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Original Research Article

The use of NAAT- PCR to determine asymptomatic chlamydia and gonorrhoea infections in infertile patients undergoing hysterosalpingogram at the federal medical centre, Yenagoa, South-South Nigeria

Chibuzor P. Oriji^{1*}, Kelvin E. Kiridi², Dennis O. Allagoa¹, James E. Omietimi¹,
Idowu B. Orisabinone¹, Olakunle I. Makinde¹, Chidiebere Njoku¹

¹Department of Obstetrics and Gynecology, Federal Medical Centre, Yenagoa, Bayelsa State, Nigeria

²Department of Radiology, Niger Delta University Teaching Hospital, Okolobiri, Bayelsa State, Nigeria

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***Correspondence:**

Dr. Chibuzor P. Oriji,

E-mail: chibuzor54@gmail.com

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ABSTRACT

Background: The roles of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in the aetiology of infertility due to tubal occlusion have been established by various studies. These organisms may lead to pelvic infection by ascending into the upper genital tract through any instrumentation like hysterosalpingography. The objectives were to determine the prevalence of asymptomatic chlamydial and gonorrhoeal infections of the genital tract among women being investigated for infertility referred for hysterosalpingography; the relationship of these infections with tubal pathologies; and if routine endo-cervical screening and prophylactic antibiotics be recommended for these patients.

Methods: This was a descriptive cross-sectional study. The study population consisted of consecutive 220 infertile women that met the inclusion criteria for this study. Consent was obtained. Endo-cervical swab was taken for NAAT-PCR for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. Hysterosalpingography was carried out. Data was analyzed using SPSS (version 22).

Results: Amongst the 220 women, 9 (4.1%) had asymptomatic chlamydia infection. None had gonorrhoea infection and 211 (95.9%) had none of these two organisms. Forty-eight (21.9%) of the 220 women had bilateral tubal blockage and 9 (18.8%) out of these 48 women had asymptomatic infection with *Chlamydia trachomatis*.

Conclusions: There is a statistically significant association between tubal blockage and chlamydia infection ($p = 0.00$) [RR 4.31 (3.37-5.50)]. There was no evidence to recommend routine screening/antibiotics considering the low prevalence of microbes and the absence of post-HSG pelvic infection. Results from a multicenter randomized controlled trial will be more representative.

Keywords: Chlamydia, Gonorrhoea, Hysterosalpingogram, Infertility, Nucleic acid amplification tests-polymerase chain reaction, Tubal blockage

INTRODUCTION

Hysterosalpingography is an important radiological procedure in gynaecology used for evaluation of women

with infertility.¹ It outlines the uterine cavity, fallopian tubes, and adjacent peritoneal cavity following the injection of contrast material through the cervical canal.² HSG is the most common method of ascertaining tubal

patency in our environment and perhaps the most common form of uterine instrumentation in infertile women.³ HSG breaches the endocervix and may seed the endometrium and fallopian tubes with microorganisms found in the upper vagina and endocervix, which may lead to increased risk of pelvic infection after the procedure.⁴ Genital infections contribute significantly to infertility in our environment by causing tubal disease.² Infertility is defined as the inability to conceive after 12 months of regular unprotected sexual intercourse. Infertility is primary if a couple is unable to achieve pregnancy, while secondary infertility is the inability to achieve pregnancy after a previous pregnancy.

Worldwide, the prevalence of infertility is highest in Eastern Europe, North Africa/Middle East, Oceania, and Sub-Saharan Africa.² Generally, 6%-15.7% of couples are affected by infertility, worldwide.⁵⁻⁸ In Sub-Saharan Africa, the prevalence of infertility varies. It is 14.3% in The Gambia, 10.4% in Sudan and 15.7% in Nigeria.⁶⁻⁸ In the United Kingdom and the United States of America, infertility is estimated to be 6% and 10% respectively.⁹

Neisseria gonorrhoeae and *Chlamydia trachomatis* are among the leading causes of pelvic inflammatory disease (PID), which can lead to tubal factor infertility.⁴ Ten to twenty percent of women with endo-cervical gonorrhoea or chlamydia infections have salpingitis if untreated, thereby contributing significantly to tubal factor infertility, and 12% of women present with infertility after their first experience with PID.⁹ Women with secondary infertility have more infections with *Chlamydia trachomatis* and *Neisseria gonorrhoeae* when compared with women that have primary infertility.⁴

About two-thirds of the cases of infertility in Nigeria are from previous reproductive tract infections that led to severe damage to the female pelvic organs.⁴ Laparoscopic investigation of infertility in Nigerian hospitals has demonstrated the presence of pelvic infection and bilateral tubal occlusion in 35% of infertile women in Ibadan, South-West, Nigeria, 44% in Ile-Ife, South-West Nigeria and 65% of women in Jos, North-Central, Nigeria.¹⁰⁻¹² The objective of this study was to determine the types of tubal pathologies in infertile women undergoing HSG.

