International STD Research & Reviews
4(3): 1-9, 2016; Article no.ISRR.27420
ISSN: 2347-5196, NLM ID: 101666147

SCIENCEDOMAIN *international*
www.sciencedomain.org

Chlamydia trachomatis Infection: Serological Evidence in Women with Ectopic Pregnancy in Port Harcourt

Vitalis Chinonye Ibe[1], Israel Jeremiah[2*] and Eugene Ikeanyi[2]

[1]Department of Obstetrics and Gynaecology, University of Port Harcourt Teaching Hospital, Port Harcourt, Rivers State, Nigeria.
[2]Department of Obstetrics and Gynaecology, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author VCI designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors IJ and EI performed the statistical analysis and managed the analyses of the study. Author IJ managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ISRR/2016/27420
Editor(s):
(1) Jose Eduardo Serrao, Department of General Biology, Federal University of Viçosa, Brazil.
Reviewers:
(1) Suncanica Ljubin-Sternak, University of Zagreb, Croatia.
(2) Edith Angel-Muller, Universidad Nacional de Colombia, Colombia.
(3) Guadalupe García-Elorriaga, Mexican Social Security Institute, Mexico.
Complete Peer review History: http://www.sciencedomain.org/review-history/16886

Original Research Article

Received 31st May 2016
Accepted 18th October 2016
Published 11th November 2016

ABSTRACT

Background: Genital chlamydial infection is the most commonly reported bacterial sexually transmitted disease worldwide. It poses a public health problem of epidemic proportions. Untreated or poorly treated cases result in endosalpingeal damage and destruction of tubular architecture with multiple sequelae including tubal infertility and ectopic pregnancy.
Aims: To determine serological evidence of prior chlamydial infection in patients with ectopic pregnancy and to investigate associations between prior Chlamydia infection, its risk factors and ectopic pregnancy in Port Harcourt.
Materials and Methods: This was a case-control study carried out at the University of Port Harcourt Teaching Hospital, involving 64 women treated for ectopic pregnancy who served as subjects and the 64 women with normal intrauterine pregnancies drawn from the antenatal clinic

**Corresponding author: E-mail: dr.israel.jeremiah@gmail.com;*

who served as controls. Information on their socio-demographic characteristics and sexual behaviour were obtained using a semi-structured questionnaire. Serological evidence of prior chlamydial infection was determined in both groups by testing for the presence of Chlamydia immunoglobulin G antibodies in their blood. Statistical analysis was done using SPSS 20 statistical software.

Results: Chlamydia antibiodies were found in 53.1% of patients with ectopic pregnancy and 28.1% of the control (p=0.03). The presence of Chlamydia antibodies was associated with a two-fold risk of ectopic pregnancy with an Odd's ratio (O.R 2.25; CI 1.02-5.03). Two-thirds of the patients with Chlamydia antibodies had pelvic adhesions at laparotomy. Early coitarche, previous history of pelvic inflammatory disease, history of induced abortion and single women were the risk factors of *Chlamydia trachomatis* infection found to be positively associated with ectopic pregnancy.

Conclusion: There was a risk association between *Chlamydia trachomatis* infection and ectopic pregnancy.

Keywords: Chlamydia trachomatis; antibodies; pelvic inflammatory disease; ectopic pregnancy.

