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RESEARCH ARTICLE

CHLAMYDIA ANTIBODY TITRE AS SCREENING TEST FOR TUBAL FACTOR INFERTILITY– A CASE-CONTROL STUDY

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ABSTRACT

Background: Infertility is a socially distressing medical condition that remains a public health concern in many developing countries including Nigeria. Tubal disease is the most common cause of female infertility in Africa. Sero-epidermiological studies have indicated that Chlamydial infections account for a large proportion of asymptomatic genital tract infections by demonstrating a strong link between tubal pathology and the presence of chlamydial antibodies.

OBJECTIVE: To compare the serum Chlamydia antibodies between infertile women with tubal and non-tubal factor infertility.

Methodology: Prospective case control study involving one hundred and forty seven subjects with tubal factor infertility (cases) and similar number of subjects with non-tubal factor infertility (controls) following hysterosalpingography. Exclusion criteria were previous clinical or laboratory evidence of pelvic infection in the previous 6 months, history of endometriosis, tubal surgery and abdominal or pelvic surgeries like ruptured appendix and ruptured ectopic. Structured interviewer-administered questionnaire was applied to obtain information on socio-demographic data and relevant gynaecology history. The weight and height were measured and body mass index calculated. Five milliliters of venous blood samples was collected from each subject to assay antibodies to *Chlamydia trachomatis* using ELISA Ig G kit. The data obtained was processed and analyzed using the Statistical Package for Social Sciences (SPSS), version 20.0 Chicago Illinois.

Result: The prevalence of positive *Chlamydia trachomatis* antibodies was 48% giving a sensitivity of 70.1% and specificity of 74.1%. The accuracy of Chlamydia antibody test to distinguish subjects with bilateral tubal blockage diagnosed with HSG was 72.1%. One hundred and three cases (70.1%) and 38 (25.9%) controls tested positive to the Chlamydia antibody test (P of <0.001). A higher proportion of subjects with induced abortion (54.7%) compared with 42.8% without induced abortion had positive Chlamydia antibody index (p = 0.043) while 51.2% of subjects with secondary infertility compared with 29.8% subjects with primary infertility had positive Chlamydia index (p = 0.007). The median Chlamydia IgG index of subjects with tubal blockage was significantly higher (1.06 IQR 0.59, 1.65) than those without tubal blockage (0.47 IQR 0.29, 0.82) p <0.001. Median Chlamydia IgG index of subjects with history of induced abortion and secondary infertility was significantly higher than subjects with no history of induced abortion and primary infertility respectively (p <0.05).

Conclusion: The predictability of tubal blockage using *Chlamydia trachomatis* antibody was relatively high with sensitivity and specificity of 70.1% and 74.1% respectively. Subjects with secondary infertility had a two-fold chance of having positive Chlamydia index than primary infertility.

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INTRODUCTION

Infertility is a socially distressing medical condition that remains a public health concern in many developing countries including Nigeria (Adekunle, 2002). Infertility is a disease of the reproductive system, defined as the failure to achieve clinical pregnancy after 12 months of regular unprotected sexual intercourse (Zegers-Hochschild, 2009).

In contrast to an average prevalence rate of 10-15% in the developed world like Europe and America, the prevalence of infertility in sub-Saharan Africa ranged from 20-46% (Alvarez Nieto, 2006). The prevalence of infertility in Nigeria is 20-25% among married couples and about 40-50% of all consultations in gynaecology clinics are infertility related (Okonofua, 2003). It accounts for 14.8 – 38.8% of gynaecological consultations in teaching hospitals in southwest

