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A STUDY ON AMOEBIASIS AS AN OPPORTUNISTIC INFECTION IN HIV/AIDS PATIENTS AT KORLE-BU TEACHING HOSPITAL

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ABSTRACT

Background: The immuno-compromised state of HIV/AIDS patients makes them susceptible to various infections. Amoebiasis, caused by *Entamoeba histolytica* is believed to be an opportunistic infection in HIV/AIDS patients. However in Ghana there is no data to ascertain this assertion.

Objective: To determine the opportunistic status of amoebiasis in HIV/AIDS patients through determination of amoebic antibody sero-prevalence among diarrhoea and non-diarrhoea patients in relation to their blood $CD4^+$ cell count.

Study design and methodology: A sample size of 95 HIV patients consisting of 43 non-diarrhoea and 52 diarrhoea patients were involved. They constituted 65 females and 30 males aged 20 to 60 years old. A rapid test kit, INSTANT™CHEK-amoeba was used to determine the presence of anti-*E. histolytica* antibodies in patients' sera. Their blood $CD4^+$ cell count was also determined using a FACS counter.

Results were analyzed by using the Chi square test statistic with a 95% confidence limit and a significant level of 0.05, to determine the significant difference between anti *E. histolytica* antibodies positive in diarrhoea and non-diarrhoea HIV patients.

Results: The overall prevalence of anti-*E. histolytica* antibodies in the study population was 13.7%. About 77.0% of these had low $CD4^+$ cell count. The highest anti-*E. histolytica* antibody prevalence of 17.3% was found in diarrhoea patients mostly, females and almost all of them 20-50 years old. There were no amoebic antibody sero-positives in patients with high $CD4^+$ cell counts. There was no significant association between the detection of anti-*E. histolytica* antibodies in diarrhoea and non-diarrhoea HIV/AIDS patients, considering their CD4+ cell counts.($p=0.258$).

Conclusion: Amoebiasis could not be confirmed as an opportunistic infection in HIV/AIDS patients using amoebic antibody seroprevalence.

KEY WORDS: Opportunistic infections, HIV/AIDS patients, *Entamoeba histolytica,* diarrhoea, $CD4^+$ T-cells, Korle Bu Teaching Hospital.

INTRODUCTION

Widespread amongst all human chronic infections the world over are Intestinal Parasitic Infections (IPI). In sub-Saharan Africa the rate of infection is remarkably high where the majority of HIV/AIDS patients/cases are concentrated (1). IPI includes amoebiasis, a very common infection caused by *Entameoba histolytica*. There are two forms of the disease namely, intestinal (amoebic dysentery) and extra-intestinal (liver abscess).

Around 480 million people worldwide are infected by the protozoan *E. histolytica* (2).

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Although only about 10% are symptomatic, it is the third leading parasitic cause of death after malaria and schistosomiasis with an annual mortality of approximately hundred thousand (3). *Entamoeba histolytica* is endemic in many parts of tropical and sub-tropical Africa, Asia, Mexico South America and China (4). Inadequate environmental sanitation and poor personal hygiene impacts on its distribution more than the climate. Severe infection occurs in pregnant women, the malnourished and people on steroids. (5). The symptoms of amoebic dysentery include diarrhoea, abdominal cramps, bowel movements streaked with blood or mucus, nausea or vomiting and occasionally, fever.

In a study conducted at the Korle-Bu Teaching Hospital (KBTH) by Ayeh-Kumi *et. al.*; unpublished data)., examination of stool samples from 550 out-patients visiting the central laboratory of the hospital revealed 9.1% *E. histolytica* and of 7.4% *E. dispar* infections. Comparison of the laboratory results with presumptive diagnosis showed amoebic infection in more of the patients suspected of amoebic dysentery (15.4%), than other infections such as helminthiasis (13.2%), gastroenteritis (11.0%), anaemia (7.0%) diarrhoea (4.4%) and enteric fever (2.2%). Patients with presumptive diagnosis of cyesis had the highest prevalence of amoebic infection (27.0%).

HIV infected individuals are predisposed to several parasitic diseases as a result of the opportunistic behaviour of these parasites due to the immuno-compromised state of the patients. The aim of the study thus is to determine the opportunistic status of amoebiasis in HIV/AIDS patients through determination of amoebic antibody sero-prevalence among diarrhoea and non-diarrhoea patients in relation to their blood $CD4^+$ cell count.

MATERIALS AND METHODS

STUDY DESIGN

The study was conducted from February to April, 2006 among HIV/AIDS patients reporting to the Fevers Unit of the KBTH in Accra. First ethical clearance for this study was obtained and then study participants were recruited following education and informed consent.

