Prevalence and Coinfectivity of *Chlamydia Trachomatis* and Syphilis Infections among HIV Seropositive Women in Nsukka, Enugu State, Nigeria

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Abstract: Objectives: **To study the prevalence of *Chlamydia trachomatis* infection as well as its coinfectivity with Syphilis among HIV-seropositive women(test group) and sero-negative women (control group) of child-bearing age in Nsukka, Enugu State, Nigeria.**

Study Design: **A total of 100 tests and 100 control groups attending antenatal and routine medical services were used in the study Informed consents were obtained from the participants attending antenatal and routine medical care in Bishop Shanahan Hospital, All Saints Medical Centre, Primary health Centre, Nsukka, Adonai Medical Laboratory and Nsukka District Hospital.**

Methods: **Questionnaire was used in the first phase to obtain demographic information of the sample population. Two ml of the participants' plasma samples were used in the phase two of the study. HIV antibodies were detected using the Determine (Alere, Japan) and the Gold (Trinity, Ireland). *C. trachomatis* antibodies were detected using CT IgG EIA kit (Xema, Russia) while syphilitic antibodies were detected using Syphilis Ultra Rapid Test Strip Package Insert, (Global USA). Statistical significance was determined using SPSS 16.0. Those at risk were established using attributable and relative risks.**

Results: **A preponderance of HIV infection among individuals aged 26-40 years (63%). Single infection with *C. trachomatis* and Syphilis were 6% and 2% respectively for test group, and 11% and 8% respectively for control group. A high prevalence of the dual infection was observed in the test group with no statistical significance ($\alpha = 0.05$). Age distribution of both infections indicated preponderance among 16-35 years age bracket.**

Conclusion: **HIV infection with low $CD4^+$ count and high viral load is a strong risk factor for the infections in the study area.**

***Key words: Chlamydia trachomatis*, Syphilis, HIV/AIDS, Women, Reproductive age, Nsukka, Nigeria.**

I. INTRODUCTION

Chlamydia trachomatis is one of the most dreaded sexually transmitted bacterial infections in the world, with an estimated global incidence of about 92 million (Isiabor *et al*, (2005). The Centers for Disease Control and Prevention (CDC), reported an approximately one million global cases of *C. trachomatis* infections among sexually active young people aged 15-25 years, more than half of these were in females. In Nigeria, prevalence rates of about 38-51% among pregnant and non-pregnant women and their spouses were reported (Tutur *et al*, 2006). Lower prevalence however is being reported in developed countries such as Italy (6.3%) and North India (19.9%) (Malhotra *et al*, 2011; Maragoni *et al*, 2011).

The risk factors for the disease transmission include age, personal hygiene, illiteracy, poverty, multiple or irregular sex partnering, non-use or erratic use of contraceptive devices, overcrowding, previous exposure to STIs as well as poor access to health care (Kucinskiene *et al*, 2006; Malhotra *et al* 2011; Maragoni *et al*, *2011*)

A number of gynaecological problems have been strongly linked to CT and Syphilis infections in both single and pair-wise cases. In men, urethritis and occasionally epididymitis have been reported while some deadly outcomes such as miscarriage, ectopic pregnancy, tubal infertility, preterm delivery and still birth are being reported in women. These result from the blockade of fallopian tubes and a complex immunological reaction emanating from autoimmune inflammatory damage of the bacterial 60-kD heat-shock protein-10 or rupture of uterine endometrium (Mathew *et al*, 2004; . Ikeme, *et al*, 2011; Manju *et al*, 2011).

C. trachomatis and HIV infections have mutual beneficial outcomes and common portal of entry (urogenital tracts). *C. trachomatis* upon invasion, damages the genital epithelial layer by an enzymatic action of the invasin which facilitates HIV infection. HIV is an immunosuppressive virus destroying $CD4^+$ cells, and inhibiting T-cell activation. The T-cells play a significant role in the recognition of epitopes present in the *Chlamydia trachomatis* omp1 variable domain, hence, the severity of the infection in immuno-compromised individuals (Ward and Ronn 2010; Ikeme *et al*, 2011). Apart from being biologically intertwined, the two infections and indeed all STIs share the same social behaviors which facilitate sexual transmission of one another (Alvares-Travassos *et al*, 2012; Parikh, 2013).

