

Original Research Article

***Chlamydia trachomatis* infection among pelvic inflammatory disease patients attending the gynaecology clinic of a private tertiary hospital in Ogun State, Nigeria.**

ABSTRACT

Background: Pelvic inflammatory disease (PID) is one of the very serious complications arising from sexually transmitted infections (STIs) and *Chlamydia trachomatis* has been implicated as one of the commonest causes of STI. Considering the adverse sequelae of PID, there is a need for locally relevant data which will guide preventive and therapeutic efforts. Detection of a combination of immunoglobulin G (IgG) and immunoglobulin A (IgA) has been described as an indicator of an actively chronic infection

Aims: The aim of this study was to determine the prevalence of *Chlamydia trachomatis* infection by the use of IgA and IgG and evaluate the associated risk factors among females that presented with Pelvic inflammatory disease at the gynaecology clinic of Babcock University Teaching Hospital. Ilishan-Remo, Ogun State, Nigeria. (BUTH)

Materials and methods: This was a hospital-based, case-controlled study involving 44 patients diagnosed with PID and 44 age-matched controls at the gynaecology clinic of BUTHI. Interviewer-administered questionnaires were used to obtain information on socio-demographic characteristics, and risk factors for PID, from consenting participants. Blood samples were collected from each participant and analysed, using the enzyme-linked immunosorbent assay, for *Chlamydia trachomatis* type-specific for IgA and IgG. Analysis was done by SPSS, IBM version 23.0

Results: Both IgG and IgA were present in 15 cases (34.1%) as compared to none of the controls. The difference between *Chlamydia* IgG, IgA and (IgG+IgA) among the cases and the controls were statistically significant. Majority of the participants positive for the immunoglobulins were aged 25 years or younger (11, 73.3%). *Chlamydia trachomatis* infection, number of lifetime sex partners and age of first sexual intercourse being 18 years or younger were statistically associated with *Chlamydia trachomatis* causing PID.

Conclusion: *Chlamydia trachomatis* remains an important causative pathogen of PID and more prevalent among the young people. Screening is advocated among the young in resource limited countries.

Keywords: Pelvic inflammatory diseases, *Chlamydia trachomatis*, IgG, IgA

Comment [CO1]: Is this part of the risk factor?

INTRODUCTION

Pelvic inflammatory disease (PID) is a polymicrobial inflammatory lesion of the female upper genital tract and its adjoining structures.^[1] It describes a wide range of diseases such as salpingitis, salpingo-oophoritis, endometritis tubo-ovarian abscess and pelvic peritonitis.² Its clinical presentations range from being asymptomatic through having mild, nonspecific symptoms to being a severe, life-threatening disease.^{[2]-[3]} PID can be complicated by chronic pelvic pain, ectopic pregnancy and tubal infertility amongst others.^[4] Although PID requires no notification of incidence, it is a major disease syndrome of public health importance among women of reproductive age group.^{[3],[5]-[6]} In the United States, an estimate of over a million females report an incidence of PID with resultant 125,000–150,000 hospitalizations every year.⁵⁻⁶ In the high income countries, about 10-20 per 1000 females in the reproductive age groups are recorded to have PID yearly while in low and middle income country like Nigeria, the prevalence of PID has been reported to be much higher at 58% and 70%.^{[3],[7]-[8]}

The most frequent bacteria, among the sexually transmitted infections (STI) pathogens, causing PID are *Chlamydia trachomatis* and *Neisseria gonorrhoea*.^{[2],[4]} However, *Chlamydia trachomatis* is the commonest bacteria pathogen of STI that infects 131 million people and cause 73.7 million new infections every year.^{[9]-[10]} In Africa, about 15 million new cases of *Chlamydia trachomatis* is reported yearly while in Nigeria, prevalence of between 7.3% and 33% have been reported by different authors^{[11]-[14]}

Chlamydia trachomatis infection is mostly asymptomatic in females or present with mild symptoms until it results in sequelae such as PID. and the pathogenicity of the bacteria is due to its ability to cause active chronic or persistent infection which has been proven to be a major factor for developing PID.^{[10],[15]} A previous study from Canada noted that 55% of those infected with *Chlamydia trachomatis* have increased risk of developing PID while another study observed that 20% of PID cases was attributed to Chlamydia infection.^{[10],[16]}