and north-eastern part of Nigeria (Adegbola, 2013; Olatunji, 2003 and Idrisa, 2000). Infertility in women is ranked 5th highest serious global disability (WHO, 2003). Parenthood is one of the major transitions in adult life for both men and women. The stress of the non-fulfillment of a wish for a child is associated with emotions like anger, depression, anxiety, marital problems and feeling of worthlessness (Nachatigall, 1992). Marital discord often develops among infertile couples, especially when they are under pressure to make medical decisions. Couples experience stigma, sense of loss and diminished self-esteem as a result of infertility (Nachatigall, 1992). The causes of female infertility include ovulatory dysfunction (40%), tubal and pelvic pathology (40%), unexplained factors (10%) and unusual factors (10%) (National, 1990). Tubal disease is the most common cause of female infertility in Africa (Okonofua, 2003). There is an estimated 340 million new cases of "curable" infections occurring each year worldwide in men and women aged 15 - 49 years (Global strategy for the prevention and control of STIs, 2007). Most of these causes arising from tubal blockage are due to pelvic inflammatory disease from sexually transmitted infections, post-abortal and puerperal sepsis (Umeora, 2007). Endometriosis is also a cause of tubal factor infertility accounting for 7-14 % of cases (Patil, 2009). Other causes of tubal factor infertility include pelvic tuberculosis, scarring from abdominal and pelvic surgeries. Polyps near the tubal ostium can occlude the cornua and interstitial portions of the fallopian tube, causing proximal fallopian tube blockage (Patil, 2009).

The common causes of pelvic inflammatory disease are infections from *Chlamydia trachomatis* and *niesseria gonorrhea (*Soper, 2010*)*. Worldwide, *Chlamydia trachomatis* is considered to be a leading cause of sexually transmitted infections and female infertility (Ngandijo, 2003). According to the World Health Organization (WHO, 2003)101 million Chlamydial infections are detected annually worldwide. The incidence of Chlamydial infections in women has increased dramatically from 79 to 467 per 100,000 between 1987 and 2003 (Price, 2013).This is alarming as the morbidity associated with the disease will continue to rise, resulting in tubal factor infertility. A systematic review by Vajdic *et al.* (2005), reported an overall prevalence of *Chlamydia trachomatis* pelvic inflammatory disease as 4.6% with a higher prevalence of 5.6% among adolescents and young adults in Australia. Prevalence of genital *Chlamydia trachomatis* in parts of Africa varies considerably, ranging from 3.78% in Cameroon (Ngandijo, 2003), to as high as 68.25% in female sex workers in Niger republic (Mamodou, 2006). Data from African studies suggest that prevalence is on the rise and may exceed the value reported in developed countries (Nwanguma, 2009; Okoror, 2007 and Wariso, 2012). The prevalence of *Chlamydia trachomatis* in Nigeria varies according to the study population. Prevalence rate of 11% has been reported among female undergraduates in Port Harcourt (Wariso, 2012), while prevalence rate of 9.8% and 29.4% were reported in studies amongst attendees of a fertility clinic in Abeokuta and female residents in Enugu respectively (Ogiogwa, 2012 and Ikeme, 2011). Risk factors associated with Chlamydial pelvic inflammatory disease and its sequelae are young age, sexual intercourse at an early age, multiple sexual partners, inconsistent condom use and the presence of Chlamydial antibodies (Kimani, 1996). If untreated, it can result in pelvic inflammatory disease and lead to higher risk of infertility, ectopic pregnancy and chronic pelvic pain (Inglais, 1995).

Fitz Hugh Curtis syndrome or peri-hepatitis is also a complication of *Chlamydia trachomatis* infection. The challenge being faced in the management of Chlamydial disease is that as many as 70 to 80 percent of women and 50 percent of men have asymptomatic infection (Stamm, 1999).This results in a large reservoir of unrecognized, infected individuals who are capable of transmitting the infection to their sexual partners especially with rampant sexual promiscuity and lack of use of barrier protection. Sero-epidermiological studies have indicated that Chlamydial infections account for a large proportion of asymptomatic genital tract infections by demonstrating a strong link between tubal pathology and the presence of Chlamydial antibodies (Akande, 2003 and Thomas, 2000).Thus, Chlamydia immunoglobulin G (IgG) antibodies are associated with the development of late sequelae and are markers for previous exposure or endogenous reactivation of a previous Chlamydial infection. In chronically infected patients who are negative for endocervical *Chlamydia trachomatis*, a positive serological test may be the only indication of Chlamydial involvement (Den Hartog, 2004). An average of 4 out of 10 new gynaecological clinic patients attends for infertility. Since tubal factor infertility is very common in our environment with Chlamydia being a likely cause, this necessitates the need to assess the predictability of Chlamydia trachomatis infection in patients diagnosed with tubal factor infertility in our facility.