Untreated early Syphilis in pregnant women results in perinatal death in up to 40% of cases and, if acquired during the 4 years before pregnancy, can lead to infection of the fetus

in 80% of cases. In 2010, the rate of primary and secondary syphilis was highest among younger population especially persons aged 20–24 years (Parikh, 2013).

Co-infection of *C. trachomatis and T. pallidium* in HIV/AIDS cases has been widely reported in different part of the world with up to 3% prevalence (Apea-Kubi *et al*, 2004; Malhotra *et al*, 2011). Higher prevalence rate of up to 73% has been recorded with Hepatitis-C virus infection due mainly to mutual biological and immunologic activities (Krznaric 2012; Chun 2013).

II. METHODS

Focus Group Discussion and Questionnaire Administration:

Focus group discussion: This constituted the preliminary part of this study, and it was an interactive session. Discussions were based on previously structured objective of the study: sexual behaviour and exposure, including multiple sex partnering, knowledge of sexually-transmitted infections including HIV, *Chlamydia trachomatis* and Syphilis infections, socio-economic status, age, previous infertility history and/or history of abortion, preterm delivery or miscarriage, previous exposure to other sexually transmitted infections (STIs) as well as mode of spread of the diseases.

Questionnaire administration: Information obtained from the structured oral discussions and/or interviews formed the basis of the questionnaire formulation. Five hundred (500) structured questionnaires were then administered to participants following informed consent and promise of confidentiality. The completed questionnaires were then collated and analyzed using percentages.

Laboratory investigation:

Five (5) ml of blood samples were collected from each of two hundred (200) consenting women: 100 HIV seropositive (test) and 100 seronegative (control group) on routine medical check-up at Bishop Shanahan Hospital, Nsukka (20 each for test and control), Primary Health Center, Nsukka (10 each for test and control), District Hospitals Nsukka (20 each for test and control) and Ogrute (20 each for test and control), Adonai Medical Diagnostic Laboratory Nsukka (10 each for test and control) and All Saints Medical Center Nsukka (10 each for test and control). The blood samples were dispensed into sterile vacutainer and centrifuged at 1000xg to separate blood cells from plasma. The plasma was then stored at 2^0C and used for subsequent tests.

HIV-1 and HIV-2 antibodies were screened by using two HIV test kits: the Determine^{TM} (Alere medical Co., Ltd. Japan) which differentiates between HIV-1 and HIV-2 antibodies and the Gold^{TM} (Trinity Biotech Plc Bray, Co. Wicklow, Ireland) which tests for consistency of positive samples. Samples that did not test positive with the two kits were not used in the study.

C. trachomatis antibodies was screened for using *C. trachomatis* IgG EIA kit (Xema Co. Ltd., Russia) according to manufacturer's instructions

The syphilis Ultra Rapid Test Strip (Whole Blood/Serum/Plasma) Package Insert, Global USA was used for this screening according to manufacturer's instruction.

IV. RESULTS

Table 1: Demographic Characteristics of the Sample Population (Questionnaire Responses).

Age group	No. Interviewed	Educational level		Settlement		Number of Sex Partners		Prev. Case of STI	Prev. Case of CT Inf.	Previous case of Child-bearing problems				
		Higher Sch. level	Lower Sch. Level	Rural Dwellers	Urban Dwellers	Multiple Sex Partner	Single Sex Partner			Sa	Pd	Sb	Ep	Cd
16-20	75	1 (1.3%)	74(98.7%)	60(80.0%)	15(20.0%)	0(0.0%)	75(100%)	57(76%)	0(0.0%)	3	1	3	2	4
21-25	105	12(11.4%)	93(88.6%)	73(69.5%)	93(31.5%)	0(0.0%)	105(100%)	68(64.8%)	1(0.95%)	6	2	5	6	5
26-30	117	25(21.4%)	92(78.6%)	77(65.8%)	40(34.2%)	0(0.0%)	117(100%)	73(62.4%)	0(0.0%)	5	3	4	6	12
31-35	133	21(15.8%)	112(84.2%)	72(54.1%)	61(45.9%)	2(1.5%)	131(98.5%)	70(52.6%)	2(1.5%)	18	6	16	13	20
36-40	52	8(15.4%)	44(84.6%)	32(61.5%)	20(38.5%)	3(5.8%)	49(94.2%)	50(96.2%)	5(9.6%)	9	3	7	9	12
41-45	18	3(16.7%)	15(83.3%)	11(61.1%)	7(38.9%)	1(5.6%)	17(94.4%)	13(72.2%)	0(0.0%)	2	1	1	2	5
Total	500	70(14.0%)	430(86.0%)	325(65.0 %)	175(35.0 %)	6(1.2%)	494(98.8%)	331(66.2 %)	8(1.6%)	43	16	36	38	58

