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Occurrence of Chlamydial Infection Based on Clinical Symptoms and Clinical History among Pregnant Women Attending Clinics in Zaria Metropolis, Kaduna State, Nigeria

Shango Patience Emmanuel Jakheng [a,b], Emmanuel Ifeanyi Obeagu [c*], Emmanuel William Jakheng [d], Onyekachi Splendid Uwakwe [c], Gloria Chizoba Eze [e], Getrude Uzoma Obeagu [f], S. Vidya [g] and Sunil Kumar [g]

[a] Department of Science Laboratory Technology, Microbiology Unit, Nigerian Institute of Leather and Science and Technology, Zaria, Kaduna State, Nigeria.
[b] Department of Microbiology, Kampala International University, Western Campus, Uganda.
[c] Department of Medical Laboratory Science, Kampala International University, Western Campus, Uganda.
[d] Department of Science Laboratory Technology, Physics Unit, Nigerian Institute of Leather and Science and Technology, Zaria, Kaduna State, Nigeria.
[e] Department of Ophthalmology, Enugu State University of Science and Technology, Enugu State, Nigeria.
[f] Department of Nursing Science, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria.
[g] Department of Microbiology, Faculty of Biomedical Sciences, Kampala International University, Western Campus, Uganda.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Chlamydia trachomatis also known as the "Silent Epidemic" is a major threat to the reproductive health of women in Africa. This study was aimed at determining the seroprevalence of *Chlamydia trachomatis* based on clinical symptoms and clinical history among women attending clinics in Zaria metropolis, Kaduna State. Each participant completed a researcher-devised questionnaire

**Corresponding author: E-mail: emmanuelobeagu@yahoo.com;*

and quasi design was used in the selection of hospitals. Subsequently about 5mls of peripheral blood for serological analysis was obtained after informed consent. Presence of antibodies to *Chlamydia trachomatis* was determined using Enzyme Linked Immunosorbent Assay (ELISA) to detect IgG. Out of the two hundred and seventy (270) samples collected, 32(11.9%) were positive for *Chlamydia trachomatis* IgG of the total population. There was no significant association between chlamydial infection and based on clinical symptoms. Hence, there is an urgent need for a national policy on routine screening for *Chlamydia trachomatis* as treatment is cheap and effective, while the attendant morbidity resulting from delayed diagnosis is more difficult to manage and associated with severe sequelae.

Keywords: Occurrence; chlamydial infection; clinical history; clinical symptoms; pregnant women.

1. INTRODUCTION

The genus *Chlamydia* belongs to the taxonomic family *Chlamydiaceae*, which also contains the genus *Chlamydophila* [1]. The genus *Chlamydia* includes the human *Chlamydia trachomatis*, the mouse pathogenic *Chlamydia muridarum*, the avian *Chlamydia psittacis,* the swine *Chlamydia suis* and *Chlamydia pneumoniae*. *Chlamydia trachomatis* (CT) is a small Gram-negative bacterium that is an obligate intracellular parasite [2].

Genital infections with *Chlamydia trachomatis* are the most common sexually transmitted bacterial infections in European countries [3,4], in the United States [5,6] and worldwide, with more than 90 million cases annually [7]. The Centres for Disease Control and Prevention [8] estimates that there are approximately 2.8 million new cases of *Chlamydia* in the United States each year and that about one million individuals in the United States are infected with *Chlamydia* [8]. It has also been reported as an established cause of pelvic inflammatory disease (PID), ectopic pregnancy and infertility among women [9]. Although the major impact of the disease is on the female genital tract [3,4], men may suffer from urethritis, prostatitis, infertility and Reiter's syndrome [4]. *Chlamydia trachomatis* is also the causative agent of trachoma, a chronic infection of the conjunctiva characterized by extensive scarring and blindness.

Sexually transmitted infections (STIs) contribute to a variety of obstetric and gynaecologic complications in women, including increased risk of tubal infertility and have been associated with chronic pelvic pain. They are also significantly associated with adverse pregnancy outcomes such as spontaneous abortion, preterm delivery, ectopic pregnancy, premature rupture of membranes, intrauterine infection of the foetus, and low birth weight in infants (Mardh, 2002).

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted among pregnant and non-pregnant women attending clinics in Zaria metropolis, Kaduna State, Nigeria.

2.2 Study Design

The study was a descriptive cross-sectional survey which combines the use of administered structured questionnaires and the analysis of serum samples collected from consented patients. Quasi design was adopted for the purpose of this study.