MATERIALS AND METHODS

This was a prospective case control study involving women with tubal factor infertility (cases) diagnosed by hysterosalpingography compared with women with non-tubal factor infertility (controls). This study was conducted on women attending the Gynaecological clinic of the Department of Obstetrics and Gynaecology, Lagos State University Teaching Hospital (LASUTH), Ikeja, Lagos in South Western part of Nigeria who were being managed for infertility. The sample size formula for case control study was used to calculate the sample size for this study using the prevalence of tubal factor infertility of 30-35% from previous study (National, 2012). One hundred and forty seven women (with tubal factor infertility) cases and 147 women (with non-tubal factor infertility) controls were recruited for the study. Approval for the study was obtained from the Ethics Committee of Lagos State University Teaching Hospital, Ikeja, Lagos State. The subjects were assured of confidentiality. Inclusion criteria were women of reproductive age group (between 20 and 40 years) with infertility attending our gynaecology clinic diagnosed of having bilateral tubal occlusion with or without hydrosalpinx (cases) and those with bilateral patent tubes (controls) on hystero-salpingogram. Exclusion criteria were previous clinical or laboratory evidence of pelvic infection in the last 6 months, history of endometriosis, tubal surgery and abdominal or pelvic surgeries like ruptured appendix and ruptured ectopic. Consenting patients that met inclusion criteria were consecutively recruited as cases and controls till the desired sample size was reached. Interviewer-administered structured questionnaire was administered to all subjects to obtain information on duration of marriage, number of lifetime sexual partners, past history of abnormal vaginal discharge, PID, chronic pelvic pains, termination of pregnancy, contraceptive use and age of sexual debut. Thereafter, the weight and height of subjects were measured and body mass index calculated. Five milliliters of venous blood samples was collected from each subject in a

plain sterile bottle for analysis of antibodies to *Chlamydia trachomatis* at the Research laboratory of the department in our institution. Anthropometric measurement of the weight was done using RGY 160 scale. This was standardized and subjects' weights were measured with light clothing on and without shoes. Measurements were taken to the nearest 0.1kg. The height was measured to the nearest 0.1m and body mass index was calculated as weight in kg divided by the square of height in meters. Subjects were classified as underweight when BMI was <18.5kg/m^2, normal with BMI between 18.5 – 24.9kg/m^2, overweight with BMI of 25.0 – 29.9kg/m^2 and obese with BMI ≥ 30kg/m^2 .

Laboratory Estimation of *Chlamydia trachomatis* Antibodies

The blood specimen was allowed to clot and then centrifuged at 10,000 revolutions per minute for 5 minutes using a Hettich^R universal 320 centrifuge. The sera was then withdrawn with a pipette and placed in a labelled micro-vial and stored in the freezer at -4 °C. The samples were collected and processed over the first four months of the study and analyzed for Chlamydia antibodies in batches. Serological assay was done using ELISA Ig G kit by Perfemed Group, Inc. South San Francisco, CA 94080, USA with the test kit having a sensitivity of 91.1% and specificity of 98.5%. The Ig G kit contained microwell strips, sample diluents, calibrator, negative control, positive control, washing concentrate, enzyme conjugate, tetramethylbenzidine (TMB) chromogenic substrate and stop solution. The principle is that purified *Chlamydia trachomatis*is coated on the surface of the microwells. The diluted patient's serum is added to the wells and the *Chlamydia trachomatis* Ig G specific antibody, if present binds to the antigen. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off and TMB chromogenic substrate is added. The enzyme conjugate's catalytic reaction is stopped at a specific time. The intensity of the colour generated is proportional to the amount of IgG specific antibody in the sample. The results are read by a micro well reader compared in a parallel manner with calibrator and controls. The results were presented using IgG index. An index of 0.99 was regarded as negative while index of 1.00 was regarded as positive.

Data processing and Statistical Analysis

The data obtained was processed and analyzed using the Statistical Package for Social Sciences (SPSS), version 20.0 Chicago Illinois. Percentages, mean and standard deviation of numerical variables were determined. The histogram plot of the outcome variable (Chlamydia index) was drawn to determine the pattern of distribution. Mann Whitney U test was used to compare the median of chlamydia index of independent groups. Chi squared test and Fischers' exact test was used to compare categorical variables. Kappa was used to assess the agreement between classification of tubal blockage using chlamydia index and tubal blockage following hystero-salpingogram. All independent variables that were significant ($P < 0.05$) on bivariate analysis were entered at once to assess their predictive ability. The quality of the model was adjudged good when the Omnibus tests of model coefficients were significant ($P < .05$) and the Hosmer–Lemeshow goodness-of-fit test value was > 0.05.