Key: Sb = Spontaneous abortion, Pd = Preterm delivery, Sb = Still birth, Ep = Ectopic pregnancy, Cd = Conception difficulties.

Table 2: The effects of some demographic factors on the distribution of the diseases

Disease		Marital status		Settlement		Educational background			Previous exposure to STI		Number of sex partners	
		Single	Married	Rural	Urban	Prim. Sch.	Sec. Sch.	Higher Sch.	Yes	No	Multiple Partner	Single Partner
Test	C. trachomatis infection	2 (33%)	4 (67%)	3 (50%)	3 (50%)	3 (50%)	2 (33%)	1 (17%)	6 (100%)	0 (0%)	1 (17%)	5 (83%)
	Syphilis	8 (73%)	3 (27%)	3 (27%)	8 (73%)	2 (18%)	9 (82%)	0 (0%)	7 (64%)	4 (36%)	2 (18%)	9 (82%)
Control	C. trachomatis infection	1 (50%)	1 (50%)	0 (0%)	2 (100%)	2 (100%)	0 (0%)	0 (0%)	2 (100%)	0 (0%)	0 (0%)	2(100%)
	Syphilis	1 (13%)	7 (87%)	7 (87%)	1 (13%)	2 (25%)	5 (63%)	1(13%)	6 (75%)	2 (25%)	1 (13%)	7(87%)

Table 3: Prevalence of *Chlamydia Trachomatis*and Syphilis Infection among Different Age Groups in HIV Seropositive participants

Age groups (yrs)	Chlamydia infection						Syphilis Infection					
	Test			Control			Test			Control		
	No Screened	No Positive	% Prevalence	No Screened	No Positive	% Prevalence	No Screened	No Positive	% Prevalence	No Screened	No positive	% Prevalence
16-20	10	1	10	28	1	3.6	10	3	30	28	3	10.7
21-25	15	2	13.3	23	0	0	15	4	26.7	23	2	8.7
26-30	23	2	8.7	31	1	3.2	23	1	4.3	31	2	6.5
31-35	20	1	5	10	0	0	20	3	15	10	1	10
36-40	20	0	0	6	0	0	20	0	0	6	2	3.3
41-45	12	0	0	2	0	0	12	0	0	2	0	0
Total	100	6	6	100	2	2	100	11	11	100	8	8

Table 4: Attributable a risk Condition of Contracting *Chlamydia trachomatis*and Syphilisin HIV infected participants.

Age group	Chlamydia trachomatis Infection									Syphilis Infection								
	Test				Control				Relative risk	Test				Control				Relative risk
	CT (+)	CT(-)	Total	Attributable	CT(+)	CT(-)	Total	Attributable		TP (+)	TP(-)	Total	Attributale	TP (+)	TP (-)	Total	Attributable	
16-20	1	9	10	0.10	1	27	28	0.04	2.50	3	7	10	0.30	3	25	28	0.11	2.73
21-25	2	13	15	0.13	0	23	23	0.00	∞	4	11	15	0.26	2	21	23	0.09	2.89
26-30	2	21	23	0.09	1	30	31	0.03	3.0	1	22	23	0.04	2	29	31	0.06	0.67
31-35	1	19	20	0.05	0	10	10	0.00	∞	3	17	20	0.15	1	9	10	0.1	1.5
36-40	0	20	20	0.00	0	6	6	0.00	∞	0	20	20	0.00	0	4	6	0.00	0.00
41-45	0	12	12	0.00	0	2	2	0.00	0.00	0	12	12	0.00	0	2	2	0.00	0.00
Total	6	94	100	0.06	2	98	100	0.02	3.00	11	89	100	0.11	8	92	100	0.08	1.38
16-20	1	9	10	0.10	1	27	28	0.04	2.50	3	7	10	0.30	3	25	28	0.11	2.73