Although Polymerase Chain Reaction (PCR) is the mainstay of diagnosis of *Chlamydia trachomatis* infection, it is neither readily available nor cost-effective in resource limited countries.^[17] However, Serodiagnosis, though not routinely performed in most developing countries, is still useful and within reach especially for epidemiological purposes¹⁸. *Chlamydia trachomatis* immunoglobulin G (IgG) is known as an indicator for chronicity while IgA is now recognized as a better indicator of active infection than IgM and the detection of the combination of these two immunoglobulins (IgG and IgA) is described as good indicator of

Comment [CO2]: The introduction should begin by giving a world-wide situational analysis of *Chlamydia trachomatis*, then narrow down to Africa and Nigeria. Also search for data in Ogun state in Nigeria where the study took place.

active chronic or persistent infection and can be used as an indicator of active infection like PCR.^{[19]-[20]}

Treatment of PID is mainly empirical especially in resource limited countries, with associated problems of inadequate treatment and further complications. Considering the ability of Chlamydia to cause active chronic/persistent infection and the high prevalence of PID resulting from Chlamydia infection, it then becomes paramount to objectively establish data that is capable of guiding the formation of treatment plans for PID.^{[1],[10],[20]-[21]} Also, obtaining appropriate, additional information on the evaluation of IgG and IgA which are seromarkers of active/ chronic persistent infection among PID patients in Nigeria has the potential to make positive impact on patients' care. The aim of this study was to determine the prevalence of *Chlamydia trachomatis* by the use of IgA and IgG and evaluate the associated risk factors among females that presented with PID in the gynaecology clinic of BUTH

MATERIALS AND METHODS

Study design

This was a cross-sectional, hospital-based, age-matched case control study in ratio 1:1 conducted at the gynaecology clinic of BUTH between November 01, 2017 and September 30, 2018. The minimum sample size was calculated based on 2% prevalence of *chlamydia trachomatis* in Nigeria to give a 95% confidence level and margin of error of 5%.^[22]

Study population

The cases were 44 women with clinical diagnosis of Pelvic inflammatory diseases while the controls were 44 women that presented at the clinic with other clinical condition not related to STIs. Participants were recruited by simple random technique and balloting.

Inclusion criteria were females between the ages of 15 to 45 years, clinical diagnosis of PID for case or clinical conditions not associated with PID for control and consent for blood samples to be collected for serodiagnosis.

Exclusion criteria were females that have used antibiotics within the preceding 6 weeks and those with other clinical conditions associated with STI.

Ethical approval was obtained from the Babcock University Health Research Ethics Committee before the commencement of the study and written informed consent was obtained from all the participants before their involvement in the study.

Sample collection and laboratory diagnosis

Comment [CO3]: Is the study Cross sectional or case-control study? Is it possible to use both study designs in a single research?

About 10mls of blood samples were collected from each participant and analysed by using the qualitative sandwich third-generation enzyme-linked immunosorbent assay (ELISA), type-specific for IgG and IgA against polypeptide derived from *Chlamydia trachomatis* major outer-membrane antigen (Diagnostic Bioprobes Milano, Italy). The questionnaires were semi-structured and interviewer-administered to obtain information on socio-demographic and possible risk factors associated with *chlamydia trachomatis* infection among the participants.

Statistical analysis

Standard descriptive and inferential statistical analysis were made from the Statistical Package for the Social Sciences, version 23.0 (IBM Inc., NYC, USA). Means and standard deviations were calculated for the quantitative variables while for the qualitative variables, proportions were used. The association between categorical variables was evaluated by using the Chi-square or Fischer's exact test at statistical significance level set at 5% and logistic regression analysis as appropriate.

Comment [CO4]: Give the precise statistical test used for each analysis. Chi-Square test and Fischer's exact are used for different sample sizes and for different variables. One should not be an alternative for the other.

RESULTS

Majority of the participants were aged 15–20 years (22, 50% cases and 28, 63.3% controls). Most of them were single (34, 77.3% cases and 33, 75.0% controls), had tertiary education (38, 86.4% cases and 40, 90.9% controls), had first sex debut at age 18 years or earlier (31, 70.5% cases) and those that never used condom were 39 (88.6%) cases. Other socio-demographic factors are as presented in Table 1.

The prevalence of *Chlamydia* IgG was 72.3% (34/44) and 27.3% (12/44) while that of *Chlamydia* IgA was 34.1% (15/44) and 2.3% (1/44) among the cases and the controls respectively. Both IgG and IgA were combined in 34.1% (15/44) of the cases and in none of the controls. The difference between *Chlamydia* IgG, IgA and (IgG+IgA) among the cases to the control were statistically significant {Table 2}.

