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SERO-PREVALENCE STUDY OF PARASITIC INFECTIONS AMONG HIV POSITIVE AND NEGATIVE PATIENTS IN LAGOS, NIGERIA

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ABSTRACT

Background: Diseases caused by opportunistic pathogens are the major clinical signs of HIV infected and AIDS patients with parasitic infection being part of the common causes of morbidity and mortality.

Objectives: This was a cross-sectional study to determine the sero-prevalence of serum antibodies to three parasitic infections namely *Entamoeba histolytica*, *Schistosoma sp.* and *Toxoplasma gondii*, which are opportunistic infections among HIV/AIDS patients.

Methods: One thousand and eighty patients that attended three healthcare institutions in Lagos were recruited for the study through convenience sampling method. Venous blood was collected from the recruited patients and screened for HIV infection as well as the presence of serum antibodies to three parasitic infections. All positive sera samples were confirmed for HIV infection.

Result: The results revealed that 65/1080 (6%) of the recruited patients were HIV sero-positive. In addition, 5/65 (7.7%) of the HIV positive patients had *E. histolytica* co-infection, 1/65 (1.5%) had *Schistosoma sp.* co-infection while 2/65 (3.1%) had *T. gondii* co-infection. The results also indicated that the proportion of patients with *E. histolytica* was significantly higher among HIV sero-positive patients than the sero-negative patients ($P = 0.031$).

Conclusion: The study showed the opportunistic potential of the three parasitic infections among HIV/AIDS patients in the study area.

Keywords: [HIV, AIDS, Seropositive, Seronegative, *Toxoplasma gondii*, *Entamoeba histolytica*, *Schistosoma haematobium*]

LA SEROPREVALENCE DES INFECTIONS PARASITAIRES CHEZ LES PATIENTS SEROPOSITIFS ET LES PATIENTS VIH NEGATIFS A LAGOS, NIGERIA.

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RÉSUMÉ

CONTEXTE : Les maladies causées par les pathogènes opportunistes sont les principaux signes cliniques des patients infectés par le VIH et des Patients infectés par le VIH et le SIDA avec les infections parasitaires faisant partie des causes fréquentes de morbidité et de mortalité.

OBJECTIFS : Ceci fut une étude transversale pour déterminer la séroprévalence des anticorps sériques aux trois infections parasitaires à savoir *Entamoeba histolytica*, *Schistosoma sp.* et *Toxoplasma gondii*, qui sont les infections opportunistes chez les patients VIH/SIDA.

METHODES : Mille huit cent patients qui ont à trois établissements de sante à Lagos ont été recrutés pour l'étude à travers la méthode d'échantillonnage de commodité. Sang veineux a été recueilli des patients recrutés et dépistés pour les infections du VIH ainsi que la présence des anticorps sériques aux trois infections parasitaires. Tous les échantillons de sérums positifs ont été confirmés pour l'infection au VIH.

RESULTAT : Les résultats ont montré que des patients 65 sur 1 080 (6%) recrutés étaient séropositifs pour le VIH. En outre, 5 sur 65 (7,7%) des patients séropositifs avaient l'infection *E.histolytica*, 1 sur 65 (1,5%) avait la coinfection *Schistosoma sp.* alors que 2 sur 65 (3,1%) avaient la coinfection *T.gondii*. Les résultats ont également indiqué que la proportion de patients avec *E.histolytica* était significativement plus élevée chez les patients VIH séropositifs que les patients séronégatifs (P=0,031).

CONCLUSION : L'étude a montré le potentiel opportuniste des trois infections parasitaires chez les patients VIH/SIDA dans la zone d'étude.

MOTS CLES : {VIH,SIDA, Seropositif, Seronegatif, *Toxoplasma gondii*, *Entamoeba histolytica*, *Schistosoma haematobium*}

INTRODUCTION

Infections caused by these parasites *Toxoplasma gondii*, *Schistosoma sp.* and *Entamoeba histolytica* have been characterized as opportunistic infection in human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) patients around the world (1, 2, 3). The major clinical manifestation of HIV infection as well as in AIDS patients is diseases caused by opportunistic pathogens and parasitic co-infections constituted part of the common causes of morbidity and mortality (4, 5).

