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MOLECULAR DETECTION OF *CHLAMYDIA TRACHOMATIS* INFECTION IN INFERTILE AND PREGNANT WOMEN ATTENDING ASSISSTED CONCEPTION CLINICS IN PORT HARCOURT, NIGERIA

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ABSRACT
Chlamydia trachomatis (CT) infections remain a leading sexually transmitted disease throughout the world and studies have linked this infection to adverse pregnancy, female infertility and poor outcomes in infant health. This study was to evaluate the prevalence of this infection among infertile women attending assisted conception clinics (Case study group) and those attending antenatal clinics (Control group) in Port Harcourt, Nigeria. A total of 120 endocervical swab samples were obtained from consenting women, sixty (60) from each group. A polymerase chain reaction (PCR) based method was used for the detection of this infection. Overall prevalence rate was 60% (72 0f 120). The result further revealed a statistically non-significant (p=0.480) higher rate among the infertile women (39(65%) of 60) as against 33(55%) of the 60 pregnant women (control group). The greater percentage of the positive cases was within the 25-34 age group in both subjects and this age group is known to be more sexually active. Results also showed that 15.8% of the total subjects were asymptomatic. In conclusion, this study has revealed that the infection is significantly present in the two groups and could be a major contributor to increase in adverse pregnancy outcomes and infertility as seen presently. 15.8% asymptomatic rate is of great concern and all these therefore underscore the urgent need for more awareness in our local setting, regarding this infection, especially on early diagnosis to guide management and care.

KEYWORDS: *Chlamydia trachomatis*, Polymerase Chain Reaction, Infertile women, Pregnant women.

INTRODUCTION
Chlamydia trachomatis infections have been suspected to be the most prevalent bacterial sexually transmitted disease throughout the world and currently research, screening and even treatment are being focused on females, who are predominately considered to be the major group affected by this disease and consequently cause infertility in them.[1] More so apart from its potential to cause genital tract infections, it has been associated with long-term complications leading to infertility.[2-5] The primary site of *Chlamydial* infections of the genital tract is the columnar epithelial cells of the endocervix of women and the urogenital epithelia of men.[6]

Chlamydia infection is sometimes referred to as a silent epidemic because it is often asymptomatic in most people, and the infection can linger for months or years before being diagnosed. However, signs and symptoms when present may include vaginal bleeding or discharge, abdominal pain, painful sexual intercourse, fever, painful micturition or polyuria. Infection may also affect non-genital sites such as the lungs and the eyes.[7] It is therefore known as the common cause of urethritis, cervicitis, including Pelvic Inflammatory Disease (PID), ectopic pregnancy, tubal factor or infertility, epididymis and prostatitis (in men), and reactive arthritis.[8-12] Information from CDC, showed that 20% of women who develop PID became infertile, while 18% develop chronic PID and 9% end up with tubal pregnancy.[13] Some women may develop urethritis, and the symptoms may include dysuria without frequency while some women with Chlamydia infection end up having acute salpingitis with or without endometritis. Moreso research has linked *Chlamydia trachomatis* to adverse pregnancy and infant health outcomes including miscarriages, stillbirth, ectopic pregnancy, preterm birth, neonatal conjunctivitis, neonatal pneumonia, etc). Westron[14] reported that 10% of women with Chlamydia infection who do not conceive end up having ectopic pregnancy. *Chlamydia trachomatis* infections even though treatable, yet only a few countries have routine pregnancy screening and treatment programs for these infections. Kristina[15] affirmed that screening and treatment of sexually transmitted infections (*Chlamydia trachomatis* infection inclusive) in pregnancy is known to be an

overlooked opportunity to improving the health outcomes of women and infants worldwide. This is instructive of Nigeria where routine screening of pregnant women for *Chlamydia trachomatis* infections is not yet emphasized. According to World Health Organization (WHO), over 92 million *Chlamydia trachomatis* (CT) infections occurred worldwide as at 1999 and in the recent time their guidelines recommend that screening strategies be aimed mostly at women.[16] This study was aimed to evaluate the prevalence of *Chlamydia trachomatis* infection among infertile women and pregnant women attending assisted conception clinics in Port Harcourt, Nigeria, using the Polymerase chain reaction (PCR) technique.

