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SHORT COMMUNICATION

Detection of anti-*Chlamydia trachomatis* antibodies in Patients with Acquired Immune Deficiency Syndrome in Abuja, Nigeria

Izebe K.S.,[1] Ngwai Y.B.,[2]* Ekpeyong M.,[1] Ezeunala M.,[1] Ajoku G.A.,[1] Oladosu P.,[1] Yusuf Y.,[1] Ibrahim K.,[1] Oladepo D.[1] and Inyang U.S[1]

[1]National Institute for Pharmaceutical Research and Development, P.M.B. 21 Garki, Abuja, Nigeria
[2]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, P.M.B. 071 Yenagoa, Bayelsa State, Nigeria.
E-mail: ybngwai@yahoo.com

ABSTRACT

Chlamydia trachomatis (CT) infections are among the sexually transmitted diseases known to increase the risk for human immunodeficiency virus infection. Serum samples from 34 consenting AIDS patients which attended the Government-approved Antiretroviral Treatment (ART) Facility at the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja between April 2005 and March 2006 were screened by enzyme immunoassay (EIA) for the presence of anti-CT antibodies using ImmunoComb® Chlamydia Bivalent IgG Test kit (Orgenics, Israel). Anti-CT antibodies were detected in ten (29.4%) of the thirty-four patients tested. The detection rate was higher among the females (33.3%) than the males (23.1%). Patients of the age group 31-45 years had the highest detection of anti-*C. trachomatis* antibodies, followed by those of age group 16-30 years. The result of the present study suggests the presence of anti-CT antibodies in AIDS patients, and reinforces the need for routine screening for anti-CT antibodies as a necessary intervention to reduce the burden of chlamydial diseases and to reduce the risk of HIV and its spread in Nigeria. The outcome of this study also provides justification for the possible inclusion of anti-chlamydial agents in the National AIDS Management Plan to treat associated *C. trachomatis* infections.

Keywords: *Chlamydia trachomatis,* Antibodies, AIDS, Nigeria

INTRODUCTION

Chlamydiae are non-motile, gram-negative bacterial pathogens with an obligate intracellular life cycle in eukaryotic cells. Of all the four species of the genus *Chlamydia* known to exist, *Chlamydia trachomatis* has emerged as the most common bacterial sexually transmitted pathogen (Schachter, 1999; Joyee, 2003; Stevens *et al*., 2004). *C. trachomatis* is known to cause infections of the cervix, urethra and upper genital tract in women, infections of the urethra and epididymis in men, and conjunctivitis and pneumonia in newborns, in addition to being the causative agent of trachoma (Stamm, 1990; Cates and Wasserheit, 1991; Quinn *et al*., 1996; Cohen and Brunham, 1999; Cevenini *et al*., 2002).

Chlamydia trachomatis infections are among the sexually transmitted diseases (STDs) known to increase the risk for human immunodeficiency virus (HIV) infection (Laga *et al*., 1993; Joyee *et al*., 2005). In Nigeria, information on the prevalence of *C. trachomatis* in AIDS patients is lacking or at best scarce. In the general population (that is, subjects without HIV infection), detection of *C. trachomatis* is between 15-40% (Obunge *et al*., 2001; Ekpenyong M, personal communication). Given the high number of HIV cases in Nigeria, the third highest in the World, after South Africa and India (UNAIDS, 2006), the additional burden of *C. trachomatis* infections will further increase the risk of HIV and its spread if not routinely screened for and checked. The present study aims at the detection of anti-*Chlamydia trachomatis* antibodies in serum of AIDS patients attending a Government-approved anti-retroviral treatment center to provide a basis for the possible inclusion of routine screening for anti-*C. trachomatis* antibodies and anti-chlamydial agents in the National AIDS Management Plan in Nigeria. To our knowledge, no similar study has been carried out before now.

MATERIAL AND METHODS

Study Population and Specimen Collection

Thirty-four (34) patients (13 males, 21 females) between the age of 16 and 46 years which attended the

Government-approved Antiretroviral Treatment (ART) Facility at the National Institute for Pharmaceutical Research and Development, Abuja during April 2005 to March 2006 were enrolled for the study. Twenty-one (9 males, 12 females) of the patients were married; four (all females) were widows; and nine (5 males, 4 females) were singles. Fifteen (4 males, 11 females) of the patients had evidences of genital discharge; whereas eighteen (9 males, 9 females) did not have discharges. The CD4, packed cell volume (PCV) and urine analysis for the 34 patients is as given in Table 1. The patients gave their consent in writing, by filling and signing a questionnaire administered to them.

Five milliliters of blood was collected by venepuncture from each of the patients using a sterile syringe, then allowed to coagulate at room temperature and serum separated. The sera were kept at -20 °C until required for testing.

ImmunoComb® Chlamydia Bivalent IgG Test

Detection of anti-*C*. *trachomatis* IgG was done by a solid-phase EIA using ImmunoComb® Chlamydia Bivalent IgG (*C*. *trachomatis* and *C*. *pneumoniae*) Kit manufactured by Orgenics (Israel).