Confidence interval was set at 95% for all statistical tests. P value of less than 0.05 as considered statistically significant.

RESULTS

The socio-demographic characteristics of the cases and controls are presented in Table 1.There was no significant difference in the mean age of cases (36.5±5.6) and controls (35.4±5.7) ($p = 0.694$). All study participants were married. There was no difference in the educational status of cases and controls ($p = 0.677$) and majority of the study participants had tertiary education 92 (62.6%) cases and 88 (59.9%) controls There was no significant difference in the socioeconomic status, tribe and BMI of cases and controls ($p > 0.05$), however majority of cases and controls were middle class (75.5% vs 66.6%), Christians (70.7% vs. 79.6%), Yorubas (66.7% vs. 66.7%) and overweight (39.5% vs. 44.2%) respectively. The clinical characteristics of cases and controls were presented in Table 2a and 2b. There was no significant difference in parity, number of sexual partners, age at first sexual intercourse, history of PID, vaginal discharge, lower abdominal pain, fever, chronic pelvic pain and spontaneous abortion between cases and controls ($p>0.05$). A significantly higher proportion of cases compared to controls had history of induced abortion (53.1% vs. 38.1%), post abortal sepsis (12.9% vs. 2.0%), secondary infertility (91.7% vs. 76%), used condoms (25.9% vs. 16.3%) and oral contraceptive (21.1% vs.8.2%) $p <0.05$. The prevalence of *Chlamydia trachomatis* antibodies amongst the participants was 48% as 141 out of 294 participants were positive to the test.

The validity of the Chlamydia serology tests using HSG results as standard revealed a sensitivity of 70.1% (103/147) and a specificity of 74.1% (109/147). The accuracy of Chlamydia serology test to distinguish subjects with bilateral tubal blockage diagnosed with HSG was 72.1% as shown in Table 3. Amongst cases 103 (70.1%) had seropositivity to the Chlamydia antibody test while 38 (25.9%) of the controls had seropositivity to the test. This was statistically significant, p of <0.001. The factors associated with positive Chlamydia antibody index were shown in Table 4a and 4b. History of induced abortion and type of infertility were associated with Chlamydia index in this study. A higher proportion of subjects with induced abortion (54.7%) compared with 42.8% without induced abortion had positive Chlamydia antibody index ($p = 0.043$) while 51.2% of subjects with secondary infertility compared with 29.8% subjects with primary infertility had positive Chlamydia index ($p = 0.007$). Parity, number of sexual partners, age at first sexual intercourse, number of induced abortion, history of PID, post abortal sepsis, vaginal discharge, lower abdominal pain, fever, chronic pelvic pain use of condom, OCP and IUCD were not associated with positive Chlamydia index ($p >0.05$). The median Chlamydia Ig G index of subjects with tubal blockage was significantly higher (1.06 IQR 0.59, 1.65) than those without tubal blockage (0.47 IQR 0.29, 0.82) $p <0.001$. Median Chlamydia Ig G index of subjects with history of induced abortion and secondary infertility was significantly higher than subjects with no history of induced abortion and primary infertility respectively ($p <0.05$). Parity, multiple sexual partners, coitarche, history of PID and history of post abortal sepsis showed no association with chlamydia Ig G index ($P > 0.05$) as shown in Table 5. Table 6 illustrates multivariate analysis of predictors of positive chlamydia index.