A prospective study of 53 infertile women undergoing HSG in Ilorin, South-West Nigeria, between 2011 and 2012 revealed a pre-HSG endocervical microbial infection in 54.7% of these women.¹³ The prevalence of *Chlamydia trachomatis* infection was 38.6% among patients with infertility at the University of Calabar Teaching Hospital, Cross River State, South-South Nigeria, while the prevalence of *Neisseria gonorrhoeae* was 2% among women attending General Hospital in Calabar, South-South Nigeria.^{14,15} The prevalence for chlamydia and gonorrhoea infections were 17.6% and 2.4% respectively at the University College Hospital, Ibadan, South-West, Nigeria.²

Different studies have reported various prevalence rates of post-HSG clinical pelvic infection. 1.4% was reported by Pittaway; 3.1% by Stumpf; 3.4% by Moller; and 44% by Lema.¹⁶⁻¹⁹ Post-HSG infection occurs more in women with findings of damaged or dilated fallopian tubes during the procedure.^{2,16} Some authors have suggested that prophylactic antibiotics should be considered in all women before undergoing any instrumentation.²⁰ Prophylactic antibiotics are not routinely given before HSG. However, where they are given, the most common regimen for prophylaxis is doxycycline 100 mg orally twice daily for five days.²¹

In 2010, a Cochrane review of antibiotic prophylaxis for transcervical procedures revealed insufficient evidence to accept or reject the routine use of antibiotics in such procedures.²¹ Stumpf advocates routine endo-cervical screening of women, and treatment before undergoing HSG.¹⁷ To offer these women endo-cervical screening or prophylactic antibiotics and treat only patients that have infections, remains debatable as there is presently no consensus in the literature.

Nucleic acid amplification tests (NAATs) are powerful molecular techniques for the screening and diagnosis of infectious microorganisms.²² Traditionally, diagnostic tests for chlamydia and gonorrhoea are based on laboratory cultures of the microorganisms. These laboratory culture tests are less sensitive for detection of chlamydia and gonorrhoea and have longer turn-around time.²² NAATs are highly sensitive and specific for chlamydia and gonorrhoea testing on urine, cervical swab and urethral swab specimens.²² They are however expensive and not readily available. There are different types of NAATs. They include polymerase chain reaction, loop mediated isothermal amplification, quantitative nucleic acid sequence-based amplification, transcription-mediated amplification, strand displacement amplification, ligase chain reaction, rolling circle amplification and branched DNA signal amplification.²² The specificities of NAATs range from 97.9-99.6%.²² While the sensitivities of NAATs range from 83.3-96.7%.²² The objectives were to determine the prevalence of asymptomatic chlamydial and gonorrhoeal infections of the genital tract among women being investigated for infertility referred for hysterosalpingography; the relationship of these infections with tubal pathologies; and if routine endo-cervical screening and prophylactic antibiotics be recommended for these patients. The NAAT - PCR was used for this study.

METHODS

This study was carried out at the obstetrics and gynecology and the radiology departments of the Federal Medical Centre, Yenagoa, Bayelsa State, South-South, Nigeria between June to September, 2018. It was a descriptive cross-sectional study. The study population consisted of 220 consecutive patients being investigated for infertility sent for hysterosalpingography.

Eligible women were counselled and enrolled in the study after giving a written informed consent. Explanation of the nature of the study, procedure and likely benefits to the patient preceded the administration of written consent. Women who were menstruating, had abnormal uterine/vaginal bleeding, pelvic inflammatory disease, history of contrast hypersensitivity or who declined consent/incompletely filled consent form were excluded from the study.