Table 5: Relative risks of the infections at different demographic factors

RF	CT			TP		
	Risk in the presence of RF	Risk in the absence of RF	RR	Risk in the presence of RF	Risk in the absence of RF	RR
≤ 30 yrs	0.85	0.15	5.7	0.74	0.36	2.1
RS	0.5	0.5	1.0	0.53	0.47	1.1
LE	0.9	0.1	9	0.95	0.05	19
PE-STI	1	0	∞	0.68	0.32	2.1
SSP	0.88	0.12	7.3	0.84	0.16	5.3

Key: RF = Risk factor, CT = *C. trachomatis* infection, TP = Syphilis infection, RS = Rural settlement, LE = Lower education, PE-STI = Previous exposure to STI, SSP = Single sex-partnering RR = Relative risk.

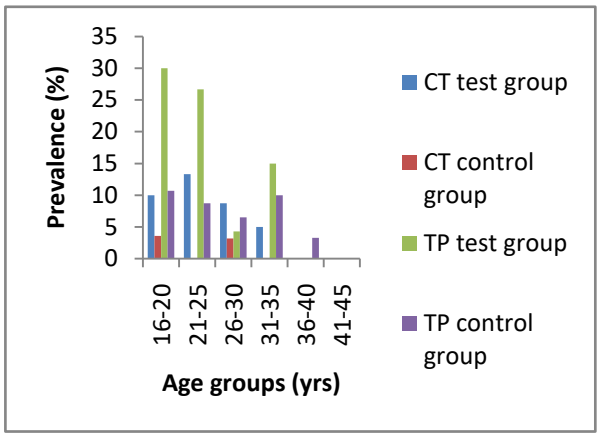

Fig. 1: Comparison of the % Prevalences of the Diseases in Different Groups of the Participants.

Key: CT = *C. trachomatis* infection, TP = Syphilis infection

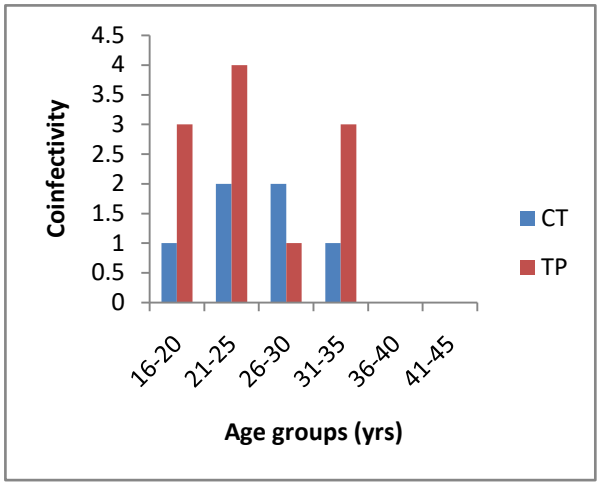

Fig. 2: Comparison of Single and Pair-wise infections of the Diseases in Test Group.

Key: CT = *C. trachomatis* infection, TP = Syphilis infection

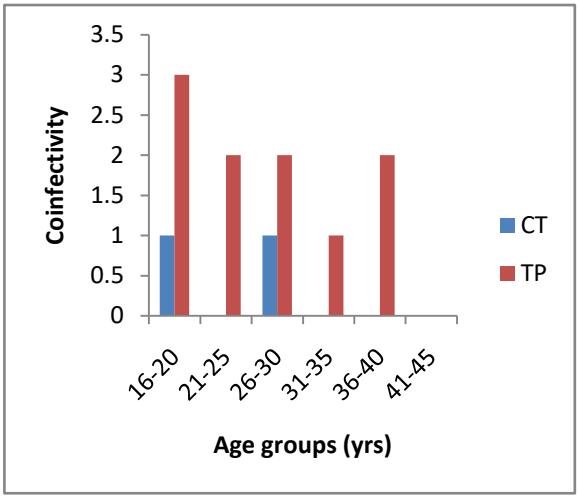

Fig. 3: Comparison of Single and Pair-wise infections of the Diseases in Control Group.

