

The Effects CD4⁺ Counts and Viral Load on the Distribution and Coinfectivity of *Chlamydia trachomatis* and Syphilis Infections among HIV Positive Women in Nsukka, Enugu State, Nigeria

Ezema, James Nnabuike¹, Dibua, Maria Esther²

¹Department of Medical Microbiology, College of Medicine, Enugu State University of Science and Technology, Enugu, Nigeria.

²Department of Microbiology, University of Nigeria, Nsukka, Nigeria

Abstract:-

Objective: To investigate the effects of CD4⁺ and viral loads of HIV on the distribution of *Chlamydia trachomatis* and Syphilis infections among HIV-positive women in Nsukka, Enugu State.

Study Design: 100 HIV-seropositive women formed the test group while 100 HIV-seronegative women were used as control. Ethical clearance was obtained from the health facilities used. The study was carried out in Nsukka area of Enugu State, Nigeria, comprising urban, semi-urban and rural communities.

Methods: Plasma samples were used in the study. HIV antibodies were detected using the Determine (Alere, Japan) and the Gold (Trinity, Ireland). *C. trachomatis* and Syphilis antibodies were detected using CT IgG EIA kit (Xema, Russia) and Syphilis Ultra Rapid Test Strip Package Insert, (Global USA) respectively. CD4⁺ counts were determined by cytometry (CyFlow®). HIV viral loads of the samples were determined using DNA amplification and hybridization protocol. Statistical significance was determined using SPSS 16.0.

Results: Single infection occurred at CD4⁺ counts less than 500 cells/μl, while pair-wise infection occurred at CD4⁺ less than 400 cells/μl. Pair-wise infection for the test group was observed at CD4⁺ count less than 200 cells/μl while that of control occurred at CD4⁺ less than 400 cells/μl. Similarly, viral loads of the CT-positive samples ranged from 106888 to 645243 copies/ml while those of CT-negative samples in the same test group ranged from 1516 to 500217 copies/ml.

Conclusion: Sexually-transmitted infections (STIs) some of which present no immediate symptoms pose great public health challenge. *Chlamydia trachomatis*/ Syphilis co-infection is a serious health condition in HIV patients. This particular research confirms the reports on *Chlamydia* epidemiology from other parts of the world and also confirms HIV status as a strong risk factor of the diseases. Incorporation of CT and Syphilis diagnosis and treatment in the management of HIV infection is necessary.

Key words: *Chlamydia trachomatis*; Syphilis; HIV/AIDS; Women of reproductive age; Risk factors.

I. INTRODUCTION

Chlamydia trachomatis causes varieties of infections in humans such as urogenital infections (urethritis, cervicitis, pelvic inflammatory disease, endometritis), conjunctivitis, ocular trachoma and abscess of batholin gland. Studies show that 50-60% of *Chlamydia* infections presented with cervical and urethral problems, 30% are single cervical infections while 5-30% are single urethral infections (Isiabor *et al*, (2005). The differences in the symptoms presented by *C. trachomatis* infections are due to the different serovars and numerous variants of antigens which the organism is endowed with. Serovars A, B/Ba, C, D/Da/E, F, G, Ga, H, I/Ia, J, K, L1, L2, L2a and L3 have been identified using polyclonal and monoclonal antibodies against their major antigens, (major outer-membranes proteins-momp). Serovars A-C are associated with trachoma, serovars D-K, with urogenital and conjunctival cases while serovars L1-L3 are associated with *lymphogranuloma venereum* (Behrouz *et al*, 2010; Fruzsina *et al* 2004; Jonsdottir *et al*, 2002; Deborah *et al*, 2010).

The obligate intracellular nature of *C. trachomatis* is a barrier to its study. Most isolates carry a plasmid which encodes eight proteins. The plasmid serves as a transcriptional regulator and a virulence factor. Plasmid-free isolates also exist naturally and do not spread in the population like the plasmid-bearing isolates. This indicates reduced biological fitness to survive in its environment (Unemo *et al*, 2010). One of these proteins called the outer membrane protein 1 (omp1) has five regions of conserved sequences that alternate with four variable regions which have high genetic variations, and can therefore be used in genotyping of isolates. The variable domains give the bacteria all necessary endowments to survival in their environments. The human immune response is directed towards these variable domains and the monoclonal antibodies mounted on these domains are able to destroy the bacteria in a cell culture (Jonsdottir *et al*, 2003). The T-cell epitopes are also coded for in these variable domains; therefore any change

in the epitopes that are coded for (which often happens) will enable the bacteria to escape monoclonal antibody detection. Efforts are then directed at sequencing these regions to determine which serovars are present in a particular case of an infection.