MATERIALS AND METHODS

Study Design/ Sample Collection

The study was conducted from two assisted conception clinics (Pristine Medical Consultants and Care Women Clinic) and one antenatal clinic (Rumuokwurushi Primary Health Centre) in Port Harcourt. The subjects were all married women aged between 15 and 44 years. The study involved randomly selected 60 infertile women who have been married and who had had continuous coital relationship for at least one year (Case study group) and 60 pregnant women (Control group). Short structured questionnaires were also administered for other demographic data.

Following ethical clearance from Rivers State Hospitals Management Board (RSHMB), informed consent was obtained from the participating subjects. Using sterile specula, a total of 120 endocervical swab samples were randomly collected from these subjects and transported to the laboratories for processing.

Sample Processing

DNA Extraction: DNA Extraction Procedure: Quick-DNA universal Extraction Kit was used.200µl of sample was added into a microcentrifuge tube, followed by 200µl of biofluid and 20µl of proteinase k. This was mixed thoroughly and incubated at 55^0C for 10 minutes. Then, 1 volume of Genomic Binding Buffer was added to the Centrifuged sample (digested sample) and mixed thoroughly, (i.e. 420µl genomic binding buffer to 420µl digested sample). The mixture was transferred to a Zymo-spin 11c-xl column in a collection tube, and then centrifuged at 12,000xg for 1minute, later the collection tube with the flow through was discarded. Into the column in a new collection tube, 400µl DNA pre-wash buffer was added and centrifuged again for 1 minute, and then the collection tube was emptied. Into the column 700µl of g-DNA wash buffer was added and centrifuged for 1 minute, and then the collection tube was emptied. Into the column 200µl of g-DNA wash buffer was added and centrifuged for 1 minute. The collection tube with the flow through was discarded. To elute the DNA, the extract was transferred to a clean micro centrifuge tube and 50µl DNA elution buffer was added into it, incubated for 5 minutes and then centrifuge for 1 minute. The extracted DNA was ready for quantification.

DNA Quantification Procedure: The machine was initialized using nuclease free water = 2µl, then blanked using Elution buffer =2µl. The Nucleic acid present was measured by adding 2µl of the extracted sample in the cuvette. Result was read and printed out. It is worthy of note that normal DNA concentration is from 5ng\dl-upwards, normal absorbance for DNA purity is (260/280), (1.70-2.0).

Amplification: The genes were amplified using an applied biosystem thermal cycler or PCR machine at final reaction volume of 30µl, ie 27µl of the master mix and 3µl of each template (Taq polymerase, dNTPs, MgCl and Buffer), DNA template, Primers, and Water. The Primers were-**(**FORWARD PRIMER; **OMP 1F:** GCCGCTTTGAGTTCTGCTTCCTC. REVERSE PRIMER; **OMP 1R:** CTTKAYTTTAGGTTTAGATTGAGC). All were mixed and loaded in the thermal cycler for amplification process which included a Primary Amplification at 24 cycles with the PCR temperature condition set at initial denaturation at 95^OC for 5minutes, denaturation at 95^OC for 60 seconds, annealing temperature at 60^OC for 1 min, extension was 72 ^{O}C for 1.30min. A secondary amplification at 30 cycles was also performed using similar PCR conditions.

Agar Rose Gel Electrophoresis: 4g of Agarose salt was added to 400ml of TBE and mixed. The mixture was boiled by microwaving for 8minutes and cooled to 45-50^oC; then 4µl ethidium bromide (EB) was added. The molten agarose was poured into moulds containing combs and allowed to set. The combs were carefully removed to obtain wells. Then 10µl of the extracted solutions were added into each well created and then a DNA ladder as a control/check. The electrophoretic machine was powered for 20mins. The Agarose gel was removed and transferred into a UV trans-illuminator for visualization and documentation.

RESULTS

Out of the 120 Infertile Women and Pregnant Women tested, 72 showed positive giving an overall prevalence rate was 60% (Table 1). The result further revealed a statistically non-significant (p=0.480) higher rate among the infertile women (39(65%) of 60) as against 33(55%) of the 60 pregnant women (control group). The greater percentage of the positive cases was within the 25-34 age group in both subjects and this age group is known to be more sexually active (Table 2). Results also showed that 15.8% of the total subjects were asymptomatic (Table 3).

Table 1: Frequency of *Chlamydia trachomatis* infection among Infertile Women and Pregnant Women.