1. INTRODUCTION

In Nigeria, in the last two decades, ectopic pregnancy has assumed epidemic proportion with significant maternal morbidity, mortality and fetal wastage [1]. The incidence of ectopic pregnancy is directly related to the prevalence of salpingitis. Endosalpingeal damage secondary to pelvic inflammatory disease caused by *Chlamydia trachomatis* infection has been found to be a major risk factor for the development of ectopic pregnancy [2,3]. Studies from Northern and South-Western Nigeria showed macroscopic evidence of pelvic inflammatory disease at laparotomy in over 60% of patients with ectopic pregnancy [4-6]. Both increased incidence of *C. trachomatis* infection and the efficacy of antibiotic therapy in preventing total tubal occlusion, after an episode of salpingitis, are related to increased incidence of ectopic pregnancy [7]. In females, up to 40% of chlamydial cervicitis may ascend to the endometrium and is responsible for the aetiology of endometritis and salpingitis [8,9]. Fallopian tube samples of patients with ectopic pregnancy have also been found positive for *C. trachomatis* deoxyribonucleic acid using the polymerase chain reaction. The frequent association between chlamydial cervicitis and the presence of vaginal clue cells or gram stain abnormalities indicative of an overgrowth of anaerobic bacteria has led to the speculation that *C. trachomatis* alters the normal vaginal ecology thereby setting the stage for a complex polymicrobial upper genital tract infection9. Untreated or poorly-treated chlamydial infection of the genital tract can therefore have serious long term reproductive consequences. Late sequelae of the disease include chronic pelvic inflammatory disease, tubal blockage and infertility, chronic pelvic pain and ectopic pregnancy [10-12]. Seroepidemiological studies have indicated that Chlamydial infections account for a large proportion of asymptomatic genital tract infection, by demonstrating a strong link between tubal pathology and the presence of chlamydial antibodies. The chlamydial immunoglobulin G antibodies are associated with the development of late sequelae and are markers for previous exposure. In chronically infected patients negative for endocervical *C. trachomatis*, a positive serological test may be the only indication of chlamydial involvement. Serum immunoglobulin G antibodies to Chlamydia have also been associated with tubal factor infertility and ectopic pregnancy [13-16].

This study therefore evaluates prior exposure to *C. trachomatis* in patients treated for ectopic pregnancy by determining the presence of immunoglobulin G antibodies in their sera. By comparing same with women carrying normal intrauterine pregnancy, it seeks to determine the risk association between prior *C. trachomatis* infection and ectopic pregnancy in the University of Port Harcourt Teaching Hospital, South-South, Nigeria.

2. MATERIALS AND METHODS

This was a case-control study carried out in the University of Port Harcourt Teaching Hospital between 1st January and 30th September 2014. The study involved 128 subjects comprised 64 patients in the gynaecological ward who had laparotomy for tubal ectopic pregnancy and served as cases, while 64 women with intrauterine pregnancy randomly selected from the antenatal clinic on the date of admission of each patient served as controls. All patients who underwent laparotomy for tubal ectopic

pregnancy and gave consent were eligible for this study. Excluded from this study were patients who conceived through *in-vitro* fertilization and embryo transfer or any other form of assisted fertilization, patients with ectopic pregnancies while on intrauterine contraceptive device or progesterone only contraceptives, patients who had had previous tubal surgery or patients with ectopic pregnancy who had had a previous history of abdomino-pelvic surgery with peritonitis.

The sample size for this study was calculated using the formula for calculating sample size for case-control study [17]. After computing for the expected 10% attrition, the sample size was calculated to be 64 cases and 64 controls giving a total sample size of 128. All patients who had laparotomy for tubal ectopic pregnancy, who consented to this study and satisfied the inclusion and exclusion criteria, were recruited until the minimum sample size was obtained. Ethical approval was obtained from the University of Port Harcourt Teaching Hospital's ethical committee.

After obtaining informed consent, the socio-demographic characteristics of both cases and controls were documented. A structured questionnaire was administered to both groups to enquire for sexual behaviour and risk factors for chlamydial infection. Serological evidence of prior chlamydial infection was determined in both groups by examining for the presence of Chlamydia immunoglobulin G antibodies in their blood using the Immunocomb Chlamydia IgG (*C. trachomatis*) kit by (Orgenics of Israel); a rapid, semi quantitative, solid phase enzyme immunosorbent assay (EIA). After obtaining informed consent, the author collected 2 millilitres of blood from the antecubital veins of each study subject. The sample was allowed to clot and the serum transferred into a cuvette and covered. It wss stored in the freezer compartment until it was ready for use.