II. METHODS

Sample collection:

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°C and used for subsequent tests.

Screening for HIV1 and HIV 2 antibodies:

HIV-1 and HIV-2 antibodies were screened by using two HIV test kits: the Determine™ (Alere medical Co., Ltd. Japan) which differentiates between HIV-1 and HIV-2 antibodies and the Gold™ (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.

Determination of CD4⁺ counts:

Flow-Cytometric method used by Alexander (1998) was employed in the CD4⁺ counting using an automated flow-cytometric machine (Cyflow®) according to instruction of the manufacturer

Screening for Chlamydia trachomatis antibodies from the blood samples:

C. trachomatis antibodies was screened for using *C. trachomatis* IgG EIA kit (Xema Co. Ltd., Russia) according to manufacturer's instructions. The principle was based on antigen-antibody reaction as described in section. The desired number of microstrips were put into the frame; eight (8) wells, 2 each were allocated for the calibrators, CAL (human IgG Ab to *C. trachomatis*) and control samples, CONTROL- (human serum without IgG), CONTROL+ (human serum with high IgG content) and serum. 80 µl of EIA buffer was pipette into each well. 20 µl of calibrators, control samples CONTROL- and CONTROL+ were pipette into the wells and were covered with plate adhesive tapes (included into the kit). The wells were then incubated for 30 minutes at 37°C. Washing solution was prepared by 21x dilution of washing solution concentrate BUF WASH (aqueous solution of sodium chloride) 21x by distilled water. At least 250 µl of washing

solution was used per well. The strips were washed 3 times. About 100 µl of CONJ HRP (monoclonal antibody + peroxidase enzyme) were dispensed into the wells. The wells were covered with adhesive tape and incubated for 30 minutes at 37°C after which the strips were then washed 5 times. Then about 100 µl of SUBS TMB (chromogen-substrate mixture) was dispensed into the wells and incubated for 10-20 minutes at +18...+25°C. 100 µl of STOP solution was dispensed into the wells and optical density (OD) was measured at 450nm.

Screening for Syphilis antibodies:

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

Measurement of viral load:

Viral load of the HIV-positive samples were measured using the method described by Paba *et al* (2011) and it involves the following steps in automated PCR machines according to the principle described in section 1.6.7:

Selective amplification:

This was achieved in COBAS AmpliPrep / COBAS AMPLICOR HIV-1 MONITOR Test, v1.5 by the use of AmpErase (uracil-N-glycosylase) enzyme and deoxyuridine triphosphate (dUTP). AmpErase enzyme destroys DNA containing deoxyuridine which is not present in naturally occurring DNA. Thereafter, PCR amplification of viral nucleic acid took place in the thermocycler where both annealing and extension occurred simultaneously.

Hybridization reaction:

Following amplification reaction, COBAS AMPLICOR Analyzer automatically added denaturation solution to chemically denature the HIV-1 amplicon and HIV-1 Quantitation Standard amplicon to form single stranded DNA. Suspension of magnetic particles coated with probes added to the amplicons and the amplicons hybridized to the target-specific oligonucleotide detection probe bound to the magnetic particles.

Detection reaction: COBAS AMPLICOR Analyzer was used to wash the detection magnetic particles to remove unbound materials and then add avidin-horseradish peroxidase conjugate which binds the biotin-labelled amplicon. Unbound conjugate was removed by washing the detection probe using COBAS AMPLICOR Analyzer. Amplicon was detected through microplate reader connected to a computer system.