The sample size for this study was calculated using the formula:²³

$$n = z^2pq/d^2$$

Where,

n = minimum sample size

z = normal standard deviation set at 95% confidence limit = 1.96

p = prevalence of asymptomatic Chlamydia and gonorrhoea infections in a previous study

q = 1 - p (complementary probability)

d = margin of error = 7% = 0.07

Prevalence of asymptomatic Chlamydia and gonorrhoea infections based on previous studies are 38.6% and 2% respectively.^{7,8}

Therefore,

For Chlamydia trachomatis

$$p = 0.386$$

$$q = 1 - 0.386 = 0.614$$

$$n = (1.96)^2 \times 0.386 \times 0.614 / (0.07)^2$$

$$n = 3.8416 \times 0.386 \times 0.614 / 0.0049$$

$$n = 185.8$$

For Neisseria gonorrhoea

$$p = 0.02$$

$$q = 1 - 0.02 = 0.98$$

$$n = (1.96)^2 \times 0.02 \times 0.98 / (0.07)^2$$

$$n = 3.8416 \times 0.02 \times 0.98 / 0.0049$$

$$n = 15.4$$

$$\text{Thus: } 185.8 + 15.4 = 201.2$$

This was rounded up to 200.

The minimum sample size was adjusted to 220, giving room for 10% attrition.

Therefore, 220 patients who met the inclusion criteria were recruited for this study.

Selected patients' bio-data and type of infertility (primary or secondary), previous gynecological and obstetric history were entered into a predesigned proforma. HSG

was carried out at the proliferative phase of the menstrual cycle. Premedication with oral Hyoscine N Butyl Bromide 10 mg and 50 mg of Diclofenac were given 30 minutes before the procedure to reduce tubal spasm and post-procedure pelvic pain respectively. Protective lead apron and eye shield were put on.

After passing urine, the patient was initially placed in the supine position on the X-ray table. The scout radiograph of the antero-posterior view of the pelvis was taken. She was then placed in the lithotomy position. After hand-washing and putting on sterile gloves, she was cleaned and draped to ensure privacy. With good light source, an un-lubricated plastic disposable sterile speculum was inserted into the vagina to expose the cervix.

The ecto-cervix was cleaned with savlon solution, and the anterior lip grasped with a tenaculum. Cohen cannula was inserted into the cervix, and the speculum was removed for patient's comfort. Water-based contrast medium (10-15 ml) was warmed to body temperature, and injected slowly into the endometrial cavity. Three radiographs to outline the endometrial cavity, fallopian tubes and intraperitoneal spillage were obtained respectively. The cannula was removed, the vulva was cleaned, and she was asked to dress up. The HSG films were reported by the consultant radiologist and same transferred into the appropriate proforma. In addition, the microbial profile from the pre-HSG swabs, and outcome of the procedure were discussed with the women. The women who required treatment were treated accordingly.

DNA extraction

The tip of the swab stick was cut into a microcentrifuge tube and 2 ml of normal saline was added into it. The microcentrifuge tube containing the cotton swab and normal saline was rocked rigorously using a vortex mixer for five minutes. Two hundred microlitres of this sample was added to another microcentrifuge tube and 200 µl of Biofluid, 200 µl of cell buffer and 20 µl of proteinase K were added to this sample. The sample was mixed thoroughly and then incubated at 55°C for 10 minutes. Then, 420 µl of genomic binding buffer was added to the 420 µl digested sample above, and mixed thoroughly. The mixture was transferred to a Zymo-Spin™ IIC-XL Column in a collection tube and centrifuged at 12,000 × g for 1 minute. The collection tube with the flow through was discarded. Four hundred microlitres of DNA pre-wash buffer was added to the column in a new collection tube and centrifuged for 1 minute. The collection tube was emptied. Seven hundred microlitres of g-DNA wash buffer was added to the column in the collection tube and centrifuged for 1 minute. The collection tube was again emptied. Another 200 µl of g-DNA wash buffer was added to the column in the collection tube and centrifuged for 1 minute. The collection tube was discarded with the flow through. To elute the DNA, the sample was transferred to a fresh microcentrifuge tube and 50 µl of DNA elution buffer was added to it,

incubated for five minutes and then centrifuged for 1 minute.

DNA quantification

The extracted genomic DNA was quantified using the nano drop 1000 spectrophotometer. The software of the equipment was launched by double clicking on the nano drop icon. The equipment was initialized with 2 µl of sterile distilled water and blanked using normal saline. Two microliters of the extracted DNA were loaded onto the lower pedestal; the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the “measure” button.