The ImmunoComb® Chlamydia Bivalent IgG is a quantitative serologic test that uses two distinct strains on two differentiated spots: L2 serovar strain (*C. trachomatis*) and IOL 207 (TWAR) strain (*C. pneumoniae*). The extraction and elimination of the common genus-specific lipopolysaccharide (LPS) antigenic fraction enables the specific and differential diagnosis of *C*. *trachomatis* and *C*. *pneumoniae* infections. The EIA was performed and interpreted in accordance with manufacturer's instructions.

RESULTS

Anti- *C*. *trachomatis* antibodies were detected in ten (29.4%) of the thirty-four patients tested. As shown in Table 2, the detection rate was higher among the females (33.3%) than the males (23.1%). In addition, patients of the age group 31-45 years had the highest detection of anti- *C*. *trachomatis* antibodies followed by those of age group 16-30 years.

DISCUSSION

The rapid spread of the HIV-1 epidemic in some areas has been explained in terms of a "bidirectional" interaction between HIV-1 and the "classic" STDs (Wasserheit, 1992). Many studies have shown that STDs and other genital-tract infections increase the risk of HIV-1 acquisition, even though only few studies have measured the effect of HIV-1 on the risk of STD acquisition (Rottingen *et al*., 2001; McClelland *et al*., 2005). An increase in the risk of acquisition of STDs could have a substantial impact on global HIV-1 transmission, since genital tract infections likely increase the infectiousness of HIV-1-seropositive individuals (Baeten and Overbaugh, 2003).

Chlamydia trachomatis infections are among the STDs known to increase the risk for human immunodeficiency virus (HIV) infection (Laga *et al*., 1993; Rottingen *et al*., 2001; Joyee *et al*., 2005). They promote HIV transmission by a variety of biological mechanisms, which are likely to affect both HIV infectiousness and susceptibility. They facilitate HIV shedding in the genital tract, which probably promotes HIV infectiousness (Eron *et al*., 1996; Cohen *et al*., 1997). In HIV negative individuals, non-ulcerative STDs appear to increase susceptibility to HIV by recruiting HIV target cells to the endocervix (Levine *et al*., 1998). In addition, in vitro data suggest that *Chlamydia trachomatis* not only recruits polymorphonuclear leucocytes, but may also interact with these cells to increase HIV replication (Ho *et al*., 1995). Furthermore, Ghinsberg and Nitzan (Ghinsberg and Nitzan, 1992) have shown that the presence, in CT patients of anti-CT IgA and high C3 fraction of the complement, may be crucial since IgA inactivates CT and C3 which may increase the invasion of HIV into the cells. On the other hand, HIV-1 may influence the susceptibility to genital-tract infections or the duration, response to treatment, recurrence rate, or severity of many genital-tract infections including *Chlamydia trachomatis* (Fleming and Wasserheit, 1999).

Genital *Chlamydia trachomatis* infection can be diagnosed by several different approaches, some of which require sophisticated costly equipment and skill not always available in developing country laboratories (Black, 1997). These include: nucleic acid amplification tests - ligase chain reaction (LCR) and polymerase chain reaction (PCR) which use vulval, cervical or urethral swab or first-void urine as specimen; gene probe which uses vulval, cervical or urethral swabs as specimen; enzyme immunoassay (EIA) which uses urethral or cervical swabs as specimen; direct immunofluorescence antibody (DFA) which uses urethral or cervical swabs as specimen; direct cytologic examination with Giemsa stain; culture which uses urethral or cervical swabs as specimen; and leucocyte esterase transferase (LET) which uses first-void urine as specimen. The gold standard is culture for chlamydia performed as described by Mardh *et al*. (1977) or chlamydia diagnosed by two non-culture tests, now known as the expanded gold standard (Stary *et al*., 1996). A meta-analysis of several studies reported using these methods was carried out by Watson *et al*. (Watson *et al*., 2002). The results showed that while specificities were high, sensitivities varied widely across the tests and were also dependent on the specimen tested. Pooled sensitivities for LCR, PCR, gene probe and EIA on urine were 96.5%, 85.6%, 92% and 38%, respectively, while on cervical swabs the corresponding sensitivities of PCR, gene probe and EIAs were 88.6%, 84% and 65%. As reviewed by Black (1997), sensitivity and

Table 1: CD4 cell count, packed cell volume and urine analysis for thirty-four AIDS patients in Abuja, Nigeria