No Tested (n)	No Positive	No. Negative	p-value	X^2
Pregnant Women n=60	33(55%)	27(45%)	0.480	0.500
Infertile Women n=60	39(65%)	21(35%)		
Total n=120	72(60%)	48(40%)		

Table 2: Age-related occurrence of *Chlamydia trachomatis* among the Women.

Type of Subject	Age Group (Yrs)	Total No of Positive (%)	No Positive in each Age Group (%)
PREGNANT WOMEN n = 60	15 – 24 25 – 34 35 – 44	33(55%)	NIL 22(37%) 11(18%)
INFERTILE WOMEN n = 60	15 – 24 25 – 34 35 – 44	39(65%)	NIL 30(50%) 9(15%)

Table 3: Detection Rates between Symptomatic and Asymptomatic Women.

	Total No of Positive Cases	Symptomatic and Positive	Asymptomatic but Positive	P-value	X^2-value
Pregnant Women n=60	33(55%)	23(38.3%)	10(16.7%)	0.024	5.121
Infertile Women n=60	39(65%)	30(50%)	9(15%)	0.001	11.308
Total n=120	72(60%)	53(44.2%)	19(15.8%)		

DISCUSSION

The prevalence rate of *Chlamydia trachomatis* in this study population was 60%. This is lower than the 61.7% rate among patients with tubal factor infertility as reported by Ojule *et al.*,[17] but higher than the 51.0% prevalence of *Chlamydia trachomatis* among pregnant and non-pregnant women in Lagos reported by Okoror *et al.*,[18], prevalence rate of 56.1% among females in Jos reported by Mawak *et al.*,[19] and the 18.2% among 77 women undergoing infertility in Lagos as reported by Oloyede *et al.*,[20] Prevalence rates vary from one geographical location to another, from one population to another, as well as influenced by the detection technique. The higher rate in this study could be due to the molecular detection technique employed, as against serological immunoassay methods used in many other studies. In most parts of Nigeria, routine screening for *C. trachomatis* does not appear to be of primary consideration for attendees at Infertility clinics and this could be attributable to oversight among health care providers and low awareness as regards this infection among the general public. Further findings from this study also revealed that whereas the infection was sufficiently present in each of the populations studied: 65% among the infertile women who were the case study group and 55% among the pregnant women who were the control group, there was no significant difference (p=0.480) between the two populations. Ojule *et al.*,[17] had previously reported a significantly higher rate of Chlamydia antibodies among patients with tubal factor infertility (61.7%) than in pregnant controls (34%) within the location of the present study. Although this study did not reveal any significant difference between the two groups of women, the prevalence rates are high and could be a major contributor to increase in adverse pregnancy outcomes and infertility as seen presently. This result is in tandem with that of Vitalis,[21], who out of 64 subjects for case study and 64 control, showed that 31(53%) of the subjects who had undergone laparatomy for tubal ectopic pregnancy (Case study) and 18(28%) of those with intrauterine pregnancy (Control) appeared positive for *Chlamydia trachomatis* infection respectively. CDC[13], reports that 20% of women who develop PID became infertile, while 18% develop chronic PID and 9% end up with tubal pregnancy. Therefore, there is need for more awareness regarding this infection, as well as more careful observation/examinations by Health Care Givers.

From Table 3, whereas 44.2% of the 72 positive cases where from symptomatic subjects, 15.8% were from asymptomatic subjects. This result is comparable to that of Vineets[22] who reported that the prevalence rate of Chlamydia infections was more among symptomatic population with a percentage of (11.11%) in comparison to asymptomatic population of (7.14%). While symptomatic cases are obvious indications for management, the asymptomatic cases go unnoticed, with their attendant complications. The 15.8% asymptomatic rate is therefore of great concern and all these underscore

the urgent need for more awareness in our local setting, regarding this infection, especially on early diagnosis to guide management and care.

Age-related prevalence revealed that a greater percentage of the positive cases were within the 25-34 years age group in the two groups of women and this age group is known to be more sexually active and at the peak of married life. Routine screening should be geared towards adolescents and women in the reproductive age as called upon by world health organization.

CONCLUSION

This study has revealed that *Chlamydia trachomatis* infection is significantly present in the two groups and could be a major contributor to increase in adverse pregnancy outcomes and infertility as seen presently. 15.8% asymptomatic rate is of great concern and all these therefore underscore the urgent need for more awareness in our local setting, regarding this infection, especially on early diagnosis to guide management and care.

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