Toxoplasmosis is a typical disease that affects humans, with most cases asymptomatic. While the prevalence of *Toxoplasma* antibodies correspond with age, it does not correspond with sex; variations and differences exist in each country. In part of sub-Saharan Africa with moist weather, variations in toxoplasmosis prevalence ranges between 50% and 70% and the estimated population risk and infection is one third and half among worldwide population (6). The incidence of sero-positivity varies in humans, showing previous exposure and location in addition to eating habits (7, 8). Previous studies demonstrated the common cause of focal lesions of the brain as a result of *Toxoplasma* encephalitis and thereby complicating the direction of AIDS due to the reactivation of a latent infection (9, 10). In advanced stage with *Toxoplasma* encephalitis recrudescence usually manifest as cerebral abscess impairing the immune responses to infections causing the infective reactivation in AIDS patients through immune suppression (2, 11, 12). Patients with *Toxoplasma* and HIV co-infections have between 30% to 40% risk of developing *Toxoplasma* encephalitis especially amongst those with significant

immunosuppression (CD4 count less than 200 cells/ μ l) (13).

Schistosomiasis is a parasitic disease cause by *Schistosoma sp.* and it is mainly found in the tropical and sub-tropical areas of the world. Due to inadequate potable water supply and limited water resource development, schistosomiasis is prevalent in sub-Saharan Africa, Asia and South America (14). Earlier studies demonstrated that urinary schistosomiasis occurs more than intestinal schistosomiasis in Nigeria (15, 16). Both HIV/AIDS and schistosomiasis causes significant disease burden in sub-Saharan Africa with frequent overlap in their epidemiological characteristics¹⁷. While few studies have examined interaction between the two diseases (3, 15, 16, 17, 18, 19), studies have shown that both diseases exert bi-directional effects on one another (20, 21).

Entamoeba histolytica primarily inhabits the large intestine where the trophozoites or active forms live. Studies have shown that morphologically identical pathogenic and non-pathogenic strains of amoebae that are genetically, biochemically and immunologically distinguishable exist (22, 23, 24). However, the same strain can change its behavior within the same host from time to time. Hence, there is no doubt that immunosuppression of the host can turn a harmless commensal infection into a dangerously invasive one (1, 25, 26). Invasive amoebiasis (IA) is a significant parasitic infection that is associated with significant morbidity and mortality world-wide in individuals that resides or travels to endemic areas and it accounts for 40,000 to 100,000 deaths annually. IA had previously been reported in HIV infected / AIDS patients (27, 28, 29, 30).

While some work had been conducted on these parasitic infections in Nigeria, few of such studies had been carried out comparatively in HIV infected and non-infected individuals in the country. Hence, the objective of this study was aimed at determining the sero-prevalence of these three parasitic infections among HIV seropositive and sero-negative patients using serodiagnostic approach to determine the presence of serum antibody to these infections.

Research Method and Design Study

This was a cross-sectional based study that was designed to carry out sero-prevalence of serum antibodies to three parasitic infections namely *Entamoeba histolytica*, *Schistosoma sp.* and *Toxoplasma gondii* using serodiagnostic approach. Details of the study design and population have been published previously³¹. Briefly, patients attending three health-care institutions in Lagos, Nigeria namely, General Hospital Ikeja, Sexually Transmitted Diseases Clinic Yaba, and the Central Public Health Laboratory Yaba were recruited for the study through the convenient sampling method between January 1996 and December 1997. The selected institutions serve as: (i) provider-based facility, (ii) referral centre for HIV and STD patients, and (iii) client based facility especially for people who intend to be familiar with their HIV and STD statuses.