Amplification of the outer membrane protein 1 of Chlamydia

Primers CT1: 5'-GCCGCTTTGAGTTCTGCTTCCTC-3' and CT2: 5'-ATTTACGTGAGCAGCTCTCTCAT-3' were used for initial amplification of omp1 gene, whereas primers CT3: 5'TGACTTTGTTTTCGACCGTGT-3' and CT4: 5'-CCGAAGATTTTCTAGATTTTCATCTTGT-3' were used for nested PCR.

The 50 µl PCR mixture contained 0.4 µl of both forward and reverse primer, x² master mix comprising of 0.2 mM dNTPs, 1.5 units of Taq polymerase, 1.8 mM of MgCl₂ and buffer. The initial PCR contained 5 µl of extract. Nested PCR was conducted with 1 µl of the initial PCR. PCR was conducted on a 9700 ABI thermocycler (Applied Biosystems). In the first round of PCR, samples were initially heated at 95°C for 7 min, with 9 cycles of denaturation (95°C for 1 min), annealing (60°C for 1 min), and extension (72°C for 1.5 min), followed by an additional 24 cycles with the annealing temperature reduced to 55°C. The nested PCR used the same conditions, except the latter part of the program which was increased to 30 cycles from 24. PCR products were resolved on agarose gel electrophoresis stained with EZ vision dye and viewed on a blue light transilluminator for an 800 bp fragment size.

Amplification of the Neisseria gonorrhoeae Orf1 gene

The primers Orf1F: 5'-CAACTATTCCCGATTGCGA-3' and Orf1R: 5'-TTATACAGCTTCGCCTGAA-3' were used for the PCR at a final volume of 40 µl other components included, x² master mix comprising of 0.2 mM dNTPs, 1.5 units of Taq polymerase, 1.8 mM of MgCl₂ and buffer.

The PCR was conducted with 7 µL of the extracted DNA on a 9700 ABI thermocycler (Applied Biosystems) at 40 cycles. Each cycle consisted of 30 seconds at 94°C, 30 seconds at 56°C, and 1 minute at 72°C. The amplified PCR product was analyzed by electrophoresis in a 2% agarose gel.

Statistical analysis

Data regarding asymptomatic chlamydia and gonorrhoea infections, report of hysterosalpingography and other relevant patients' data (age, tribe, parity, weight, height and BMI) collected with a semi-structured questionnaire, were analyzed using statistical software version 22, SPSS Inc.; Chicago, USA). p value less than 0.05 was taken as being statistically significant. Result is presented in tables, charts, frequencies and percentages.

RESULTS

The mean age of the women that participated in the study was 33.98±3.85 and ranged between 24 and 40 years. Women with tertiary level of education were 156 (70.9%), those with secondary level of education were 49 (22.3%), a few women had no formal education and there was no woman with primary level of education (Figure 1). All the women that participated were Christians. Most of the women (72.2%) were nulliparous, 36 (16.4%) were multiparous, others were primiparous, and none of the women was grand multiparous (Figure 2). More than half of the women (55%) were of the Ijaw tribe (Table 1).

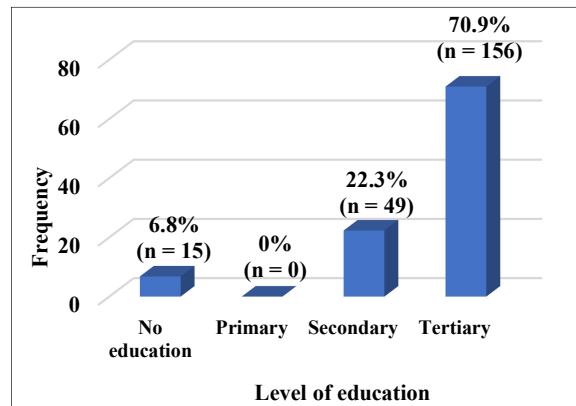


Figure 1: Distribution of level of education of the participants.

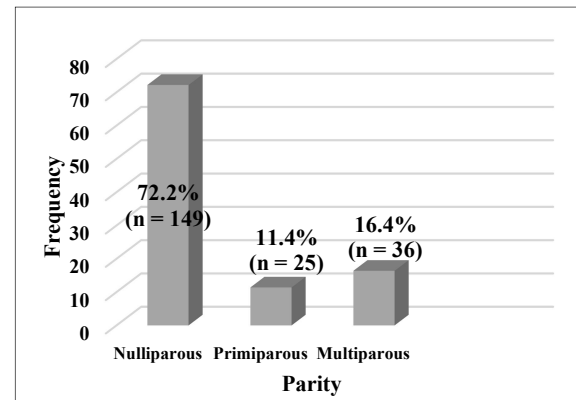


Figure 2: Distribution of parity of the participants.