The sample was analysed for chlamydia immunoglobulin G antibodies using the Immunocomb Chlamydia IgG (*C. trachomatis*) kit by (Orgenics of Isreal). This was a rapid, semi quantitative test and a solid phase enzyme immunosorbent assay (EIA). It was very sensitive in detecting immunoglobulin G *Chlamydia trachomatis* for the analysis, the serum was added to the diluent in the well of row A of the developing phase (the test spot was divided into rows). The attached card was inserted into the well of this row. Chlamydial antibodies if present in the specimen specifically bound to the respective chlamydial antigens on the lower spots on the teeth of the card. Simultaneously immunoglobulins present in the specimen were captured by the antihuman immunoglobulins present in the upper spot which served as the internal control. In the lower row (row C), the captured human IgG reacted with alkaline phosphatase-labeled anti human IgG in the next two rows, while the unbound component was removed by washing. The results were visualised as grayish-blue spots on the surface of the teeth of the card.

Data collected were entered into a spread sheet using SPSS 20.0 for Windows® statistical software which was also used for analysis. Results are presented as means with standard deviations, rates and proportions in tables. Descriptive analysis was done for socio-demographic data and tests of significance were done using Chi square (x^2) test and Fisher's exact test for bivariate statistical analysis. The regression logistic analysis was used to determine the association between risk factors for *Chlamydia trachomatis* and ectopic pregnancy. The level of statistical significance was set at P-value <0.05.

3. RESULTS

Mean age of the subjects was 27.8±5.3 and 30.7±3.4 for controls. There were significantly more [20(31.3%)] single women among the subject population with ectopic pregnancy than the controls [2(3.1%)] [p < 0.01]. The nulliparous women in the subject group were more [30 (46.9%)] than the controls [6(9.4%)], p=0.03. (Table 1).

Chlamydia andibodies were found in 34(53.1%) and 18(28.1%) of the subjects and controls respectively [p = 0.03]. The presence of chlamydia antibodies is associated with a two-fold risk of ectopic pregnancy; Odd's ratio (O.R 2.25; CI 1.02-5.03).

Table 2 shows sexual behaviour of the participants. The subjects engaged in sexual intercourse at a significantly younger age than the controls (p<0.01). The mean number of sexual partners in the subjects and controls were 3.53±2.4 and 3.11±1.96 respectively. Previous history of pelvic inflammatory disease was obtained in 28(43.8%) of the study group. This

was significantly more than 13(20.3%) among the controls (p= 0.03).

The mean number of induced abortions in the subjects and controls were 2.85 ± 1.2 and 1.6±1.02 respectively. This showed a statistical difference with a p value of 0.04.

Out of the 34 subjects that were seropositive for Chlamydia immunoglobulin G antibodies, 23 (67.6%) had peritubal adhesions at laparotomy; while 11(32.4%) had not. All the six patients who had dense pelvic adhesions were seropositive for *C. trachomatis* antibodies.

Table 1. Sociodemographic characteristics of the subjects and controls

Parameter	Subjects		Controls		p
	No.	%	No	%	
Age					
15-20	7	10.9	0	0	0.13
21-24	9	14.1	17	26.6	
25-29	22	34.4	24	37.5	
30-34	18	28	20	31.3	
> 35	8	12.5	3	4.6	
Mean age	27.8±5.3		30.7±3.4		
Marital status					
Single	20	31.3	2	3.1	0.04
Married	44	68.8	62	96.9	
Parity					
0	30	46.9	6	9.4	0.03
≥1	34	53.1	58	90.6	
Occupation					
Professionals	8	12.5	10	15.3	0.18
Non-professionals	56	78.5	54	84.7	
Education					
1^0	2	3.1			
2^0	30	46.9	26	40.6	0.07
3^0	32	50.05	38	59.4	

Table 2. Sexual behaviour of subjects and controls

Parameter	Subjects		Controls		p-value
	N	%	N	%	
Coitarche					<0.01
13-17	38	59.4	10	15.6	
≥18	26	40.6	54	84.4	
Mean age	17.03±2.58		20.66 ±3.12		
No of sexual partners					
1or 2	44	68.8	41	64.1	0.28
≥3	20	31.2	23	35.9	
Mean	3.53 ±2.4		3.11±1.96		
History of PID					
Yes	31	48.4	13	20.3	<0.01
No	33	51.6	51	79.7	
Condom use					
Yes	62	96.9	59	92.2	0.78
No	2	31	5	7.8	
Induced abortions					
Yes	43	67.2	29	45.3	0.04
No	21	32.8	35	54.7	
Previous STI in partner					
Yes	2	3.1	1	1.7	
No	62	96.9	63	98.3	0.55