Blood samples for screening of HIV, *Toxoplasma gondii*, *Schistosoma sp.* and *Entamoeba histolytica* were obtained with consent from 1080 patients out of 200 patients interviewed. Written informed consent or thumbprints were received from all recruited individuals and consent could not be obtained from 920 patients who declined to participate and were excluded from the study. Both patients who refused to be recruited for the study and those who were sero-positive in the study were given adequate clinical services.

Laboratory procedures

Blood sera of the patients were screened for HIV-1 and HIV-2 using Cambridge Biotech Corporation Recombigen HIV-1 and HIV-2 rapid test device while confirmation of positive cases was done with Immunocomb 11 and Bio-rad Novapath HIV-1 immunoblot for HIV-2 and HIV-1, respectively.

Diagnosis of parasitic infections from blood sera of sampled patients was done by Indirect Haemagglutination (IHA) method. The IHA principle involves the use of reagent made of formalized sheep blood cells, which are sensitized by a soluble antigen of the parasite and

made to react with antibodies present in the patient with the formation of a haemagglutination i.e. a reddish-brown film that is observed in the wells of a U-microplate. In the absence of specific antibodies, the sensitized red blood cells will deposit, by forming a ring in the bottom of the well. Cellognost® kits from Behring Diagnostic Inc. were used for the screening of IgG antibodies to *Schistosoma sp.* and *Entamoeba histolytica*, while Toxocell IHA kits from Biokit SA were used for screening of *T. gondii* IgG antibodies. *Toxoplasma* kits from Randox Laboratories Ltd. were used for the detection of IgM antibodies to *T. gondii* by the Enzyme Linked Immunosorbent Assay (ELISA) method. Titer values were interpreted specific to each parasite according to the manufacture's guideline for interpretation as earlier documented (32, 33, 34, 35, 36, 37).

Manufactures recommended cutoff titer of 1:16 (Low) and 1:32 was used for the evaluation of *Entamoeba histolytica* and *Schistosoma sp.*, while 1:64 (Toxo IgG) and 0.9 (Toxo IgM) was used for *Toxoplasma gondii*

Data analysis

The obtained data were analyzed using the EPI-INFO Statistical Package, version 6.0. Statistical significance of the proportions of categorical data was estimated using Fisher's exact test for contingency table. All tests were two-tailed and P-value of <0.05 was taken as statistical significant.

RESULTS

Of the sampled patients (aged 4 to 62 years), 36/1080 (3.3%) were males and 29/1080 (2.7%) were females. Titer values ranged from 1:16 to 1:4096. Out of the total patients screened for HIV in the study, 65 (6%) were sero-positive. Among the 65 patients with confirmed HIV sero-positive results, 8 (12.3%) had three parasitic co-infections while among the 1015 patients without HIV infections, 34 (3.3%) had the three infections (Table 1).

Sero-diagnosis assay from the study showed that 5 (7.7%) of patients with HIV sero-positive result had serum antibodies to *E. histolytica* (Table 1). Titer values of serum antibodies to *E. histolytica* infection ranged from 1:16 to 1:512 for the sampled patients. A significant titer value range of 1:32 to 1:512 was found in 5 (0.5%) HIV sero-negative patients, while 3 (4.6%) HIV sero-positive patients had a significant titer value of 1:256 to 1:512 for *E. histolytica* serum antibody (Table 2).

Only 4 (0.4%) HIV sero-negative patients and 1 (1.5%) HIV sero-positive patients had serum antibodies to *Schistosoma sp.* infection (Table 1). Among HIV sero-negative patients 3 (0.3%) had a

titer of 1:16 while only 1 (0.1%) patient had a higher titer of 1:256. Titer value in the HIV sero-positive patient was 1:16 (Table 2).