Table 1. Socio demographic characteristics of subjects

Variable	Case 147 (%)	Control 147(%)	x2	p
Age group (years)				
< 30	12 (8.2)	15 (10.2)	1.45	0.694
30 – 34	42 (28.6)	49 (33.3)		
35 - 39	52 (35.4)	47 (32.0)		
40-42	41 (27.9)	36 (24.5)		
Mean±SD	36.5±5.6	35.4±5.7		
Educational level				
None	1 (0.7)	0 (0.0)	1.525	0.677
Primary	10 (6.8)	13 (8.8)		
Secondary	44 (29.9)	46 (31.3)		
Tertiary	92 (62.6)	88 (59.9)		
Socio economic status				
Low	36 (24.5)	50 (34.0)	3.221	0.073
Middle	111 (75.5)	97 (66.0)		
High#	0 (0.0)	0(0.0)		
Religion				
Christianity	104 (70.7)	117 (79.6)	3.080	0.079
Islam	43 (29.3)	30 (20.4)		
Tribe				
Yoruba	98 (66.70	98 (66.7)	0.415	0.937
Hausa	3 (2.0)	3 (2.0)		
Igbo	19 (12.9)	16 (10.9)		
Others	27 (18.4)	30 (20.4)		
BMI				
Underweight	2 (1.4)	1 (0.7)	2.11	0.549
Normal	40 (27.2)	44 (29.9)		
Overweight	58 (39.5)	65 (44.2)		
Obese	47 (32.0)	37 (25.2)		

NB: # = Not included in the analysis Age range = 22 – 42years

Table 2a. Clinical characteristics of case and control

Variable	Case n = 147 (%)	Control n = 147 (%)	x2	p
Parity				
Nulliparous	103 (70.1)	106 (72.1)	0.149	0.700
Multiparous	44 (29.9)	41 (27.9)		
Number of sexual partner				
0	10 96.8)	16 (10.9)	8.451	0.076
1	41 (27.9)	49 (33.3)		
2	29 (19.7)	34 (23.1)		
3	34 (23.1)	32 (21.80		
≥4	33 (22.4)	16 (10.9)		
Mean±SD	2.69±2.4	1.94±1.3		
Coitarche (years)				
< 18	23 (15.6)	15 (10.2)	1.934	0.164
≥18	124 (84.4)	132 (89.8)		
History suggestive of PID				
Yes	58 (39.5)	65 (44.2)	0.685	0.408
No	89 (60.5)	82 (55.8)		
History of induced abortion				
Yes	78 (53.1)	56 (38.1)	6.637	0.010*
No	69 (46.9)	91 (61.9)		
Number of induced abortion				
None	69 (46.9)	91 (61.9)	7.86	0.049*
1	30 (20.4)	24 (16.3)		
2	27 (18.4)	17 (11.6)		
≥ 3	21 (14.3)	15 (10.2)		
History of post abortal sepsis				
Yes	19 (12.9)	3 (2.0)	12.578	<0.001*
No	128 (87.1)	144 (98.0)		

NB: * = Significant

Table 2b. Clinical characteristics of case and control

Variable	Case n = 147 (%)	Control n = 147 (%)	x2	p
Duration of infertility (years)				
1 – 5	70 (47.6)	96 (65.3)	9.368	0.009*
6 – 10	49 (33.3)	22 (22.4)		
>10	28 (19.0)	18 (12.2)		
Type of infertility				
Primary	12 (8.3)	35 (24.0)	13.236	<0.001*
Secondary	133 (91.7)	111 (76.0)		
Vagina discharge				
Yes	99 (67.3)	94 (63.9)	0.377	0.539
No	48 (32.7)	53 (36.1)		
LAP				
Yes	53 (36.1)	47 (32.0)	0.546	0.460
No	94 (63.9)	100 (68.0)		
Fever				
Yes	25 (17.0)	21 (14.3)	0.412	0.521
No	122 (83.0)	126 (85.7)		
Chronic pelvic pains				
Yes	12 (8.2)	17 (11.6)	0.956	0.328
No	135 (91.8)	130 (88.4)		
Condom use				
Yes	38 (25.9)	24 (16.3)	4.006	0.045*
No	109 (74.1)	123 (83.7)		
Use of OCP				
Yes	31 (21.1)	12 (8.2)	9.834	0.002*
No	116 (78.9)	135 (91.8)		
Use of IUCD				
Yes	3 (2.0)	0 (0.0)	3.031	0.247#
No	144 (98.0)	147 (100.0)		
Spontaneous abortion				
Yes	32 (21.8)	34 (23.1)	0.078	0.780
No	115 (78.2)	113 (76.9)		