Participants that were 25 years or younger had the highest incidence of *Chlamydia trachomatis* infection among the cases (11/15, 73.3%). [Table 3]

The number of lifetime sex partners and age of first sexual intercourse being 18 years or earlier were factors that were significantly associated with the presence of both IgG and IgA on bivariate analysis while the number of lifetime sex partners and age of first sexual intercourse were significantly associated with the development of PID on multivariate analysis – AOR of 9.5 (95% CI = 2.11 – 42.39) and 4.3 (95% CI = 1.37 – 13.32) respectively. {Table 4}

Table 1: Sociodemographic factors of the participants

Variables	Cases (%)	Control (%)
Age		
15-20	22(50)	28(63.6)
21-25	12(27.3)	6(13.6)
26-30	4(9.1)	6(13.6)
31-35	3(6.8)	3(6.8)
>35	3(6.8)	1 (2.3)
Marital status		
Single	34(77.3)	33(75.0)
married	10(22.7)	11(25.0)
Education		
Primary	0(0.0%)	0(0.0)
Secondary	6(13.6%)	4(9.1)
Tertiary	38(86.4)	40(90.9)
Age of first sex		
No sex	6(13.6)	24(54.5)
18years and below	31(70.5)	5(11.4)
More than 18 years	7(15.9)	15(34.1)
Past history of STI		
Yes	4(9.1)	1(2.3)
No	40(90.9)	43(97.7)
Use of Condom		
Yes	5(11.4)	2(4.5)
No	39(88.6)	42(95.5)
Hormonal contraceptive		
Yes	2(4.5)	3(6.8)
No	42(95.5)	41(93.2)
Present sex partners		
None	37(84.1)	42(95.5)
1	6(13.6)	2(4.5)
2	0(0.0)	0(0.0)
More than 2	1(2.3)	0(0.0)
Life time sex partners		
None	11(25.0)	30(68.2)
1	12(27.3)	11(25.0)
2	15(34.1)	3(6.8)
More than 2	6(13.6)	0(0.0)

Alcohol intake (16 bottles per week)			
Yes	1(2.3)		0(0.0)
No	43(97.7)		44(100.0)
Cigarette smoking(1 per day)			
Yes	1(2.3)		0(0.0)
No	43(97.7)		44(100.0)

Table 2: *Chlamydia trachomatis* Sero-markers of the participants

Immunoglobulin Markers		Cases (N=44)	Control (N=44)	Odds Ratio (95% CI)	P value
IgG	Yes	34	12	9.067 (3.44 – 23.87)	<0.0001
	No	10	32		
IgA	Yes	15	1	22.241 (2.78 – 177.74)	0.0034
	No	29	43		
IgG+IgA	Yes	15	0	46.763 (2.69 – 811.99)	0.0083
	No	29	44		

Table 3: Factors associated with *Chlamydia trachomatis* among the participants (Bivariate analyses of cases)

Variables	Chlamydia IgA+ IgG		X ²	P-value
	Yes	No		
Age				
15-20	7(31.8)	15(68.2)	3.47	0.63
21-25	4(33.3)	8(66.7)		
26-30	2(50.0)	2(50.0)		
31-35	1(33.3)	2(66.7)		
36-40	1(100)	0(0.0)		
41-45	0(0.0)	2(100.0)		
Marital status				
Single	11(32.4)	23(67.6)	0.20	0.65
Married	4(40.0)	6(60.0)		
Education			0.78	0.38
Primary	0(0.0)	0(0.0)		
Secondary	3(50.0)	3(50.0)		
Tertiary	12(31.6)	26(68.4)		
Age of first intercourse				
No sex	0(0.0)	6(100.0)	9.54	0.008
18years and below	15(48.4)	16(51.6)		
More than 18 years	0(0.0)	7(100.0)		
Past history of STI				
Yes	3(75.0)	1(25.0)	3.8	0.070
No	12(30.0)	28(70.0)		
Use of Condom				
Yes	1(25.0)	4(75.0)	0.49	0.48
No	14(35.9)	25(64.1)		
Hormonal contraceptive				
Yes			0.24	0.63
No	1(50.0)	1(50.0)		
Present sex partners				
None	14(37.8)	23(62.2)	5.27	0.072
1	0(0.0)	6(100.0)		
2	0(0.0)	0(0.0)		
More than 2	1(100)	0(0.0)		
Lifetime sex partners			16.51	0.002
None	1(9.1)	10(90.9)		
1	2(16.7)	10(83.3)		
	6(40)	9(60.0)		