PID = Pelvic inflammatory disease, STI = Sexually transmitted infection

Tables 3 and 4 show further analysis of subjects and controls who were seropositive for chlamydial antibodies. Those who were positive for chlamydial antibodies in the study group were more likely to be single 16 (47.1%) than the controls 2 (11.1%) [p < 0.01]. They were also more likely to be nulliparous 15(44.1%) than the pregnant controls 3 (16.7%) [p =0.04]. This analysis also shows the patients with ectopic pregnancy seropositive for chlamydia antibodies were more likely to have coitarche at an earlier age between 13–17 years [20 (58.8%)] than their pregnant controls [5 (27.8%)] [p < 0.01]. They were also more likely to have a history of pelvic inflammatory disease 18 (52.9%) and 5 (27.7%) [p<0.01]. A history of induced abortion was also

Table 3. Socio-demographic characteristics of chlamydia seropositive women

Parameter	Subjects		Controls		p value
	Frequency		Frequency		
	N	%	N	%	
Age					
15-20	4	11.8	0	0	
20-24	5	14.7	4	22.2	
25-29	13	38.2	7	38.9	
30-34	8	23.5	7	38.9	0.1
≥35	4	11.8	0	0	
Marital status					
Single	16	47.1	2	11.1	<0.01
Married	18	52.9	16	88.9	
Parity					
0	15	44.1	3	16.7	0.04
≥1	19	55.9	15	83.3	
Occupation					
Professionals	4	11.8	3	16.7	0.15
Non professionals	30	88.2	15	83.3	
Education					
Primary	0	0	0	0	
Secondary	16	47.1	7	38,9	0.11
Tertiary	18	52.9	11	61.1	

Table 4. Sexual behaviour of chlamydia seropositive women

Parameter	Subjects		Controls		p value
	Frequency		Frequency		
	N	%	N	%	
Coitarche					
13-17	20	58.8	5	27.8	<0.01
≥18	14	41.2	13	72.2	
Number of sexual partners					
1or 2	23	67.6	12	66.7	0.78
≥3	11	32.4	6	33.3	
History of pelvic inflammatory disease					
Yes	18	52.9	5	27.7	0.03
No	16	47.1	13	72.3	
History of condom use					
Yes	33	97.1	18	100	0.50
No	1	2.9	0	0	
History of induced abortion					
Yes	21	61.8	7	38.9	0.04
No	13	38.2	11	61.1	
Previous sexually transmitted infections in partner					
Yes	2	6.1	1	5.9	0.90
No	33	93.9	17	94.1	

significantly higher in the subjects than controls 21(61.8%) and 7 (38.9%) [P=0.04]. There was no statistical difference between the two groups in terms of age, educational status and occupation, number of sexual partners, condom use and history of a partner with previous sexually transmitted infection.

Logistic regression model analysis of risk factors for ectopic pregnancy showed a four-fold increase in the risk of ectopic pregnancy in those with *C. trachomatis* antibodies when controlling for previous history of pelvic inflammatory disease (O.R 4.2; 95% CI 2.15-8.26). However, the association between Chlamydia antibodies and ectopic pregnancy was reduced (O.R 2.08; CI 1.43-4.87) when the effects of sociodemographic characteristics, previous history of pelvic inflammatory disease, marital status, number of sexual partners, condom use and partner with history of previous sexually transmitted disease were controlled for. With this control, early coitarche (O.R 5.28; CI 2.33-12.06), previous history of induced abortion (OR 4.14; CI 1.25-9.4), single women (OR 3.20; CI1.87-6.28) and previous history of pelvic inflammatory disease (O.R 3.22; CI2.28-8.40) were shown to be more likely to be associated with ectopic pregnancy than Chlamydia antibodies.

4. DISCUSSION

Serum immunoglobulin G antibodies have been associated with endosalpingeal damage and ectopic pregnancy resulting from pelvic inflammatory disease [13-16].