III. RESULTS

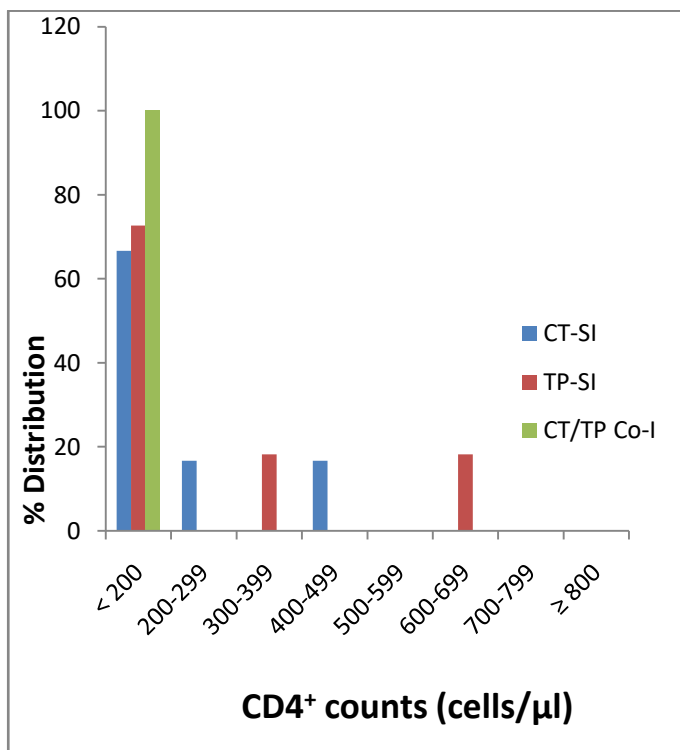


Fig. 1: Percentage Distribution of CT and TP Single and Pair-wise at Different CD4+ Counts in the HIV-Seropositive Women (Test Group).

Key: CT-SI = *C. trachomatis* single infection, TP-SI = *T. pallidum* single infection, CT/TP-Co-I = Co-infection of both.

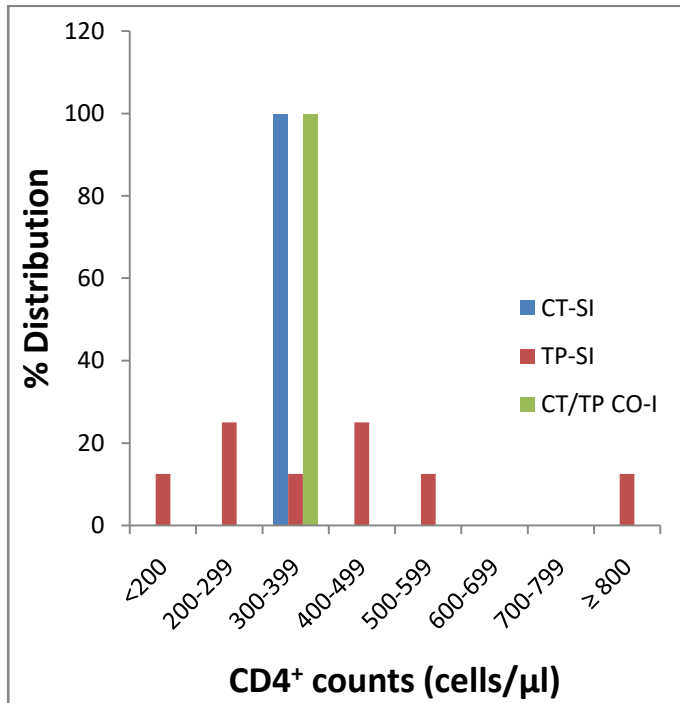


Fig. 2: % Distribution of CT And TP Single and Pair-Wise Infections At Different CD4+ Counts in the HIV-Seronegative Women (Control Group).

Key: CT-SI = *C. trachomatis* single infection, TP-SI = *T. Syphilis* single infection, CT/TP-Co-I = Co-infection of both.

Table 1: Viral Load of CT-Positive Samples in the Test Group.

Sample	Age (yrs)	Viral load (copies/ ml)	CD4+ counts (cells/ μl)	Co-infection with Syphilis
T5	41	136895	159	Yes
T24	40	106888	270	No
T28	36	645243	104	No
T58	35	328103	119	Yes
T75	42	158497	405	No
T84	29	200701	145	Yes

Key: CT = *Chlamydia trachomatis*, Tn = Test sample serially numbered accordingly.