2	6(100.0)	0(0.0)		
More than 2				
Alcohol intake (16 bottles or more per week)				
Yes	0(0.0)	1(100.0)	0.53	0.47
No	15(34.9)	28(65.1)		
Cigarette smoking (1 or more per day)				
Yes	0(0.0)	1(100.0)	0.53	0.47
No	15(34.9)	28(65.1)		

Table 4: Logistic regression analysis of the participants (Multivariate analysis of cases)

Variables	IgG+IGA	AOR (95% CI)	P value
Age of first intercourse	0(0.0)		
*No sex	15(48.4)		
18years and below	0(0.0)	4.3(1.37-13.32)	0.013
More than 18 years			
Lifetime sex partner			
*None	1(9.1)		
1	2(16.7)		
2	6(40)	9.5(2.11-42.39)	0.003
More than 2	6(100.0)		

P-value less than 0.05 is significant, *reference category

DISCUSSION

Pelvic inflammatory disease is a clinical syndrome that cannot be diagnosed by using a single laboratory test although clinicians depend mostly on clinical features to make diagnosis and treat empirically.^[5] *Chlamydia trachomatis* has been implicated as the predominant pathogen of PID, therefore a local data to determine association of *Chlamydia trachomatis* and PID will go a long way in its management.^{[23]-[24]}

Participants that were 25 years or younger had the highest prevalence of *Chlamydia trachomatis* infection among the cases. This observation is in tandem with a previous report on PID and STIs where participants aged 25 and younger had the highest prevalence of PID

compared to those aged above 25 years.^{[25]-[29]} This age group is known for their curiosity about sex-related matters, increased sexual activities and practice of risky sexual behaviours.^[30]

There is a need for developing countries like ours to start screening programme for *Chlamydia trachomatis* infections in order to facilitate early diagnosis and prompt treatment which will prevent severe sequelae such as PID.^[31]

In tandem with the findings of Price et al in 2016, about one-third of the participants in this study were positive for the combined IgG and IgA and this is indicative of active chronic or persistent infection.^[23] However, the rate is higher than previous rates of 21.7%, 19% and 14.2% reported from different parts of Nigeria.^{[32]-[34]}

Outside of Nigeria, varying rates of 22.7%, 47.9%, 6%, and 14.7% were observed in Malaysia, Sudan, Nepal, and Ethiopia respectively.^{[24- [26], [35]-[36]}

From this study, about three-quarters of the participants were positive for IgG which implied that a larger percentage of them had previously been exposed to *Chlamydia trachomatis*. This observation is consistent with the findings of Jeremiah and Odule from Port Harcourt, Nigeria in 2011 and 2015 respectively.^{[37]-[38]} High prevalence of *Chlamydia trachomatis* is a marker of high burden of diseases caused by this pathogen especially in developing countries.

The significant difference between *Chlamydia trachomatis* infection among the study population and the control is a proof that *Chlamydia trachomatis* plays major roles in the development of PID and this is similar to previous report by Ravindran and Monita and colleagues respectively.^{[23],[27]}

Moreover, age of first sexual intercourse less than 18 years was strongly associated with *Chlamydia trachomatis* infection. The reason for this association might be related to the fact that these age groups are young and involved in experimenting different sexual practises that might predispose them to infection.^[5]

Life time sex partner that is more than 2 is another factor linked with *Chlamydia trachomatis* in our study and which similarly, corresponds to previous findings^{[5],[39]} although Tandasse and colleagues found no association between the number of lifetime sex partners and Chlamydia infection in Southern Ethiopia.^[26] However, this difference might be associated with recall bias or the stigma associated with having many lifetime sex partners which might make participants give false information.

Although other authors have found past history of STI to be associated with PID, the association was found to be insignificant in our study possibly because the participants chose not to divulge such history for the fear of stigmatization.^{[10],[40]}

About 93.3% of participants with *Chlamydia trachomatis* infection in this study were without a recent sex partner and this observation further buttressed the chronic nature of *Chlamydia trachomatis* which make it easier to cause an ascending infection such as PID. This study is majorly limited by the lack of PCR-based technique for definitive comparison as it is the mainstay of diagnosis of Chlamydia trachomatis.

Conclusion : the main findings in this study was the revelation that Chlamydia trachomatis remained an important pathogen in the development of PID and that it is chronically active in nature while being more prevalent among young people. Therefore, there is a crucial need for screening programmes and sex education especially among the young population in the developing world which will be targeted towards early diagnosis and prompt management

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Comment [CO5]: This reference is not correct, it is a secondary reference. The materials are gotten from a published article on the website with in-text citations.

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UNDER PEER REVIEW