In this study, the prevalence of chlamydial antibodies was significantly higher (53.1%) in patients with ectopic pregnancy than women with intrauterine pregnancy (28.1%). This finding confirms the report of other researchers that patients with ectopic pregnancy are more likely to have immunoglobulin G antibodies against *C. trachomatis* when compared with women with intrauterine pregnancy [12,18,19]. This suggested relationship was further strengthened by the finding in this study of a two fold increase in the risk of ectopic pregnancy in women with chlamydial antibodies. The prevalence of prior *C. trachomatis* infection, as evidenced by the presence of Chlamydial antibodies in this and other studies underscores the reason ectopic pregnancy has remained a major complication of reproductive health of women and why the incidence is increasing in parallel with the rise in the rate of chlamydial infection [20].

This study also found that women with ectopic pregnancy were more likely to be single, corroborating the findings in another Nigerian study [21]. This can be explained by the fact that single women are more likely to have multiple sexual partners than married women.

In examining the risk factors for Chlamydia infection among patients with ectopic pregnancy and normal pregnancy this study looked critically at the sexual behaviour of both groups. The results of bivariate analysis showed that women with ectopic pregnancy engaged in sexual intercourse at a significantly younger age than women with intra uterine pregnancy. This finding is in keeping with previous studies which linked early coitarche with *Chlamydia trachomatis* infection [22]. Adolescents are prone to sexually transmitted disease (STD) because of high risk sexual behaviour. Biologically, the adolescent is particularly at risk of STD because the columnar epithelium, which is susceptible to Chlamydia and gonococcal organisms extend from the endocervical canal to the ectocervix making it fully exposed to pathogens. They also have difficulties using barrier methods of contraception and may have less access to STD care because of limited facilities, negative peer pressure, concealment and restrictive policies especially in the developing countries [20].

In contrast to other studies [22-25], this study did not find any difference in the number of lifetime sexual partners. Majority of both subjects and controls in this study reported one or two sexual partners. This can be explained from a disadvantage of self-reporting where women will not want to be associated with multiple sex partners since it is an indicator of sexual promiscuity.

Previous history of pelvic inflammatory disease was significantly higher in patients with ectopic pregnancy when compared with women with normal pregnant controls. This agrees with the findings of previous studies in this regard [13-16].

In terms of barrier contraception, many of the study subjects and controls reported condom use eliminating it as a significant risk factor for Chlamydia infection in this study. This finding is a departure from a wide variety of studies [22-24] where inconsistent condom use was recognised as a risk factor for Chlamydia infection. This

disparity could have resulted from the participants' misconception of that aspect of the questionnaire or to identify with the current increased campaign in the media about condom use and its relationship with safe sex.

Induced abortions were significantly higher in patients with ectopic pregnancy in this study. This finding agrees with a study done in Kenya [25] but is at variance with another study done in Norway [26]. This discrepancy can be explained by the fact that in Norway which is a developed nation, abortion is legalised but Nigeria has restricted abortion laws and terminations are carried out clandestinely in unsafe conditions resulting in an increased risk of ascending infection and post-abortal sepsis.

The characteristics of the participants who were seropositive for Chlamydia antibodies among the subjects and controls were critically examined. The bivariate analysis showed similar trends in terms of risk factors of Chlamydia infection and predictors of ectopic pregnancy. However, when these were subjected to logistic regression model (multivariate) analysis, patients with Chlamydia antibodies showed a four-fold increase in the risk of ectopic pregnancy when controlling for previous history of pelvic inflammatory disease. But when other factors like socio-demographic characteristics, history of previous pelvic inflammatory disease, marital status, number of sexual partners, condom use, partner with previous STD were controlled for, the strength of association between chlamydial antibodies and ectopic pregnancy was reduced by half (O.R from 4.2 to 2.08) while women with early coitarche, previous history of pelvic inflammatory disease and history of induced abortion became more associated with ectopic pregnancy. The effect of parity on ectopic pregnancy was no longer significant. This finding suggests that any effects of Chlamydia antibodies on ectopic pregnancy are probably modulated through combined effects of these factors.