Two hundred and fourteen (97.3%) of the women were married in a monogamous family setting, while 6 (2.7%) of the women were married in a polygamous family setting. Most of the women that participated in this study were traders (Table 2). Figure 3 shows the distribution by type of infertility; most had secondary infertility. The average body mass index of the participants was $26.49 \pm 4.38 \text{ kg/m}^2$. The range was $17.22\text{-}37.73 \text{ kg/m}^2$. Forty-five (20.5%) of the women were obese and 5 (2.3%) were underweight (Table 3). Majority (76%) of the women had at least one premarital termination of pregnancy.

Table 1: Distribution of tribe of the participants.

Tribe	Frequency	Percent
Igbo	45	20.5%
Ijaw	122	55.5%
Yoruba	5	2.3%
Others	48	21.8%
Total	220	100%

Table 2: Distribution of occupation of the participants.

Occupation	Frequency	Percent
Trader	100	45.5%
Civil Servant	60	27.3%
Engineer	1	0.5%
Banker	3	1.4%
Lawyer	3	1.4%
Student	22	10.0%
Military officer	5	2.3%
Caterer	10	4.5%
Teacher	6	2.7%
Unemployed	10	4.5%
Total	220	100%

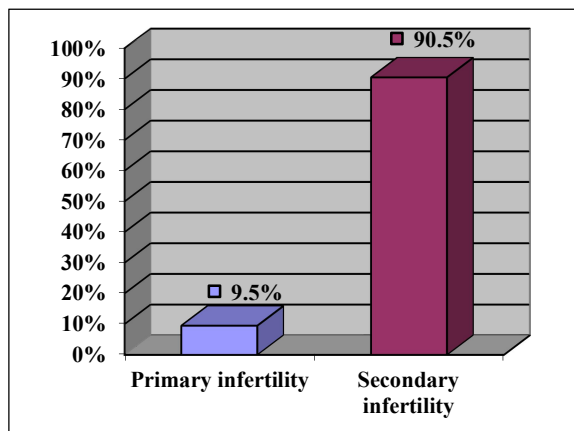


Figure 3: Distribution of the type of infertility in the participants.

Nine (4.1%) of the 220 women that participated in this study had asymptomatic chlamydia infection. None had

gonorrhoea infection and 211 (95.9%) had none of these two organisms.

Table 3: Distribution of body mass index of the participants.

BMI	Frequency	Percent
Underweight	5	2.3%
Normal	75	34.1%
Overweight	95	43.2%
Obese	45	20.5%
Total	220	100%

Table 4: Pattern of tubal pathology as seen on hysterosalpingography.

Pathology	Number	Percent
Both normal	136	61.8%
Right normal tube, left blocked	15	6.8%
Right normal tube, left hydrosalpinx	1	0.5%
Left normal tube, right blocked	6	2.7%
Left normal tube, right hydrosalpinx	4	1.8%
Bilateral tubal blockage	48	21.9%
Bilateral hydrosalpinx	10	4.5%
Total	220	100%



Figure 4: Bilateral tubal blockage.



Figure 5: Bilateral hydrosalpinx.

Table 4 shows types of tubal blockage. Bilateral normal tubes characterised by free intraperitoneal spillage with normal size tubes were present in 136 patients (61.8%). Bilateral tubal blockage was observed in 48 patients (21.9%) (Figure 4). Nine (18.8%) of these 48 women had asymptomatic infection with *Chlamydia trachomatis*. This association was statistically significant ($p=0.00$) [RR 4.31 (3.37-5.50)].

All the participants with asymptomatic chlamydia infection had bilateral tubal blockage. There was no participant with unilateral tubal blockage that had asymptomatic infection with *Chlamydia trachomatis*.

Hydrosalpinx, either bilateral or unilateral was observed in 15 patients (6.8%) (Table 4). Bilateral hydrosalpinx was observed in 10 patients (4.5%) (Figure 5). Right hydrosalpinx alone was more common than left hydrosalpinx alone. There was no patient with a combination of tubal blockage and hydrosalpinx.

Ten (4.5%) of the women had peritubal adhesions. Seven (3.1%) had right and 3 (1.4%) had left peritubal adhesions. None of them was associated with asymptomatic chlamydia infection.