2.3 Inclusion Criteria

Women receiving antenatal care and non-pregnant women at the various Health Clinics during the period of study who gave consent to participate in the study aged <15-50 years with or without HIV were eligible for inclusion).

2.4 Exclusion Criteria

Women receiving antenatal care and non-pregnant women at the various health Clinics during the period of study who do not give their consent to participate in the study aged <15- 50 years with or without HIV were not eligible for inclusion.

2.5 Sample Size

The sample size was determined using the following equation as described by Naing et al. (2006).

$$(n = z^2 p (1 - p)) / d^2$$

Where

n = sample size
z = z score for a level of 95% confidence interval = 1.96

p = prevalence rate at 10.5% Zaria, Nigeria [10].
d = allowable error = 5% = 0.05

Therefore:

$n = ((1.96)^2 \times 0.105 \times (1 - 0.105)) / (0.05)^2$
= 144.406
= 144 samples

Attrition rate = 10% of 144 sample size
= 158.4
= 158 samples

A total of 270 samples were collected (equal number of samples were collected to make good comparism between the subjects).

2.6 Questionnaire Administration

A structured questionnaire was administered after informed consent was obtained from the study population in order to access the risk factors associated with the prevalence of *Chlamydia trachomatis* among pregnant and non- pregnant women. Parameters considered included occupation, husband's occupation and educational level

2.7 Sample Collection

Blood samples were collected from women of reproductive age <15-50 years who gave their consent. Approximately two hundred and seventy (270) blood samples were collected from one hundred and thirty five (135) pregnant and one hundred and thirty five (135) non- pregnant women attending clinics from each of the Clinics under study in Zaria.

All materials required for the collection of venous blood was assembled and labelled appropriately with the subject's identification number (ID) and date. Five mililitre of blood was collected through venepuncture using sterile 5ml needles and syringes (CHANGZHOU HUICHUN MEDICAL EQUIPMENT CO., LTD, CHINA - Expiry date: 02-2016). The blood sample in the syringe was released into the labelled container and allowed to clot for 30 minutes. This was followed by centrifugation of the collected blood sample to separate the serum at 3000 r.p.m. for 5 minutes. The serum was thereafter carefully separated with a transfer pipette in order to avoid extracting red cells and aseptically transferred into a sterile labelled serum storage screw-capped container. The serum was stored in the Microbiology laboratory in a freezer at a temperature of -20°C until analysed.

2.8 CT Screening Using Enzyme Linked Immunosorbent Assay (ELISA)

2.8.1 Assay procedure

All reagents for the assay were brought to room temperature (20^0C-25^0C) from a temperature of 2^0C-8^0C and thoroughly mixed by swirling gently before use. Samples were numbered according to the microtitre wells. The desired number of coated strips was placed into the holder. Five microlitre (5µl) of the test samples, negative control, positive control and calibrator control were added to 200 µl of the sample diluents to make 1 in 40 dilutions. One hundred microlitres (100 µl) of diluted sera, calibrator and controls was dispensed into appropriate wells. For the reagent blank, 100µl sample diluents were dispensed into 1A well position. The holder was tapped so as to remove air bubbles, mixed well and incubated for 30 minutes at room temperature. The liquid from all the wells were removed and wells washed three times with washing buffer.

One hundred microlitres (100 µl) of the enzyme conjugate was also dispensed into each well and the plate incubated for 30 minutes at room temperature. Excess enzyme conjugate was removed by washing each well with washing buffer three times. Approximately, 100µl of TMB chromogenic Substrate was dispensed into each well and incubated at room temperature for 30 minutes. One hundred microlitres (100 µl) of 2 N HCl was finally added to stop the reaction. There was a colour change from blue to yellow and within 15 minutes the plates were read at an absorbance of 450nm using a microwell reader (WKEA MED SUPPLIES, CHANGCHUN, CHINA – Expiry date: 08-2013).

2.8.2 Data analysis

Results and data from questionnaires were presented on tables and figures. All statistical analysis was done using a computer software program, SPSS Version 19. Associations and relationship between the various risk factors were obtained using chi-square, fisher's exact, analysis of variance (ANOVA) and the student t-test. Two tailed P values ≤0.05 was considered to be statistically significant.