Key: CT = *C. trachomatis* infection, TP = Syphilis infection

IV. DISCUSSION

Phase one of this study carried out using focus group discussion and questionnaire administration highlighted some demographic factors influencing the distribution of the infections which include the age of the participants, the level of education, settlement, number of sex partners and previous exposure to sexually-transmitted diseases (Table 1). Rural settlement was associated with poor socio-economic conditions such as poor hygiene, poverty and low literacy level, which are risk factors for these infections as earlier reported by Sheringham*et al* (2011)

Prevalence of the diseases:

The observed higher prevalence at younger age groups 16-35 yrs could be attributed to the high level of promiscuity among the younger people, especially the rural dwellers. Syphilis infection occurring at age group 41-45 yrs and pair-wise infection (co-infection) occurring at 26-35 yrs could be due to immune suppression which gradually took place over time especially if HIV infection occurred at younger age (Table 3, Fig. 1, 2 and 3). This agrees with the reports that HIV infection increases the chance of multiple STI in an individual (Alvares-Travassos et al, 2012; Parikh et al, 2013). A comparison of the prevalence of the infections between the two groups, however, showed no significant difference at 95% confidence interval. Single and pair-wise infections of the two agents were examined in terms of their distribution among different age groups.

Demographic Factors Affecting the Distribution of the Infections:

*Age as a factor***:** Preponderance of CT and TP infections among younger age groups, 16-30 years were indicated in this study, which is in agreement with the report by the Centre for Disease Control and prevention in 2005, that the disease affects mostly younger people aged between 16 -25 years. Also, there is a little coherence among the 4.3% prevalence in 26-30 years, 5% prevalence in 31-36 years and the reports of Maragoni *et al* and Malhotra *et al* which have 26.8±5.5 and 20-30years respectively as the age affected (Malhotra *et al*, 2011; Maragoni *et al*, 2011). The coinfectivities as shown in Figs 2 and 3 clearly brought out age as a significant risk factor. The risk ratio between the younger age group (≤ 30 yrs) and the older age group (> 30 yrs) determined the extent of risk which age poses to the spread of the infections (Table 5). Similar pattern of distribution is observed in the control but extended to 40 yrs of age (Table 4)). This is in agreement with Parikh's report in 2003. Age was observed as a significant risk factor for CT, which has a predilection for the columnar cells of the cervix of young women. According to research findings, the incidence of CT infection in women decreases substantially after 30 years of age, likely because the target cell for CT (i.e., the columnar epithelial cell, which is present on the ectocervix of young women (cervical ectopy) is replaced by squamous epithelium through the process of squamous metaplasia that occurs with age (Jacobson *et al*,

2000) This trend is also not unconnected with sexual promiscuity associated with most young people.

HIV as a risk factor: The immuno-compromised condition of the HIV positive women was observed in this study as a contributory factor in their susceptibility to the infections, hence, higher prevalence in the test group (Table 3 and Fig. 1 and 2). HIV destroys $CD4^+$ cells, and inhibits T-cell activation. The T-cells play a significant role in the recognition of epitopes present in the *Chlamydia trachomatis* omp1 variable domain, hence, the severity of the infections in immuno-compromised individuals. Another reason is the ability of both infections to infect monocytes/ macrophages and have an obligate intracellular replication cycle. The present study underscores a significant association between STIs and HIV spread. A mutual and/or synergistic interaction was therefore observed in the co-infectivity of both infections (as in other STIs, especially the ulcerative STIs of the lower genital tract which facilitate the transmission of HIV) It is suggested that the joint epidemiology of the deadly trio (HIV CT and Syphilis infections) could be partly due to their common transmission modes; namely, sexual transmission and associated high risk behaviour including premarital sex and other forms of sexual promiscuity. An aspect of the biologic interaction that could also be responsible for the co-infectivity include the invasive intracellular pathogenesis of CT and Syphilis by which they induce aggressive genital epithelial layer and mucus membrane damage, which potentiate HIV entry and invasion, as well as some other subtle immunological modifications resulting from HIV subsequent invasion and colonization, which drastically encourages CT and Syphilis co-infections. On the other hand, immunosuppression resulting from HIV invasion could dispose infected persons to aggressive chlamydial diseases including pelvic inflammatory diseases and other associated adverse CT outcomes (Ikeme *et al*, 2011) These biologic interactions therefore increased the risk of the infections in the test group which agrees with the report by Parikh, (2013), that HIV increases the risk of STI and vice-versa.