Venous blood samples safely collected from each patient was divided into anti-coagulant added and anti-coagulant-free tubes. The whole blood samples were processed for CD4 cell count using a Fluoresce Activated Cells Sorter Count (FACS Count) System. Serum samples prepared from the other portions of blood were tested for anti-*E. histolytica* antibodies using a rapid test kit, INSTANT™CHEK-amoeba. The test results were analysed and correlated with medical history and relevant literature to draw conclusions and make recommendations.

STUDY SITE

The Fevers unit of KBTH, Accra, Ghana was the study site. The KBTH is the leading tertiary hospital in Ghana with seventeen (17) departments. It serves the city of Accra, the surrounding urban areas and the southern part of Ghana. The KBTH also serves the entire West African sub-region through referral cases. The Fevers Unit is a special unit in the KBTH which attends mostly to HIV/AIDS patients and caters for the training of medical staff as well.

STUDY POPULATION

A total of 95 HIV/AIDS patients aged between 18 and 60 years who reported to the Fevers Unit during the study period (February to April) were sampled. They were made up of 65 females and 30 males with certain clinical characteristics as described below. HIV-positive patients of all ages and both sexes who are not on Anti- Retro viral drugs, HIV-positive patients who were showing clinical symptoms of diarrhoea as well as those who were not showing any such symptoms clinical signs of diarrhoea were included in the study.

The World Health organization's criterion for diarrhoeal episodes which is defined as a total of three or more unformed stools in one 24-hour period was applied in selecting diarrhoeal patients. HIV-positive patients on Anti-Retro viral drugs were excluded from the study.

BLOOD SAMPLE COLLECTION AND ANALYSIS

Blood Sample Collection

Using sterile disposable syringe and hypodermal needle, approximately 5 ml of venous blood samples were taken from each patient. The needle was removed and about half the volume of drawn blood was dispensed into

tubes containing ethylene diaminetetracetate (EDTA) to prevent coagulation and later processed for CD4 cell count using the FACS Count system.

The remaining blood was dispensed into a separate tube, allowed to clot and spun at 3,000 rpm for 5 min. The serum was carefully transferred into a fresh tube, aliquoted into eppendorf tubes and kept at -20°C until used.

Sample Analyses

Cd4 Cell Count

Appropriately labelled reagent tubes were vortexed individually and 50 µl of thoroughly mixed patients' whole blood was added to each corresponding tube. Each filled tube was capped and vortexed again for 5 seconds. After incubation for 60 min at room temperature, a volume of 5 µl of fixative solution was added. The tubes were re-capped with fresh caps and vortexed again for a few seconds. The tubes were run and read on the FACS counter within 48 hours of processing. The counter then gave the CD4 counts of each of the samples.

Detection Of Anti-Amoebic Antibodies

The stored serum samples were retrieved and tested for anti-*E. histolytica* antibody using a rapid test kit, INSTANT™CHEK-amoeba. The frozen samples were allowed to thaw and warm to room temperature. Serum samples were mixed gently and 20 µl taken from the clear part was mixed with 60 µl sample diluent to make a four-fold dilution. Lyophilized propriety Gold Conjugate (GCP) was also re-constituted by adding 500 µl of GCP diluent to one vial. All reagents and solutions were gently shaken for 10 minutes before use. One INSTANT™ CHEK-amoeba kit was used per test sample.

A drop of pre-wet buffer was added to the test area of device, allowed to soak in, and the diluted test sample (20 µl sample and 60 µl sample diluent) was added and allowed to be absorbed as well. Two drops of reconstituted GCP were added followed by three drops of wash buffer immediately after the applied reagent had completely soaked in to wash off excess reagents.

Finally, three drops of clear solution were added and allowed to soak in and the results were then read immediately by visual assessment. Presence of a distinct red/pink spot in the test area and a control spot indicated positive result (i.e. *E. histolytica* antibodies detected). The result was interpreted as negative when there was no visible spot in the test area and control spot was present. The test was discarded if there was no control spot.

RESULTS

A total of ninety-five (95) HIV/AIDS patients were sampled. Forty-three (43) patients comprising of fourteen (14) males and twenty-nine (29) females were non-diarrhoea patients and fifty-two (52) diarrhoea patients made up of sixteen (16) males and thirty six (36) females. They constituted 65 females and 30 males aged 20 to 60 years old. The rapid test, INSTANT™ CHEK- amoeba was used to determine the presence of anti *Entamoeba histolytica* antibodies in the patients' sera and the $CD4^+$ cell counts of the patients were also determined using a FACS counter.