Table 2: Viral Load of Some Randomly-Selected CT-Negative Samples in the Control Group

Sample	Age	Viral Load (copies/ml)	CD4+ counts (cells/ μl)	Syphilis single infection
T1	27	500217	65	No
T18	28	80609	218	No
T39	31	5206	807	No
T55	20	1516	479	No
T59	33	6690	130	No
T89	31	81205	350	Yes

Key: CT = *Chlamydia trachomatis*, Tn = Test sample numbered accordingly.

IV. STATISTICAL ANALYSIS

Despite the difference in the percentage prevalence, there is no significant difference in the prevalence of both infections at 95% confidence interval. Also at 95% confidence interval, there is no significant difference in the mean viral load of the CT-positive and the randomly-selected CT-negative samples.

V. DISCUSSION

This study investigated the prevalence of single and pair-wise infection with *Chlamydia trachomatis* and *Treponema palladium* (Syphilis) infections among HIV-seropositive women of reproductive age in Nsukka zone, Enugu State of Nigeria, necessitated by the increasing incidence of adverse pregnancy outcomes that are usually associated with these infections. The difficulties in child-bearing as well as previous cases of STI and CT are the indicators of the infections and these cases underscored the preponderance of the infections in the population studied. These findings were however not further authenticated by laboratory diagnosis as a result of non availability of diagnostic equipments. The observed prevalence in this study could be attributed to the mutual biological activities of the etiologic agents. This is in consonance with the previous report by Ikeme *et al*, 2001 and

Ward and Ronn, 2010 who indicated similar finding on the mutual beneficial outcomes of the disease causative agents.

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 of each of the infections in the test group. 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*, 2001; Joyee *et al*, 2004). 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.

CD4⁺ Counts and the Distribution of the Infections:

CD4⁺ counts were observed in this study as a significant factor in the susceptibility of individuals to the infections (Figs 5 and 6). The occurrence of all CT and Syphilis infections in both test and control groups at CD4⁺ count below 500 cells / μ l is in agreement with the report in San Francisco where infection occurred at CD4⁺ counts below 500 cells / μ l (Alvares-Travassos, 2012). HIV is an immunosuppressive virus and reduces CD4⁺ counts upon infection thereby increasing the susceptibility of the individual to the infections. When the CD4⁺ count is low, the body immunity becomes weak that it cannot offer protection against infections. Furthermore, these STIs have mutual biological activities with HIV infection. 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 infection in immuno-compromised individuals. *Chlamydia trachomatis* on the other hand creates tiny holes that aid HIV entry into the body. Similarly, Syphilis has been reported to disrupt mucosa and the influx of CCR5⁺ cells while HIV-1 infection adversely affects the serologic response to syphilis treatment at low CD4⁺ counts (Chun *et al*, 2013; Ikeme *et al*, 2001; Joyee *et al*, 2004).

Viral Load and Distribution of the Infection:

This study underscores the influence of viral load on the chances of contracting the infection (Tables 9 and 10). A careful observation shows that among CT-positive individuals, viral loads were appreciably high while among CT-negative ones, the loads were relatively lower. The observed inverse relationship between the viral loads and CD4⁺ counts was due to the immunosuppressive nature of HIV and other STIs. This could be attributed to the mutual biological interactions of the disease causative agents as discussed in the preceding section. HIV destroys CD4⁺ cells thereby weakening the body defense mechanism and increasing the susceptibility of the individuals to the infections. Sample T5 with CT/Syphilis co-infection had low viral load and low CD4⁺ count and this underscores the fact that infections such as CT and Syphilis can reduce CD4⁺ even at low viral load. Syphilis for instance disrupts mucosa and the influx of CCR5⁺ cells while HIV-1 infection adversely affects the serologic response to Syphilis treatment especially at lower CD4⁺ cells (^{count} < 200 cells/ μ l) (Chun *et al*, 2013). The extremely low viral load observed in sample T1 could be as a result of immunosuppressive role of other infections not screened for in this study.

VI. 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. This particular research confirms the reports on Chlamydia epidemiology from other parts of the world and also confirms HIV status as a strong risk factor of the diseases. Incorporation of CT and Syphilis diagnosis and treatment in the management of HIV infection is necessary.

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