NB: * = Significant # = Fischers' exact test

Table 3. Sensitivity and specificity of Chlamydia index

Chlamydia index	HSG REPORT		
	Tubal blockage n = 147 (%)	Patent tubes n = 147 (%)	Total
Positive	103 (70.1%)[a]	38 (25.9) [b]	141 (48.0)
Negative	44 (29.9) [c]	109 (74.1) [d]	153 (52.0)
Total	147 (100.0)	147 (100.0)	294 (100.0)

a = 103, b = 38, c = 44, d = 109
x2 = 57.579 $p < 0.001$
Sensitivity – $\frac{a}{a+c}$ = 103/147 = 70.1%
Specificity = $\frac{d}{b+d}$ = 109/147 = 74.1%
Positive predictive value = $\frac{a}{a+b}$ = 103/141 = 73.0%
Negative predictive value = $\frac{d}{c+d}$ = 109/153 = 71.2%
Accuracy = $\frac{a+d}{a+b+c+d}$ = 103 + 109 / 294 = 72.1%
Kappa = 0.442

Table 4a.Factors associated with Chlamydia index

Variable	Chlamydia index		x2	p
	Positive n = 141 (%)	Negative n = 153 (%)		
Parity				
Nulliparous	95 (45.5)	114 (54.5)	1.817	0.178
Multiparous	46 (54.1)	39 (45.9)		
Multiple sexual partners				
Yes	129 (48.10	139 (51.9)	0.037	0.847
No	12 (46.2)	14 (53.8)		
Age at Coitarche (years)				
< 18	20 (52.6)	18 (47.4)	0.382	0.537
≥18	121 (47.3)	135 (52.7)		
History of induced abortion				
Yes	70 (54.7)	58 (45.3)	4.112	0.043*
No	71 (42.8)	95 (57.2)		
Number of induced abortion				
None	70 (49.6)	90 (58.8)	4.887	0.180
1	27 (19.1)	27 (17.6)		
2	21 (14.9)	23 (15.0)		
≥3	23 (16.4)	13 (8.6)		
History of PID				
Yes	61 (49.6)	62 (50.4)	0.226	0.234
No	80 (46.8)	91 (53.2)		
History of post abortal sepsis				
Yes	13 (59.1)	9 (40.9)	1.181	0.277
No	128 (47.1)	144 (52.9)		
Type of fertility				
Primary	14 (29.8)	33 (70.2)	7.262	0.007*
Secondary	125 (51.2)	119 (48.8)		

Table 4b. Factors associated with Chlamydia index

Variable	Chlamydia index		x2	p
	Positive	Negative		
Vagina discharge				
Yes	93 (48.2)	100 (51.8)	0.012	0.914
No	48 (47.5)	53 (52.5)		
LAP				
Yes	50 (50.0)	50 (50.0)	0.253	0.615
No	91 (46.9)	103 (53.1)		
Fever				
Yes	22 (47.8)	24 (52.2)	0.000	0.984
No	119 (48.0)	129 (52.0)		
Chronic pelvic pains				
Yes	12 (41.4)	17 (58.6)	0.558	0.455
No	129 (48.7)	136 (51.3)		
Use of condom				
Yes	33 (53.2)	29 (46.8)	0.873	0.350
No	108 (46.6)	124 (53.4)		
Use of OCP				
Yes	23 (53.5)	20 (46.5)	0.617	0.432
No	118 (47.0)	133 (53.0)		
Use of IUCD				
Yes	3 (100.0)	0 (0.0)	3.289	0.109#
No	138 (47.4)	153 (52.6)		