The $CD4^+$ cell values were categorised as low level ($CD4^+$<200cells/mm³ of blood) average level (200<$CD4^+$<500cells/mm³ of blood) and high level ($CD4^+$?500cells/mm³ of blood). The results were statistically tested using the CHI square test with a 95% confidence limit and a significant level of 0.05, to determine the significant difference between the presence of anti *Entamoeba histolytica* antibodies in diarrhoea and that of non-diarrhoea HIV patients.

The over all results of anti-*E histolytica* antibody test in age groups of HIV/AIDS patients as categorized by $CD4^+$ cell counts are summarized in Table 1.

Table 1.Overall Results of Anti-Entamoeba histolytica Antibody Test in Age Groups of HIV/AIDS Patients a s Categorized by CD4⁺ Cell Counts

Age range (yrs)	INSTANT™ CHEK – Amoeba Test						Overall Total
	CD4+<200cells/ mm³ of blood (n = 54)		200<CD4+<500 cells/mm³ of blood (n = 25)		CD4+?500cells/mm³ of blood (n = 16)		
	Positive	Negative	Positive	Negative	Positive	Negative	
20-30	4	6	1	5	0	6	22
31-40	4	29	0	6	0	4	43
41-50	2	7	0	8	0	3	20
51-60	0	2	2	3	0	3	10
Total	**10**	**44**	**3**	**22**	**0**	**16**	**95**

Out of the ninety-five (95) patients, fifty-four (54) had a low $CD4^+$ cell count ($CD4^+$<200 cells/mm^3), and twenty-five (25) had an average $CD4^+$ cell count (200<$CD4^+$<500 cells/mm^3) whereas sixteen (16) had a high $CD4^+$ cell count (CD4?500 cells/mm^3). Patients with low $CD4^+$ cell count recorded ten (10) sero-positives for anti-*E. histolytica* antibodies, those with average cell count had 3 sero-positives, and none of the high cell count patients was sero-positive for *E. histolytica* antibodies.

In Table 2, the overall results of anti *E .histolytica* antibody test in male and female HIV/AIDS patients as categorized by $CD4^+$ cell counts are shown.

Table 2.Overall Results of Anti-Entamoeba histolytica Antibody Test in Male and Female HIV/AIDS Patients as Categorized by CD4⁺ Cell Counts

CD4⁺ Cell Count (cells/mm³ of blood)	INSTANT™ CHEK – Amoeba Test				Overall Total
	Male (n = 30)		Female (n = 65)		
	Positive	Negative	Positive	Negative	
CD4⁺<200	3	17	7	27	54
200<CD4⁺<500	0	5	3	17	25
CD4⁺?500	0	5	0	11	16
Total	3	27	10	55	95

Three (3) males in the low cell count category were positive for anti-amoebic antibodies. A total number of ten (10) females, seven (7) in the $CD4^+$<200cells/mm^3 group and the remaining three (3) in the 200<$CD4^+$<500 cells/mm^3 category, were positive for anti-*E. histolytica serum antibody*. None of the patients in the $CD4^+$?500 cells/mm^3 category had antibodies to *E. histolytica*. Males showed anti-*E. histolytica* antibody prevalence of 3.2% whilst the females recorded 9.5% for both the low and average cell count categories.

The overall prevalence of *E. histolytica* antibody sero-positivity was 13.7% of the study population. By $CD4^+$ cell counts categories, the highest prevalence of 18.5% was estimated in the $CD4^+$<200cells/mm^3 group with more of the patients in the 20-30 and 31-40 years age range followed by the 41-50, and none in the 51-60 years age ranges, respectively. In the 200<$CD4^+$<500 cells/mm^3, group, 12.0% of patients had anti-amoebic antibodies 8.0% of whom were in the age range of 51-60 years and the remaining 4.0% in the 20-30 age range. In the $CD4^+$<200 cells/mm^3 category, none of the

patients had anti-amoebic antibodies.

In the non-diarrhoea HIV/AIDS patients, only those in the $CD4^+$ cell counts of below 200cells/mm³ and between 200 and 500 cells/mm³ in the age ranges of 20-30 and 31-40 age ranges showed positive antibody test results. No positives were recorded for patients in the $CD4^+$?500 cells/mm³ group.

Amoebic antibody prevalence of 10.0% was recorded in the $CD4^+$<200 cell/mm³ group, 5.0% each from the 20-30 and 31-40 age ranges. In the 200<$CD4^+$>500 cells/mm³ group of patients, amoebic antibody prevalence was 14.3% among the 20-30 and 51-60 age ranges. The summary is shown in Figure 1. The chi-square test statistic calculated to determine the association between the presence of antibodies and the cell counts with age was p=0.315.