TABLE 1: SAMPLED PATIENTS WITH SERUM ANTIBODIES TO PARASITIC INFECTIONS

Parasitic infection	Patients with serum antibody		Total
	HIV+ N = 65 (%)	HIV- N = 1015 (%)	N = 1080 (%)
<i>E. histolytica</i>	5 (7.7)	24 (2.4)*	29 (2.7)
<i>Schistosoma sp.</i>	1 (1.5)	4 (0.4)**	5 (0.5)
<i>T. gondii</i>	2 (3.1)	6 (0.6)***	8 (0.8)
Total	8 (12.3)	34 (3.3)	

*P = 0.031, ** P = 0.27, ***P = 0.082

Serum antibodies to *T. gondii* infection were found in 2 (3.1%) of HIV sero-positive patients and in 6 (0.6%) of the sero-negative patients with titer value range of 1:16 to 1: 128 (Table 1). Significant titer value of $\geq 1:64$ Toxo kit and >0.9 Toxo IgM kit interpretations was recorded in 1 (0.1%) HIV sero-negative patient and 2 (3.1%) HIV sero-positive patients (Tables 2).

Result from the study also showed that more patients within the age range 21-30 years in both HIV sero-positive and sero-negative patients had serum antibody to *T. gondii* infection (Table 3). Also, more females than males presented with antibodies to *T. gondii* infection in HIV sero-positive 2 (3.1%) and HIV sero-negative 5 (0.5%) patients. (Table 4).

TABLE 2: TITER VALUES OF SAMPLED PATIENTS SHOWING SERO-POSITIVITY FOR PARASITIC INFECTIONS

Parasitic infections	HIV Status	Titre values									Total
		1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096	
<i>E. histolytica</i>	sero-	19 (1.9)	1 (0.1)		2 (0.2)	1 (0.1)	1 (0.1)				24
	sero+	2 (3.1)				2 (3.1)	1 (1.5)				5
<i>Schistosoma sp.</i>	sero-	3 (0.3)				1 (0.1)					4
	sero+	1 (1.5)									1
<i>T. gondii</i>	sero-	2 (0.2)	3 (0.3)	1 (0.1)							6
	sero+			1 (1.5)	1 (1.5)						2

TABLE 3: AGE DISTRIBUTION OF SAMPLED PATIENTS SHOWING SERO-POSITIVITY FOR PARASITIC INFECTIONS

Age group (Years)	Sero-positive for invasive <i>E. histolytica</i>		Sero-positive for <i>Schistosoma sp.</i>		Sero-positive for <i>T. gondii</i>	
	HIV sero-	HIV sero+	HIV sero-	HIV sero+	HIV sero-	HIV sero+
1-10	3 (0.3)					
11-20	5 (0.5)	1 (1.5)	2 (0.2)		1 (0.1)	
21-30	5 (0.5)	2 (3.1)	2 (0.2)	1 (1.5)	3 (0.3)	2 (3.1)
31-40	6 (0.6)	2 (3.1)		2 (0.2)		
41-50	3 (0.3)					
>50	2 (0.2)					

TABLE 4: SEX DISTRIBUTION OF SAMPLED PATIENTS SHOWING SERO-POSITIVITY FOR PARASITIC INFECTIONS

Sex	Sero-positive for invasive <i>E. histolytica</i>		Sero-positive for <i>Schistosoma sp.</i>		Sero-positive for <i>T. gondii</i>	
	HIV sero-	HIV sero+	HIV sero-	HIV sero+	HIV sero-	HIV sero+
Males	14 (1.4)	3 (4.6)	4 (0.4)		1 (0.1)	0 (0)
Females	10 (1.0)	2 (3.1)		1 (1.5)	5 (0.5)	2 (3.1)

DISCUSSION

The presence of significant serum antibody to *E. histolytica* and *T. gondii* among HIV sero-positive patients in the study has further exposed these two parasitic organisms as possible opportunistic infections in HIV infected / AIDS patients as previously reported (1, 2). Medical practitioners in this region should focus attention on the diagnosis of these organisms and clinical management of infection with these organisms as well as other endemic diseases like malaria and tuberculosis that may interact or be opportunistic in HIV infected and AIDS patients.