NB # = Fischers' exact test

Table 5. Association between tubal blockage and Chlamydia IG antibodies

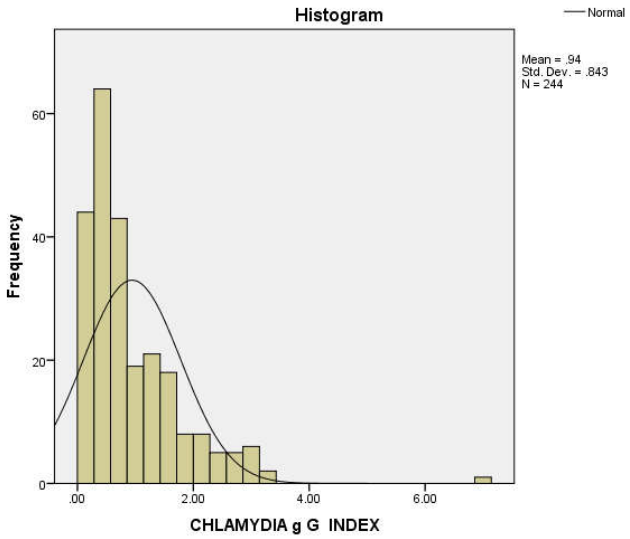
Variable	Chlamydia Ig Index Median (IQR)	U	p
Tubal blockage			
Yes	1.06, (IQR 0.59, 1.65)	4066.5	<0.001*
No	0.47, (IQR 0.29, 0.82)		
Parity			
Nulliparous	0.64, (IQR 0.36, 1.34)	5820.5	0.741
Multiparous	0.73 (IQR 0.37, 1.32)		
Multiple sexual partners			
Yes	0.67 (IQR 0.37, 1.34)	2310.5	0.677
No	0.25 (IQR 0.25, 1.41)		
Coitarche			
<18	0.76 (IQR 0.41, 1.17)	2998.5	0.559
≥ 18	0.65 (IQR 0.35, 1.34)		
History of PID			
Yes	0.72 (IQR 0.36, 1.46)	6957.0	0.514
No	0.62 (IQR 0.37, 1.31)		
Previous history of induced abortion			
Yes	0.74 (IQR 0.40, 1.47)	6202.0	0.045*
No	0.64 (IQR 0.33, 1.22)		
History of postabortal sepsis			
Yes	0.85 (IQR 0.40, 1.31)	1543.5	0.511
No	0.66 (IQR 0.35, 1.34)		
Type of infertility			
Primary	0.47 (IQR 0.28, 0.85)	3112.0	0.009*
Secondary	0.72 (IQR 0.39, 1.38)		

NB = * Significant U = Mann Whitney U test Chlamydia index range = 0.02 – 7.0

Table 6. Logistic regression of predictors of positive Chlamydia index

Variable	p	odds ratio*	95%CI
Primary infertility	1		
Secondary infertility	0.043	2.044	1.024 – 4.080
No induced abortion	1		
Induced abortion	0.488	1.192	0.725 – 1.960

NB = * Adjusted odds ratio



A Histogram plot showing the distribution of Chlamydia antibody index. The chlamydia antibody index was not normally distributed among the study participants.

Figure 1. Histogram plot showing the distribution of Chlamydia antibody index

Type of infertility made significant contribution towards positive Chlamydia index. Subjects with secondary infertility had two-fold chance (OR 2.044 95%CI 1.024 – 4.080) of having positive Chlamydia index compared to subjects with primary infertility after accounting for history of induced abortion.

DISCUSSION

Chlamydia trachomatis infections result in the development of clinical pelvic inflammatory disease in 10-16% of untreated cases (Svensso'n, 1991). Tubal infertility is a known sequel of pelvic inflammatory disease with the risk increasing after repeated episodes; 8% first episode; 23% second episode; 43% three or more episodes (Mol, 1999). Chlamydia trachomatis has been isolated in the upper genital tract of about a quarter of pelvic inflammatory disease patients (Haggerty, 2003). The overall prevalence of *Chlamydia trachomatis* Ig G antibody in this study was 48%. This finding is higher than values reported from Lagos (35.3%) (Olaleye, 2016), Ibadan (20.5%) (Morhason-Bello, 2014), Benin (20.5%) (Omo-Aghoja, 2007), Egypt (37.5%) (Ahmed, 2012) and India (5%) (El-Guindy, 2016). It was lower than 62.5% reported by Israel et al in Port Harcourt (Israel, 2011). The reason for the high prevalence of chlamydial antibodies in this study was unknown, however, the rising incidence of *Chlamydia trachomatis* infection globally may be a possible factor (Price, 2013). In addition, the infection is largely asymptomatic so poor diagnostic facilities in our environment leading to under and mis-diagnosis of most infections may also account for the finding in this study. The sensitivity of Chlamydia antibodies as a diagnostic marker for tubal factor infertility in this study was 70.1%. This is comparable to 64.2%, 78.4% and 72.7% sensitivity obtained from from Benin (Omo-Aghoja, 2007), Port Harcourt (Israel, 2011) and India (Surana, 2012) respectively. Studies from Egypt, the United kingdom and India showed lower sero-positivitites of 30% and 45%, 20% respectively compared to value obtained in this study and others (El-Guindy, 2016; Surana, 2012; Perquin, 2007). The sample size used in these studies and other infections such as tuberculosis which also cause tubal blockage could account for these findings.