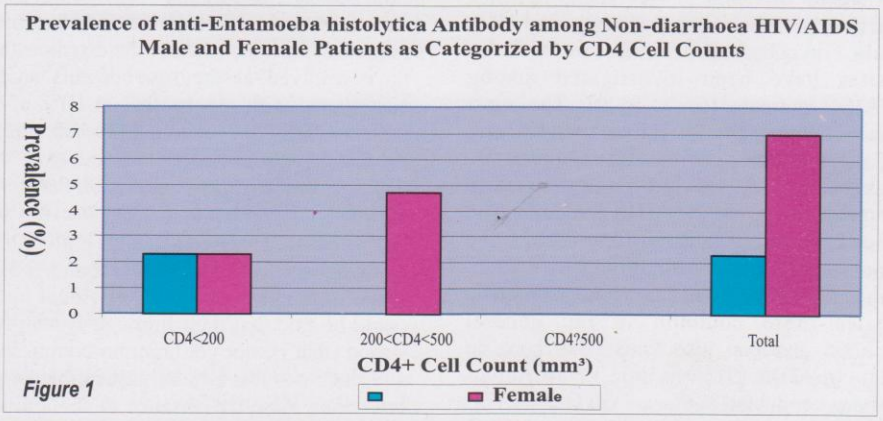

Figure 1

In the diarrhoea HIV/AIDS patients, the highest number of anti-*E.histolytica* antibodies positives was found in the $CD4^+$<200 cells/mm³ group. All of them were in the 20-50 years age range. For the 200<$CD4^+$<500 cells/mm³ group of patients only one (1) in the age range of 51-60 years was positive for *E. histolytica* antibodies and none of the patients in the $CD4^+$?500 cells/mm³ group showed sero-positivity.

The prevalence of anti-*E. histolytica* antibodies in diarrhoea patients is shown in Figure 2.

The prevalence was highest (24.0%) in the $CD4^+$<200 cells/mm³ group and consisted of about 9.0% each in the age ranges of 20-30 and 31-40, and about 6.0% in the 41-50 age group. The antibody prevalence in the 200<$CD4^+$<500 cells/mm³ group was 9.0% in the 51-60 years age range only. There were no anti-amoebic antibodies in any of the patients in the high cell count category. The chi-square test statistic calculated to determine the association between

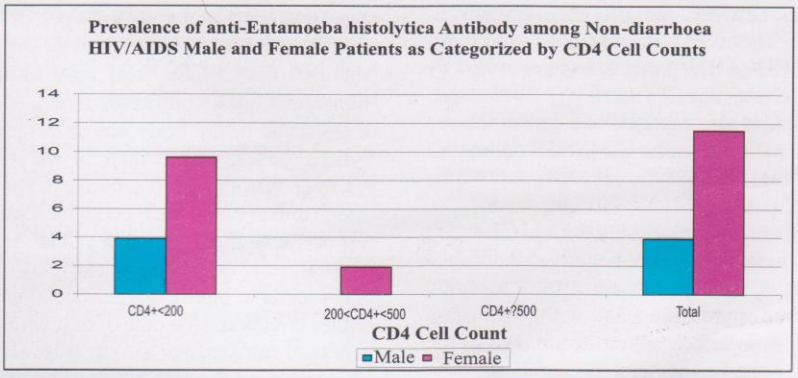

Figure 2

the presence of antibodies and the cell counts with age was p=0.048.

DISCUSSION

HIV infection has been a major threat to people living in Sub-Saharan Africa, with Ghana being no exception. HIV/AIDS has chronic diarrhoea as a major clinical symptom in a considerable number of infected individuals. Diarrhoea has many causative organisms including *E. histolytica* which has been suspected to be an opportunistic infection in HIV/AIDS patients. These diarrhoea causative organisms including bacteria, mycobacteria, viruses as well as parasites have been investigated among HIV/AIDS patients (6; 7; 8; 9). The most common pathogens isolated from patients with AIDS continue to be new and unusual parasitic organisms that have rarely been implicated in human disease before the AIDS epidemic. The most notorious of these is *Cryptosporidium parvum* and *Isospora belli* (10). Although *E. histolytica* has been listed among the pathogenic parasites more common in the general population and can also cause diarrhoea in patients in AIDS (9) very little investigations have been conducted in this area in Ghana. This study sought to determine the status of *E. histolytica* in HIV/AIDS infection by determining the sero-prevalence of the parasite in diarrhoea and non-diarrhoea patients.