The relationship between Chlamydia sero-positivity and the finding of peritubal adhesions at laparotomy among patients with ectopic pregnancy was also examined. Of the 34 women with ectopic pregnancy who were seropositive, 23(67.6%) had peritubal adhesions at laparotomy. Specifically all the six patients whose pelvises were described as having dense adhesions were seropositive for Chlamydia antibodies. This finding is relevant because all other potential causes of peritoneal adhesions like previous tubal surgery, abdominal or pelvic surgery, peritonitis and previous history of tuberculosis formed part of the exclusion criteria for this study, thereby restricting the cause of this important finding to possibly *Neisseria gonorrhoea* or *Chlamydia trachomatis*. This finding is consistent with those of previous studies which had linked increased Chlamydia antibody titre with pelvic adhesions and tubal damage [14,27].

5. CONCLUSION

This study has shown that a greater proportion of women with ectopic pregnancy had serological evidence of prior *C. trachomatis* infection than women with intrauterine pregnancy. It also demonstrated a two-fold risk association between prior *C. trachomatis* infection and ectopic pregnancy. Furthermore, early coitarche, previous history of pelvic inflammatory disease and induced abortion are positively associated with ectopic pregnancy. There is therefore a need for a public enlightenment on safe sexual practices including barrier contraception.

CONSENT

All authors declare that 'written informed consent was obtained from the patients in the course of the conduct of this study.

ETHICAL APPROVAL

Ethical approval was obtained from the University of Port Harcourt Teaching Hospital's ethical committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rose IA, Ayodeji O, Olalekan OA, Slyvia AA. Risk for ectopic pregnancy in Lagos, Nigeria. Acta Obstetrica et Gynaecologica Scandinevica. 2005;84:184-186.
2. Ugboma HAA, Nwagwu VO, Jeremiah I. Genital *Chlamydia trachomatis* infection among female undergraduate students of University of Port Harcourt, Nigeria. Nigerian Medical Journal. 2014;55(1):9–13.

3. Li C1, Zhao WH, Zhu Q, Cao SJ, Ping H, Xi X, et al. Risk factors for ectopic pregnancy: A multi-center case-control study. BMC Pregnancy Childbirth. 2015;15:187.
DOI: 10.1186/s12884-015-0613-1
4. Omokanye LO1, Balogun OR, Salaudeen AG, Olatinwo AW, Saidu R. Ectopic pregnancy in Ilorin, Nigeria: A four year review. Niger Postgrad Med J. 2013; 20(4):341-5.
5. Cornelius AC, Onyegbule A, Onyema, Uchenna ET, Duke OA. A five year review of ectopic pregnancy at Federal Medical Centre, Owerri, South East, Nigeria. Niger J Med. 2014;23(3):207-12.
6. Agholor K1, Omo-Aghoja L, Okonofua F. Association of anti-chlamydia antibodies with ectopic pregnancy in Benin City, Nigeria: A case-control study. Afr Health Sci. 2013;13(2):430-40.
DOI: 10.4314/ahs.v13i2.33
7. Sy T, Diallo Y, Toure A, Diallo FB, Baide AA. Management of ectopic pregnancy in Conakry, Guinea. Med Trop (Mars). 2009; 69(6):565-8.
8. Sziller I. *Chlamydia trachomatis*: Area under the iceberg. International Journal of Infectious Diseases. 2008;12(1):25.
9. Hafner LM. Pathogenesis of fallopian tube damage caused by *Chlamydia trachomatis* infections. Contraception. 2015;92(2):108-15.
DOI: 10.1016/j.contraception.2015.01.004
(Epub 2015 Jan 13)
10. Lanjouw E, Ouburg S, de Vries HJ, Stary A, Radcliffe K, Unemo M. 2015 European guideline on the management of *Chlamydia trachomatis* infections. Int J STD AIDS. 2016;27(5):333-48.
DOI: 10.1177/0956462415618837
(Epub 2015 Nov 24)
11. Moore MS1, Golden MR, Scholes D, Kerani RP. Assessing trends in chlamydia positivity and gonorrhea incidence and their associations with the incidence of pelvic inflammatory disease and ectopic pregnancy in Washington State, 1988-2010. Sex Transm Dis. 2016;43(1):2-8.
DOI: 10.1097/OLQ.0000000000000352
12. Omo-Aghoja L, Okonofua F, Onemu S, Bergstrom S. Association of *Chlamydia trachomatis* serology with tubal infertility in Nigerian women. J Obstet Gynaecol Res. 2007;33(5):688-95.
13. Steiner AZ, Diamond MP, Legro RS, Schlaff WD, Barnhart KT, Casson PR, et. al. *Chlamydia trachomatis* immunoglobulin G3 seropositivity is a predictor of reproductive outcomes in infertile women with patent fallopian tubes. Fertil Steril. 2015;104(6):1522-6.
DOI: 10.1016/j.fertnstert.2015.08.022
(Epub 2015 Sep 25)
14. Olaleye O, Olamijulo JA. The value of chlamydial antibody level for predicting tubal blockage among women undergoing hysterosalpingography in Lagos, Nigeria. Int J Gynaecol Obstet. 2016;pii: S0020-7292(16)30083-2.
DOI: 10.1016/j.ijgo.2015.12.009
[Epub ahead of print]
15. Jeremiah I, Okike O, Akani C. The prevalence of serum immunoglobulin G antibody to *Chlamydia trachomatis* in subfertile women presenting at the University of Port Harcourt Teaching Hospital, Nigeria. Int J Biomed Sci. 2011; 7(2):120-124.
16. Den Hartung JE, Land JA, Stassen JRA. The role of chlamydia genus-specific and species-specific IgG antibody testig in predicting tubal disease in subfertile women. Human Reproduction. 2004; 19(6):1380-1384.
17. Kirkwood BR, Sterne JAC. Calculation of required sample size. In: Kirkwood BR, Sterne JAC (eds.). Essential medical statistics 2nd edition. Oxford, UK: Blackwell science Ltd. 2003;413-428
18. Moses AO, Munirdeen AI, Adegboyega FA, Abdulgafar JA. A study of serological evidence of prior *Chlamydia trachomatis* infection in patients with ectopic pregnancy in Ilorin Nigeria. European Journal of Scientific Research. 2015;16(2):461-6.
Available:http://www.popline.org/node/189451#sthash.Pt0XcFS4.dpuf
19. Okunlola MA, Owonikoko KM, Adeyemi AS, Adeyemi-Doro FAB. Chlamydia serology and ectopic pregnancy in Ibadan. Nigerian Medical Practitioner. 2009;55(1-2):13-15.
20. Coste J, Bouyer J, Ughetto S, Gerbaud L. Ectopic pregnancy is again on the increase. Recent trends in the incidence of ectopic pregnancy in France (1992-2002). Hum Reprod. 2004;19(9):2014-2018.
21. Musa J, Daru PH, Mutihr JT, Ujah IA. Ectopic pregnancy in Jos, Northern

