared to age groups 40-49 (7.9%) and > 50 years (3.7%). Sero-positivity for *C. trachomatis* antibodies was significantly associated with oligozoospermia (22.5%) and azospermia (61.5%) than with teratozoospermia (7.3%) and asthenozoospermia (6.3%) (*P<0.001)*. The prevalence of *C. trachomatis* among infertile males was high; there was an association between *C. trachomatis* infection and poor semen characteristics and infertility. There is need for routine screening for the pathogen in males with complaints of infertility so as to rule out this potentially correctable/reversible cause of infertility.

Keywords: *Chlamydia trachomatis*, infertility, oligozoospermia, epididimytis, semen, Nigeria

Scores of individuals infected with *Chlamydia trachomatis* is increasing worldwide (Andersen *et al*., 2002). *Chlamydia trachomatis* is now known to be the commonest cause of sexually transmitted diseases that is not of viral origin (Stock & Henrichfreise, 2012). *C. trachomatis* is an obligate intracellular organism and can cause numerous disease states in both men and women (Budai, 2007). Both sexes can display urethritis, proctitis (rectal disease and bleeding), trachoma, and infertility. The bacterium can cause prostatitis and epididimytis in men. In women, cervicitis, pelvic inflammatory disease (PID), ectopic pregnancy, and acute or chronic pelvic pain are frequent complications (Gottlieb *et al*., 2010). Infection with *C. trachomatis* is usually asymptomatic in both men and women (Fadawa, 2011), as such the pathogen are able to establish silent but continued destruction of spermatozoa and in the case of women gradual blocking of the uterine tubes (Silveira *et al*., 2010).

Recent research evidence strongly incriminates *C. trachomatis* to be a significant cause of abnormal semen characteristics among infertile males (Eley *et al*., 2005). It has also been hypothesized that the pathogen produces differential effect on semen parameters (sperm motility,

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sperm morphology and sperm count) (Eley *et al.*, 2005). *C. trachomatis* is known to cause epididimytis, which leads to inflammation and obstruction of passage ways for semen (Eley *et al.*, 2005; Weidner *et al.*, 2011). It is also known that *C. trachomatis* can effectively attach to the spermatozoa, and this can be a source of transmission of the bacteria to the female counterparts of the infertile couples whom are known to have far reaching consequences when infected (Karaer *et al.*, 2012).

In view of known consequences of *C trachomatis* infection on the fertility status of males, we thought it germane to carry out this study. The study thus aimed to determine the prevalence of *C. trachomatis* among infertile males and to evaluate their association with fertility status and abnormal semen characteristics in Warri, Delta State, Nigeria.

This cross-sectional descriptive study was carried out in Lily Fertility Centre in Warri, Delta State, Nigeria. The study was carried out from November 2011 to November 2012. Warri is a major oil city in Delta State, in the southern "Niger delta area' of Nigeria with a population of over 300,000 people according to the national population figures for 2006 (NPC, 2006). The study recruited 215 males attending the Centre's clinic with complaints of infertility. Forty apparently healthy males without complaints of infertility were used as controls. All participants (subjects and control) were asymptomatic for *C. trachomatis* infection and were having no prior complains of genito-urinary tract infections.

About 5 ml of venous blood samples were collected from subjects and controls via veni-puncture into plain capillary containers (clean disposable containers without anticoagulants or coagulant) and they were safely and securely transferred to the laboratory for analysis for antibodies to *C. trachomatis*. Semen samples were collected only by infertile male subjects by masturbation into sterile universal containers after 3-5 days of abstinence. Subjects were taught essential process in collection of semen that prevents contamination of the sample.

The qualitative Dot rapid Assay Kit flow through (Colloidal Gold Immuno-filtration Assay) Ct cassettes (Weifang Kuanghua Biotech co Ltd) was used to detect *Chlamydia trachomatis* IgG in serum of subjects studied. This kit employs purified Ct polytypic mixed antigen, and the principle of Gold Immuno-filtration assay (GIFA) to detect *C. trachomatis* antibody in serum indirectly. In brief, venous blood in disposable plain containers were incubated at 37°C for more than 1 hour. It was then centrifuged at the sped of 4000 revolution per minute for at least 5 minutes and the serum were separated for detection of *C. trachomatis* antibodies. One drop of wash buffer was dispensed onto test window, waiting for the liquid to wet the membrane well, 150µl of serum into the test window waiting for the liquid to be completely absorbed 3 drops of colloidal Gold conjugate and Wash buffer was then added in three minutes intervals. The result were then read based on characteristic double red dots in control and test area produced in the case of a positive results or a single red dot on control area for a negative result. A test is said to be invalid when a red dot does is not present in the quality control area.

Serum samples found to be positive for *C. trachomatis* IgG antibodies using the qualitative dot rapid assay kit were further screened using an enzyme immunoassay test kit (Immunocomb Chlamydia test kit, Inverness medical innovations, ORGENICS, Israel). The Immunocomb *C. trachomatis* test is an indirect solid-phase enzyme immunoassay. The solid phase is a card with 12 projections ('teeth'). Each tooth is sensitized at two spots for the internal control and *C. trachomatis* synthetic peptides. The developing plate has 6 rows (A-F) OF 12 wells, each row containing a reagent solution ready to use at a different step in the assay. The test is performed stepwise by moving the card from row to row, with incubation at each step. The kit includes a positive control (containing antibodies to *C. trachomatis*) and a negative control to be included in each assay run.

Semen samples were analyzed following the world health organization guidelines (World Health Organization, 1999). Briefly, semen samples were diluted 1/20 with 10% formalin and read

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microscopically using the improved neubaeur counting chamber to determine the sperm count and Negrosin eosin stained semen samples were read microscopically to determine sperm morphology. Presence of leukocyte was observed for; significant leukocytospermia was defined as 10^6 peroxidase positive leukocyte/ml ejaculate.