S/No	CD4 (cells/µl)	PCV (%)	Urine analysis									
			Color	Bil	Uro	Ket	Glu	Prot	Blood	Nit	pH	SG
1	1248	40	C_Y	-	-	-	-	-	-	-	6	1.020
2	460	34	C_Y	-	-	-	-	-	-	-	6	1.010
3	402	36	C_Y	-	-	-	-	-	+++	-	7	1.025
4	1343	38	C_P	-	-	-	-	-	-	-	7	1.015
5	978	40	C_Y	-	-	-	-	-	-	-	5	1.015
6	584	40	C_P	-	-	-	-	-	-	-	6	1.010
7	300	34	C_P	-	-	-	-	-	-	-	5	1.015
8	197	40	C_W	-	-	-	-	-	-	-	5	1.015
9	980	45	C_A	-	-	-	-	-	-	-	5	1.025
10	Nd	47	C_A	-	-	-	-	-	-	-	7	1.005
11	412	38	C_Y	-	-	-	-	-	-	-	5	1.015
12	Nd	34	C_Y	-	-	-	-	-	-	-	7	1.005
13	1299	40	C_Y	-	-	-	-	-	-	-	5	1.010
14	522	40	C_Y	-	-	-	-	-	-	-	6	1.010
15	627	45	C_Y	-	-	-	-	-	-	-	5	1.010
16	247	41	C_Y	-	-	-	-	-	-	-	7	1.005
17	304	45	C_Y	-	-	-	-	-	-	-	7	1.010
18	396	38	C_Y	-	-	-	-	-	-	+++	6	1.010
19	240	40	C_W	-	-	-	-	-	-	-	7	1.005
20	206	40	C_A	-	-	-	-	-	-	-	5	1.010
21	293	38	C_A	-	-	-	-	+	++	-	5	1.020
22	139	46	D_A	-	-	-	-	-	-	-	5	1.015
23	201	37	C_A	-	-	-	-	-	-	-	5	1.010
24	Nd	41	C_A	+	++++	-	-	++	-	-	6	1.015
25	Nd	41	C_Y	-	-	-	-	-	-	-	6	Nd
26	35	40	C_Y	-	-	-	-	-	+	-	6	Nd
27	489	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
28	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
29	98	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
30	591	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
31	230	Nd	S_A	-	+	-	-	-	-	-	6	1.020
32	313	Nd	S_A	-	-	-	-	-	-	-	6	1.020
33	470	Nd	C_Y	-	-	-	+	-	-	-	6	1.010
34	1460	Nd	C_P	-	-	-	-	-	-	+	6	1.020

PCV, packed cell volume; Bil, bilirubin; Uro, uronic acids; Ket, ketones; Glu, glucose; Prot, protein; Nit, nitrate; SG, specific gravity; C_Y, clear yellow; C_P, clear pale; C_W, clear white; C_A, clear amber; D_A, dirty amber; and Nd, not determined

Table 2: Detection of anti-*Chlamydia trachomatis* antibodies in sera of thirty-four AIDS patients in Abuja, Nigeria

Age (Years)	No. examined		No. positive		Percentage detection	
	Male	Female	Male	Female	Male	Female
16-30	2	8	0	2	0.0	25.0
31-45	10	11	3	5	30.0	45.5
> 45	1	2	0	0	0.0	0.0
Total	13	21	3	7	23.1	33.3

specificity are as follows - (sensitivity, specificity): culture (70-85%, 100%); DFA (80-90%, 98-99%); EIA (73-83%, 97-99%); DNA probe (85%, 98-99%); PCR (90%, 99-100%); LCR (94%, 99-100%); direct cytologic examination with Giemsa stain (>90%, 100%); LET (31-100%, 83-100%); and serologic tests such complement fixation, microimmunofluorescence (MIF) and EIA for Chlamydia antibodies with varying sensitivities and specificities . The more convenient and yet highly sensitive is the serological detection of antibodies to chlamydiae, the "reference standard" being the microimmunofluorescence (MIF) test of Wang and Grayston (1970).

The presence of antibodies to *C*. *trachomatis* in the sera of AIDS patients as observed in our study may be indicative for a past chlamydial infection or for the active status in acute, chronic and recurrent chlamydial infections. The detection rate is somewhat lower than the 29.5%, 31.2% and 95% reported in India (Joyee *et al*., 2005), Mexico (Cravioto Mdel *et al*., 2003) and Jamaica (Dowe *et al*., 1997) respectively. Serological detection of specific IgG antibodies usually facilitates diagnosis for chlamydial infections. Clinical studies have shown a high correlation between the serological detection of IgG for *C*. *trachomatis* in sera and the presence of chlamydial antigen (Csángo *et al*., 1988). Since ImmunoComb test results have been shown in an earlier study to correlate well with those of the MIF test (Clad *et al*., 1994), detection of anti-*C*. *trachomatis* IgG using ImmunoComb assay provides a valid reason to suspect past or active chlamydial infections in those patients. Further study that employs a larger sample population size and isolates *C*. *trachomatis* in cell culture is required to confirm this observation.

CONCLUSION

The detection of anti-*C*. *trachomatis* IgG in sera of AIDS patients attending the ART Center in Abuja provides rationale for possible inclusion of routine screening for anti-*C*. *trachomatis* antibodies and anti-chlamydial agents in the National AIDS Management Plan in Nigeria. Programs directed at HIV-1-seropositive individuals with STDs may have a substantial impact on the spread of HIV-1. Operational research is needed to determine the ideal frequency of screening and appropriate treatment regimens for specific populations. The findings presented here highlight the need for both research and program support to implement targeted screening and treatment for genital-tract infections in HIV-1-seropositive individuals, as an HIV-1 prevention strategy.

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