Amongst the 220 participants, 70 (31.8%) had uterine fibroids. Forty-nine (70%) of these women with uterine fibroids had bilateral tubal blockage, 9 (12.9%) had right sided tubal blockage and 12 (17.1%) had left sided tubal blockage. Sixty-three (28.6%) of these 220 women had uterine fibroids of ≥ 12 weeks' pregnancy size.

Fifteen (6.8%) of the 220 women had intrauterine adhesions, and none of these was associated with asymptomatic chlamydia infection.

All the participants were followed up at the gynaecological clinic and none of them developed post-HSG pelvic infection.

DISCUSSION

The mean age of the women that participated in the study was 33.98 ± 3.85 and ranged between 24 and 40 years. This range is within the reproductive age group. Sexually transmitted infections which predispose to tubal blockage occur more among women of reproductive age group. Majority of the women were older than 30 years. With advancing female age, there is increase in the number of women with age-related infertility, tubal blockage, uterine fibroids and endometriosis.¹¹ In this study, 70.9% of the women had tertiary level of education, which is consistent with studies from various parts of Nigeria.⁴ There were more traders compared to other professionals. This is as a result of the fact that this environment is not industrialized. Therefore, more people go into trading rather than wait for availability of white-collar jobs.

This study revealed that women with secondary infertility were more and accounted for 90.5% of the participants. This group of women had all the cases of asymptomatic chlamydia infection identified in this study and this is similar to reports from various parts of the world.⁴ This observation is probably due to the fact that most of the women with secondary infertility would have had a previous pelvic infection causing bilateral tubal blockage.⁴ Majority of the women in this study had at least one premarital termination of pregnancy which is a risk factor for infection with chlamydia and gonorrhoea.

The tubal abnormalities observed were similar to reports from some other studies.^{12,13} Laparoscopy, and dye test is the gold standard for evaluation of tubal occlusion, and is done to confirm tubal occlusion after hysterosalpingography. Other tests for tubal patency are hysterosalpingo - contrast - sonography, selective salpingography and tubal catheterization, transvaginal hydrolaparoscopy, salpingoscopy, falloposcopy and fertiloscopy.

In this study, 4.1% of the participants had asymptomatic infection with *Chlamydia trachomatis* which was associated with bilateral tubal blockage. This corroborates what has been documented in the literature about chlamydia infection and tubal blockage.²⁴ The reason for this is that chlamydia infection causes scarring (damage) of the fallopian tubes, which increases the risk of pelvic inflammatory disease (salpingitis) and tubal blockage.²⁴

Hydrosalpinx is defined as fluid-filled dilatation of the fallopian tube, and it appears as a contrast filled and dilated fallopian tube, often without free spill of contrast into the peritoneum on hysterosalpingography.¹⁶ Bilateral hydrosalpinx was present in 10 (4.5%) patients, while 5 (2.3%) patients had unilateral hydrosalpinx. More patients had right hydrosalpinx, which correlates with the report from an earlier study.¹⁷ This increased incidence of right hydrosalpinx was explained in another study to be due to the presence of the appendix.¹⁸ For better visualization of hydrosalpinx, the film taken 30 minutes after the end of the procedure is preferred.¹⁸ This delay allows for more accumulation of contrast media within the dilated fallopian tube(s).¹⁸

None of the women with peritubal adhesions had asymptomatic chlamydia infection. This is contrary to what is known and documented about chlamydia infection and pelvic adhesions. It is possible that the infection has not stayed long enough in these women to cause pelvic adhesions.

Amongst the 220 women, 31.8% had uterine fibroids, and 70% of these women with uterine fibroids had bilateral tubal blockage. The relationship between uterine fibroid and infertility is more of casual rather than causal. Therefore, before infertility can be attributable to the presence of uterine fibroids, all other causes of infertility

should have been ruled out. However, in this study, 22.3% of the women with uterine fibroids had bilateral tubal blockage in the absence of chlamydia infection. It is possible that the tubal blockage was as a result of the fibroids in these particular patients.

The limitation of this study was that it was a hospital-based study. The results may not reflect the findings in other tertiary institutions in Nigeria or the West African sub-region.

CONCLUSION

There is a statistically significant association between tubal blockage and chlamydia infection ($p = 0.00$) [RR 4.31 (3.37-5.50)]. This association appears to be strongly related between bilateral tubal blockage and asymptomatic chlamydia infection, as there was nobody with unilateral tubal blockage that had asymptomatic chlamydia infection. There was no one with asymptomatic chlamydia infection that had patent fallopian tubes.

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