Form of seminal infertility; oligozoospermia (sperm count <15 × 10^6/ml, teratozoospermia (normal forms of 4.0%), asthenozoospermia (progressive motility < 32 % (a + b), and azospermia (no sperm cells in ejaculate), was grouped based on the currently approved World Health Organization guidelines on reference values for human semen characteristics (Cooper *et al*., 2010). The WHO reference values at 5th percentile for semen characteristics puts normozoospermia at semen volume of 1.5 ml, sperm count of 39 and 15 million per ejaculate and per ml respectively; vitality of 58% alive; progressive motility of 32% and morphologically normal forms (strict criteria) of 4.0% (Cooper *et al*., 2010).

Bio-data and test results were analyzed using the SPSS v. 16 and statistical significance was said to exist when *P<0.05*. Written or verbal informed consent was received from subjects before inclusion of subjects. Ethical approval for this study was received from the Lily Hospitals Ethical Committee before commencement of the study.

The result of this present study shows that *C. trachomatis* antibodies was found positive in 42 (19.5%) out of 215 infertile males studied. All subjects sero-positive with the dot rapid assay were also found positive with the enzyme immunoassay technique (Immunocomb). The prevalence of *C. trachomatis* antibodies among infertile men were significantly different when compared to apparently healthy controls used for the study (*P*<0.001) (Table 1).

Table 1: Prevalence of *C. trachomatis* among infertile males studied

Parameters	No. studied	Number infected (%)	P value
Fertility status			
Infertile males	215	42 (19.5)	
Controls	40	2 (5.0)	<0.001
Age (years)			
20-29	30	13 (43.3)	
30-39	95	21 (22.1)	
40-49	63	5 (7.9)	
≥50	27	1 (3.7)	<0.001

There was an association between significant leukocytospermia and sero-positivity for *C. trachomatis* Age based distribution of *C. trachomatis* IgG antibody prevalence is shown in Table 1, the age groups 20-29 and 30-39 significantly had the highest prevalence of *C. trachomatis* antibodies (*P*<0.001) (Table 1).

Table 2 summarizes the distribution of *C. trachomatis* IgG antibodies among the various forms of seminal male infertility among subjects, *C. trachomatis* antibodies was found in all forms of seminal infertility, but there were significantly more associated with oligozoospermia and azospermia.

Table 2: Distribution of *C. trachomatis* among forms of abnormal semen characteristics

Form of seminal infertility	Number studied	Number infected (%)	P value
Oligozoospermia	129	29 (22.5)	
Teratozoospermia	41	3 (7.3)	

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Asthenozoospermia	32	2 (6.3)	
Azospermia	13	8 (61.5)	*<0.001*

The prevalence of *C. trachomatis* among infertile males in our study was high (19.5%). *C. trachomatis* is known to be highly endemic in Nigeria with variation in distribution within various regions of the country (Okoror, 2010). The prevalence report of this study is close to 24 % among infertile males in Benin City (Ibadin *et al*., 2009), but quite less than 62.6% recorded by Okoror & Agbonlahor (2012), among infertile males in some parts of Nigeria. The prevalence in the current study is quite higher than 9.8% in Enugu, Nigeria (Ikeme *et al*., 2011). Varying prevalence of *C. trachomatis* among infertile male subjects has been documented from other parts of world, 4.5% in Amiens, France (Hamdad-Daoudi *et al*., 2004) and 35.9% in Tunisia (Gdoura, 2001). The high prevalence of *C. trachomatis* in this study shows that the pathogen poses to be a continuous threat to the fertility of men in our locality.

Whether or not there exists a negative effect of *C. trachomatis* infection on semen parameters has been a subject of controversy among researchers. In this study, *C. trachomatis* infections were significantly associated with poor semen characteristics and male infertility. *C. trachomatis* antibodies were higher in prevalence among infertile males with oligozoospermia (22.5%) and azospermia (61.5%) than other forms of abnormal semen characteristics (teratozoospermia and asthenozoospermia). *C. trachomatis* are known to be capable of attaching to sperm cells thereby destroying them by altering of its integrity and also may act as a vector for the transfer of the bacteria to the uterus of women (Okoror *et al*., 2007; Cunningham & Beagley, 2008). From the findings of this study, it can be argued that *C. trachomatis* represent a major factor to be considered when finding the root cause of infertility in men, therefore screening for *C. trachomatis* among males with complaints of infertility should be made a routine practice.

There was an observed difference in the distribution of *C. trachomatis* antibodies among the various age groups studied. The age groups 20-29 years and 30-39 years significantly had the highest prevalence of the pathogen. *C. trachomatis* has been found to be more commonly associated with younger age (Schillinger *et al*., 2005; Ikeme *et al*., 2011), were sexual activity is usually at its highest peak.

Our study has been able to provide a baseline data of an association between *C.trachomatis* infection and poor semen characteristics among infertile males who visited our fertility centre. Prevalence of asymptomatic carriage of this pathogen by males in the general population Warri, Nigeria is not known; further epidemiologic studies are needed to improve our knowledge of the prevalence, pattern and distribution of *C. trachomatis* in the general population of males whom are fertile and in individuals with genital tract infections and other urologic diseases in our locality.

In conclusion, a high prevalence of *C. trachomatis* (19.5%) antibodies was found among infertile subjects in Warri, Nigeria, there was a significant association between C. *trachomatis* infection and male infertility among the subject studied. *C. trachomatis* infection was also more significantly associated with azospermia and oligozoospermia. Routine screening for *C. trachomatis* among infertile males should be made a routine in view of its known deleterious effect on spermatozoa.

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