In this study, the prevalence of the *E. histolytica* antibody among diarrhoea and non-diarrhoea HIV/AIDS patients was determined using *E. histolytica* antibody detection test kit. Generally, it was observed that anti-amoebic antibody prevalence was slightly higher in diarrhoea patients than in non-diarrhoea patients and for both categories the antibodies were mostly found in patients with low $CD4^+$ cell counts. However, there was no significant association between the presence of anti *E. histolytica* antibodies in diarrhoea and non-diarrhoea HIV/AIDS patients as categorized by $CD4^+$ cell counts and age. ($p>0.05$). *Cyclospora cayetanensis* is also reported to cause diarrhoea in AIDS patients with CD4 cell counts of $<100mm^3$ (9).

It has been established that the normal $CD4^+$ cell count in a healthy, HIV-negative individual varies but is usually between 500 and 1,500 cells/mm^3 of blood. The $CD4^+$ cell count of an individual is an indication of the strength of the individual's immune system.

A $CD4^+$ cell count of about 200cells/mm^3 or less puts one at risk of infection with any opportunistic pathogen. In a study among immuno-competent pediatric diarrhoea and non-diarrhoeal cases from Southern India the results showed a significant correlation between acquisition of sero-positivity and age (11) whilst incidence of amoebiasis was found less in extremes of ages (12).

For the *E. histolytica* sero-positive non-diarrhoea patients they did not exhibit the clinical symptoms because the diarrhoea might have resolved at the time of this study in accordance with reports that in HIV infected patients with $CD4^+$ cell counts >180 cell/mm^3, the diarrhoea resolves spontaneously in 7 to 28 days (13).. It was also observed that in both categories of patients there were no serum antibodies to *E. histolytica* in the high $CD4^+$ cell count patients. Their $CD4^+$ cell counts fall in the range of healthy non-HIV individual hence it could be said that their immune system is still strong (that is, not yet immuno-compromised) and does not leave them susceptible to some infections. Also the absence of the antibodies could be as a result of non-exposure of the patients to the causative organism. This shows that even though one may be infected with the virus the state of the immune system of the individual could be of great help.

The average prevalence (16.3%) in diarrhoea patients was higher than in non-diarrhoeal patients (11.8%) ($p=0.048$). These results show that more diarrhoea patients have been infected with the causative agent than non-diarrhoea patients. In spite of the fact that patients with a low $CD4^+$ cell count seem susceptible to the infection and vice versa, a $p=0.315$ show that the presence of antibodies is non-dependent on the $CD4^+$ cell count or on ages of the non-diarrhoea patients. This could be attributed to the fact that a $CD4^+$ test only indicates the number of $CD4^+$ cells in the blood. Most of the $CD4^+$ cells in the body are in tissues such as lymph nodes, rather than in the blood and various factors can encourage $CD4^+$ cells to move into or out of the blood which would show up as a higher or lower $CD4^+$ cell count respectively, even though the total number of $CD4^+$ cells in one's body remained unchanged. Other infections, the time of day, whether or not a person smokes, and the stress levels can have an impact on the test level. In addition, some

people can remain well despite having a low $CD4^+$ cell count and others can develop symptoms and problems despite having a higher $CD4^+$ count.

However, for diarrhoea patients a p=0.048 shows that there is an association between the diarrhoea and the $CD4^+$ cell counts and age. This finding in part agrees with the findings by Kartalija and Sande (9) that patients with a low cell count level are susceptible to organisms which cause diarrhoea. It is also reported that in 15% to 46% of HIV-infected patients with diarrhoea, no pathogen can be identified and HIV itself may be important (14).

It was observed that the age group 20-40 years were more susceptible to *E. histolytica* infection than the other age groups which could be compared with a study conducted in India where maximum incidence of amoebiasis was found in age group 21-40 years (12).

LIMITATION

The study was limited by a small sample size. Also the factors which affect $CD4^+$ cell counts such as stress levels, time of the day and other factors as stated in the discussion above were not considered when measuring the $CD4^+$ cell counts. Other diarrhoea causative pathogens were not considered.

CONCLUSION

Amoebiasis could not be confirmed as an opportunistic infection in HIV/AIDS patients within the limitations of this study using amoebic antibody sero-prevalence.

RECOMMENDATION

A similar study could be carried out with a larger sample size and results compared with this finding. The fact that some patients had been exposed to the *E. histolytica* and suffer from their effect means that the awareness should be created so that patients are careful the way they live. This is because the infective stage of the organism is transmitted through oral faecal route and through oral and anal sex contact and untreated human faecal matter especially when used as fertilizer. A study could be conducted to investigate the amoebiasis exposure promoting factors in the life style of the 20-40 years age group.

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