The specificity of 74.1% obtained in this study as lower than 76.2% and 77.7% reported by (MorhMorhason-Bello, 2014) and Malik et al (Malik, 2009), respectively. The use of fertile controls in the studies from Omo-Aghoja in Benin and El-Guindy et al in Egypt could have accounted for the higher specificity of 83.7 and 93.3% respectively (Omo-Aghoja, 2007 and El-Guindy, 2016). This study, however, used infertile subjects who were likely to have similar characteristics as the cases. Studies that also use laparoscopy to define tubal blockage also demonstrated higher specificities of 83% (Ahmed, 2012), 84.6% (Perquin, 2007) and 100% (Swapnil, 2016) respectively. In this study, the degree of agreement of Chlamydia index and HSG in the diagnosis tubal blockage beyond chance measured by Kappa was low. In addition, the ROC curve showed that Chlamydia index cannot discriminate subjects with tubal blockage (AUC = 0.278). Though the prevalence of Chlamydial Ig G antibodies was significantly higher (70.1%) among cases than controls (25.9%), other factors such as *nieserria gonorrhea, mycoplasma hominis, mycobacterium tuberculosis*, endometriosis, tubal blockage from tubal surgeries can also cause tubal blockage which may explain the inability of Chlamydia index to serve as a good predictor of tubal blockage. Though some workers opined that Chlamydia antibody testing is not a strong diagnostic test for tubal factor infertility, it was suggested that it could be used as a screening test for tubal infertility before more expensive and invasive investigative procedures like hysterosalphingogram and laparascopy are carried out (Inglais, 1995; Olaleye, 2016; Morhason-Bello, 2014; Ahmed, 2012; El-Guindy, 2016) especially after excluding such confounding factors. It may also serve as a viable option where access to HSG is lacking especially in developing countries (Israel, 2011 and Malik, 2009). History of induced abortion was significantly associated with positive Chlamydial index (P = 0.043) and the median chlamydial index of these subjects was significantly higher than those without induced abortion (P = 0.045). *Chlamydia trachomatis* is considered to be a leading cause of sexually transmitted infections and female infertility worldwide (STD Surveillance 2003). Contrary to findings in other regions of the world, the prevalence of secondary infertility is higher in Africa (Malik *et al.*, 2009). Secondary infertility in this study was significantly associated with tubal factor infertility ($p < 0.001$). Subjects with secondary infertility also had significantly higher chlamydial index compared to those with primary infertility (P=0.009). Multivariate analysis showed that subjects with secondary infertility had two-fold chance (OR 2.044 95%, CI 1.024 – 4.080) of having positive Chlamydia index compared to subjects with primary infertility after accounting for history of induced abortion. This was similar to findings of Okunola et al (Okunola, 2005) where women with secondary infertility had significantly higher titre for chlamydia antibodies than those with primary infertility.

This study is not without some limitations. While micro-immunoflourescence (MIF) assay regarded as the gold standard in detecting serum Chlamydia antibodies was not used in this study, enzyme linked-immunosorbent assay (ELISA) used demonstrated a high specificity. ELISA is relatively cheap, readily available and easier to perform compared to the labour intensive MIF. Cross reactivity of other species of Chlamydia with *Chlamydia trachomatis* antibodies may also give some false positive results. Laparoscopy and chemotubation which is the gold standard in diagnosing tubal factor infertility was not employed to confirm those cases tubal blockage in this study. Other causative agents for bilateral tubal blockage like endometriosis and organisms such as *nieserria gonorrhea, mycoplasma hominis, mycoplasma genitalium* and *mycobacterium tuberculosis* were also not accounted for during the study. In conclusion, the prevalence of *Chlamydia trachomatis* antibodies in this study was relatively high. The predictability of *Chlamydia trachomatis* antibodies for diagnosing tubal blockage has sensitivity and specificity of 70.1% and 74.1% respectively. Subjects with secondary infertility had a two-fold chance of having positive Chlamydia index after accounting for history of induced abortion. Chlamydia Ig G antibody testing may be adopted as a screening method for tubal infertility before more expensive and invasive investigative procedures are carried out especially in women with secondary infertility.

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