Nigeria: Prevalence and impact on subsequent fertility. Niger J Med. 2009; 18(1):35-8.

22. Matteelli A, Capelli M, Sulis G, Toninelli G, Carvalho AC, Pecorelli S, et al. Prevalence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infection in adolescents in Northern Italy: An observational school-based study. BMC Public Health. 2016;16(1):200.
DOI: 10.1186/s12889-016-2839-x

23. Gravningen K, Furberg AS, Simonsen GS, Wilsgaard T. Early sexual behaviour and *Chlamydia trachomatis* infection - a population based cross-sectional study on gender differences among adolescents in Norway. BMC Infect Dis. 2012 Nov 22;12:319.
DOI: 10.1186/1471-2334-12-319
PMID: 23174009

24. Stevensons-Simon C, Sheeder J. *Chlamydia trachomatis*: Common misperceptions and misunderstandings. Journal of Paediatric and Adolescent Gynaecology. 2005;18(4):231-243.

25. Kimani J, Maclean I, Bwayo J, et al. Risk factors for *Chlamydia trachomatis* pelvic inflammatory disease among sex workers in Nairobi, Kenya. Journal of Infectious Diseases. 1996;173(6):1437-1444.

26. Atrash HK, Strauss LT, Kendrick JS. The relation between induced abortion and ectopic pregnancy. Obstet Gynaecol. 1997;89:512-8.

27. Svenstrup HF, Fedder J, Kristoffersen SE, Trolle G, Birkelund S, Christiansen G. *Mycoplasma genitalium, Chlamydia trachomatis* and tubal factor infertility- a prospective study. Fertil Steril. 2008;90(3): 513-520.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/16886