3. RESULTS

The study subjects were compared based on abnormal vaginal discharge and the result is as

presented in Table 1. Women with abnormal vaginal discharge who tested positive for chlamydial infection had higher rate of occurrence 12(13.0%) than women without abnormal vaginal discharge 20(11.2%) who tested positive for chlamydial infection. Pregnant women with abnormal vaginal discharge had higher prevalence 5(12.5%) of chlamydial infection than their counterparts 9(9.5%) who do not have abnormal vaginal discharge. These observed differences were not statistically significant. Also, non-pregnant women with abnormal vaginal discharge had higher prevalence 7(13.5%) of chlamydial infection than their non- pregnant counterparts 11(13.3%) who had no abnormal vaginal discharge but the difference was not significant.

The prevalence of chlamydial infection of all the subjects were compared on the basis of their previous history of vaginal discharge and it was discovered that there was no significant association between vaginal discharge and the infection. The prevalence of Chlamydial infection among pregnant women was 8(17.8%) for those with previous history of vaginal discharge as against 5(5.6%) for those without previous history of abnormal vaginal discharge with statistically significant association between previous history of vaginal discharge and chlamydial infection. The non-pregnant women showed that there was no significant association between vaginal discharge and the infection (Table 1).

Assessment of the study subjects based on burning during urination showed that 1(7.1%) pregnant woman with chlamydia infection had burning sensation when urinating while 13(10.8%) of the pregnant women positive for chlamydial infection had no burning sensation when urinating. This difference was not statistically significant. Similarly, there was no association between burning on urination and chlamydial infection among non-pregnant women (Table 1).

A prevalence of 4(10.8%) of chlamydial infection was observed in pregnant women that experienced painful intercourse. This was not significantly different from the prevalence observed in pregnant women 10(10.2%) that do not experience painful intercourse. On the other hand, non-pregnant women that experience painful intercourse had higher prevalence 6(23.1%) of chlamydial infection than non-pregnant women 12(11.0%) that do not feel pain during intercourse. This perceived difference was not significant (Table 2). The relationship between lower abdominal pain as well as bleeding between periods were analysed and it was observed that there was no significant association between them. Also, test of association between bleeding between periods and chlamydial infection showed that there was no statistical association.

Table 2 shows that occurrence of chlamydial infection in pregnant women with history of stillbirth was 3(15.0%) and 11(9.6%) in those without history of stillbirth. In non-pregnant women with history of stillbirth, the prevalence rate obtained was 3(15.8%) as opposed to 15(12.9%) in non-pregnant women without history of chlamydial infection. Overall, there was no statistically significant association between chlamydial infection and history of childbirth. Investigation of the relationship between history of premature birth, history of miscarriage and history of infertility also showed that there was no statistically significant association between these factors and chlamydial infection.

4. DISCUSSION

High prevalence of abnormal vaginal discharge as well as previous history of abnormal vaginal discharge was found in both pregnant and non-pregnant mothers and the observed differences were not significant. Chlamydial infection was significantly associated with previous history of vaginal discharge among the pregnant women. Vaginal discharge is one of the symptoms associated with chlamydial infections [11] and women with this symptom are at higher risk of its potential consequences. The presence of discharge in mothers may be regarded as evidence of maternal genital infection (though the specific type of infection still requires evaluation). A report showed that babies from mothers with antenatal vaginal discharges were eight times more likely to develop conjunctivitis [12]. This was consistent with other previous reports [13]. The presence of discharge increases the chances of getting infecting organism into the infant's conjunctiva especially during passage through the birth canal, where the number of organism can multiply rapidly and so we suggest that routine screening of women (pregnant and non-pregnant) especially those with vaginal discharges be carried out in order to prevent or reduce the incidence of PID and its adverse sequelae.

Table 1. Occurrence of chlamydial infection based on clinical symptoms among women attending clinics in Zaria Metropolis,Kaduna State, Nigeria