The significant titer value of 1:32 or more recorded in this study in HIV sero-positive as well as in HIV sero-negative patients is an indication of the presence of invasive *E. histolytica*. Clinical manifestations associated with *E. histolytica* infection could result in asymptomatic and symptomatic infection with or without tissue invasion. *E. histolytica* may remain a harmless commensal in its host or may become pathogenic due to certain factors not fully understood; however, immunosuppression had been described as a factor that could turn

harmless commensal *E. histolytica* into a dangerously invasive one. Majority of infection with *E. histolytica* are asymptomatic and such individuals will have a negative or weak serologic response and will primarily pass cysts in their stools^{38, 39}. In one study, asymptomatic HIV-1 infected individuals with high anti-Eh titer were reported to be at risk of IA, perhaps as a result of exacerbation of subclinical amebiasis (27). In another study, the main presenting symptoms of IA were reported as fever, chronic diarrhea, and abdominal pain (29).

Only 1.5% of the HIV sero-positive patients in the study had serum antibody to *Schistosoma sp.* infection as compared with 0.6% of HIV sero-negative patients that had serum antibody. Both urogenital and intestinal schistosomiasis are chronic inflammatory disease caused by a water-borne parasitic blood fluke with about 220 million people infected in sub-Saharan Africa, and more people especially children at risk (17). The presence of *Schistosoma sp.* serum antibodies with titer value of 1:16 in only one HIV sero-positive patients in this study does not exclude a possible interaction between *Schistosoma sp.* and

HIV in endemic areas like Nigeria since both agents co-inhabit the blood where they both cause pathological symptoms. Further studies are progressing to investigate the effect of one on the other in infected patients. Few of such studies that examined the interaction between *Schistosoma sp.* and HIV co-infection especially in areas where dual endemicity is most prevalent tried to understand the pathogenesis and immune responses in co-infection as well as clinical studies of responses to antiparasitic and antiretroviral drugs for HIV/AIDS disease progression (3, 17, 40).

Indications for the presence of *T. gondii* infection was found in two patients that were sero-positive for HIV and one patient that was sero-negative for HIV with a significant titer value. In the diagnosis of *T. gondii* infection, a negative IgM indicates absence of infection in the past 6 months while a combination of positive IgM and IgG titer indicates acute infection (41, 42, 43). A positive IgG test with a negative IgM indicates chronic infection (12). This result thus supports the presence of *T. gondii* infection in HIV infection in the area. A sero-prevalence of 58% was reported for Toxo-IgG antibodies among HIV patients without neurological complications in Lagos, Nigeria (44). Although more females than males in this study presented with serum antibodies to *T. gondii* infection, studies have shown that there is little or no difference in the prevalence of *T. gondii* infection between sexes (6).

Limitation

Limitation of this study includes the inability to investigate factors like education, nutrition, environmental and behavioral attitudes that can influence the rate differences between HIV infected and HIV non-infected patients in the study. Furthermore, serology positive tests for parasitic infections in the study could not be confirmed with more specific test due to limited resources. Another limitation is the low sensitivity and specificity of serological diagnosis of parasitic infections (45). In addition, sample

sizes of HIV positive for uncommon parasites were very small.

Recommendation

Information on the prevalence of these diseases among HIV infected patients is scanty in the area; with a population of over 150 million and HIV prevalence of 3.1% among adults ages 15-49 reported in 2012⁴⁶, more studies are required on these diseases by location in different part of the country.

Conclusion

We have therefore been able to further determine the sero-prevalence as well as demonstrate the presence of the three parasitic infections in the area most especially among HIV infected patients using serodiagnosis technique which is considered to be the most available and affordable routine method of diagnosing diseases like Toxoplasmosis in resource poor countries (37) and as well help guide therapeutic and management policies for infected patients in the area.

Ethical

Ethical

Ethical approval for the study was obtained from the Federal Ministry of Health Authority.

Informed

Study objectives were explained to all participants. Participation was voluntary and confidentiality of study information was guaranteed.

Competing

None

considerations

clearance

consent

interests

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