Settlement as a factor: CT and Syphilis distributions between the rural and urban dwellers in test and control groups follow an irregular pattern (Tables 2). The non uniformity in the pattern of distribution suggests that the spread of Syphilis is not a function of settlement. The attributable and relative risks as shown in Table 8 buttress this fact since the risk in both rural and urban dwellers is 0.5 and the relative risk is 1.0. The settlement distribution does not agree with the report in England that the disease affects poor remote communities (Sheringham *et al*, 2011). The result of phase two of this study therefore suggests that settlement is not a factor in the distribution of the disease. The diseases are sexually-transmitted and one can think of the sexual activities being the same in both rural and urban areas.

Educational background as a factor: Educational backgrounds of the subjects were defined in terms of their highest qualifications (Prim Sch., Sec. Sch. and Higher Sch.). All post Secondary Schools were grouped as higher school. The distribution of the infections was observed to decrease with increasing educational status (Tables 2). The high risk ratio between the higher and lower education categories is not a surprise as the educated people are better informed on the danger, route of spread as well as prevention of the infection which is in agreement with the report by Sheringham *et al* (2011). Education also improves on the socio-economic conditions of the people which are also determinants in the disease distribution. Syphilis infection tolled the same line except in the secondary school category in the test group where a hike in the prevalence was observed. This could be as a result of chance or other factors such as sexual behaviour and immunity level. The risk ratio here is 19 (Table 5).

Previous exposure to STI: Exposure to STIs was observed to affect the distribution of the diseases as indicated in Tables 2. The risk ratio as calculated in Table 8 tends towards infinity for *C. trachomatis* and has a value of 2.1 for Syphilis infection. This trend agreed with the reports that one STI increases the chance of getting (Apea-Kubi *et al*, 2004; Alvares-Travassos *et al*, 2012). This could be attributed to a prior attack of the genital epithelium which creates holes that make it easier for subsequent STI to invade the tissue. Furthermore, the body immune system will be weak upon the earlier infections and cannot effectively fight the subsequent infection.

Number of sex partner: Number of sex partner was observed to significantly affect the distribution **(**Tables 2). Preponderance was observed among those that have single sex partner giving the impression that those with single partners are more likely to contract the infections and this contradicts the report by Klovstad *et al* (2011) which suggested that the infections are higher among those with multiple sex partners. The risk of acquiring the infections (CT and Syphilis) are higher in those with single sex partners, so also the relative risks (Table 5). During the study, participants were hardly convinced on why they should reveal the number of sex partner they have. It was very difficult to elicit response from them and as such, the response they gave may not be sincere information. It is expected that multiple sex partnering should be a more predisposing factor as it is a common practice among teenagers and younger adults especially girls with poor socio-economic background. These young girls fall prey to their male counterparts as they find it difficult to resist psychological and financial inducement. This practice exposes individuals to several STIs hence increasing the risk of infection.

V. CONCLUSION

Sexually-transmitted infections (STIs) do more havoc than anticipated especially those that affect the upper reproductive organs. The asymptomatic nature of most of the infections which makes the diagnosis difficult poses great public health challenge. *Chlamydia trachomatis*/ Syphilis co-infection is a

serious health condition in HIV patients giving the high chance of contracting the infection. This trend is worsened by the effects of demographic factors such as age, settlement, educational level and previous exposure to other STI. This particular research not only confirms the reports on Chlamydia epidemiology from other parts of the world but also establishes epidemiologic trend of the diseases under study within the locality of Nsukka, Enugu State of Nigeria. It also underscores the significance of HIV infection as well as demographic characteristics including previous exposure to other STIs and number of sex partiners as important risk factors to the dual infections: *C. trachomatis* and Syphilis, as well as the incorporation of CT and Syphilis diagnosis in the management of HIV infection especially in the resource poor areas of the world as a result of associated interactive mutual health risks. This trend could be an insight in modeling the disease distribution pattern within the area.

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