Clinical symptoms	Pregnant		Non-pregnant		Total	
	No. of samples	No. +ve (%)	No. of samples	No. +ve (%)	No. of samples	No. +ve (%)
Abnormal vaginal discharge						
Yes	40	5(12.5)	52	7(13.5)	92	12(13.0)
No	95	9(9.5)	83	11(13.3)	178	20(11.2)
Statistics		χ^2 = 0.277, p = 0.598		χ^2 = 0.001, p = 0.972		χ^2 = 0.190, p = 0.663
Previous history of vaginal discharge						
Yes	45	8(17.8)	58	7(12.1)	103	15(14.6)
No	90	6(6.7)	77	11(14.3)	167	17(10.2)
Statistics		χ^2 = 3.985, p = 0.046*		χ^2 = 0.141, p = 0.708		χ^2 = 1.172, p = 0.279
Burning on urination						
Yes	14	1(7.1)	17	2(11.8)	31	3(9.7)
No Statistics	121	13(10.8)	118	16(13.6)	239	29(12.2)
		χ^2 = 0.300, p = 0.861		Fisher's exact = 0.598		χ^2 = 0.300, p = 0.861
Painful intercourse						
Yes	37	4(10.8)	26	6(23.1)	63	10(15.9)
No Statistics	98	10(10.2)	109	12(11.0)	207	22(10.6)
		Fisher's exact = 0.569		χ^2= 3.646, p = 0.104		χ^2 = 1.272, p = 0.259
Lower abdominal pain						
Yes	51	3(5.9)	49	10(20.4)	100	13(13.0)
No Statistics	84	11(13.1)	86	8(9.3)	170	19(11.2)
		Fisher's exact = 0.148		χ^2= 3.332, p = 0.068		χ^2 = 0.200, p = 0.654
Bleeding between periods						
Yes	6	0(0.0)	36	7(19.4)	42	7(16.7)
No Statistics	129	14(10.9)	99	11(11.1)	228	25(11.0)
		Fisher's exact = 0.512		χ^2 = 1.587, p = 0.208		χ^2 = 1.104, p = 0.293

*KEY: * = Statistically significant association exists at p ≤ 0.05; No. +ve = number of positive samples*

Table 2. Occurrence of chlamydial infection based on clinical history among women attending clinics in Zaria Metropolis, Kaduna State, Nigeria

Clinical history	Pregnant		Non-pregnant		Total	
	No. of samples	No. +ve (%)	No. of samples	No. +ve (%)	No. of samples	No. +ve (%)
History of stillbirth						
Yes	20	3(15.0)	19	3(15.8)	39	6(15.4)
No Statistics	115	11(9.6)	116	15(12.9)	231	26(11.3)
		Fisher's exact = 0.343		Fisher's exact = 0.483		χ2 = 0.545, p = 0.461
History of premature birth						
Yes	11	1(9.1)	6	3(50.0)	17	4(23.5)
No Statistics	124	13(10.5)	129	15(11.6)	253	28(11.1)
		Fisher's exact = 0.681		Fisher's exact = 0.031*		χ2 = 2.368, p = 0.124
History of Miscarriage						
Yes	31	3(9.7)	34	3(8.8)	65	6(9.2)
No Statistics	104	11(10.6)	101	15(14.9)	205	26(12.7)
		Fisher's exact = 0.594		Fisher's exact = 0.282		χ2 = 0.563, p = 0.453
History of infertility						
Yes	22	0(0.0)	22	4(18.2)	44	4(9.1)
No Statistics	113	14(12.4)	113	14(12.4)	226	28(12.4)
		Fisher's exact = 0.072		Fisher's exact = 0.331		χ2 = 0.384,

*KEY: * = Statistically significant association exists at p ≤ 0.05; No. +ve = number of positive sample*

Assessment of prevalence of chlamydial infection based on clinical symptoms observed in both pregnant and non-pregnant women that experienced burning during urination, painful sexual intercourse, lower abdominal pain as well as bleeding between periods revealed that there was no significant difference. This may account for both the symptomatic and asymptomatic nature of CT in women which shows that CT is an implicated cause of these factors, hence a major threat to the reproductive health of women [14]. Also, asymptomatic patients, unaware of their infection, may serve as a reservoir of infection to their partners.

The prevalence of CT based on history of stillbirth in the women was high compared to those without, though, not statistically significant. Similarly, the association between CT with history of miscarriage, infertility and premature birth was not significant. Premature birth represents a major problem for obstetrics and neonatology due to its increasing frequency and accompanying socioeconomic impact which we found chlamydial infection to be associated with as it indicates that *Chlamydia trachomatis* contributes relatively too early than late prematurity [15]. It should however be noted that the proportion of premature births attributable to *Chlamydia* highly depends upon the *C. trachomatis* prevalence in a given population and that some residual confounding as a result of co-infection by other genitourinary pathogens cannot beexcluded.

5. CONCLUSION

It is obvious that the infection of women of childbearing age with these silent infections portends serious danger for both heterosexual and vertical transmissions, hence posing a significant threat to the global efforts at combating the epidemic. Hence, there is an urgent need for a national policy on routine screening for *Chlamydia trachomatis* as treatment is cheap and effective, while the attendant morbidity resulting from delayed diagnosis is more difficult to manage and associated with severe sequelae.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

Approval was obtained from the ethical committee of the various Health Care Clinics before the commencement of the research in August 2012.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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