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Research Article

CRYPTOSPORIDIOSIS AND ITS CO-RELATION TO CD4 COUNTS IN HIV-INFECTED PATIENTS IN A TERTIARY CARE HOSPITAL OF PUNJAB

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ABSTRACT
In developing countries like India Cryptosporidium spp. is a major cause of diarrhoea mainly affecting children and HIV-infected individuals with low CD4 counts. The infection is self-limiting in immunocompetent hosts, but can be severe and persistent in the immunocompromised and malnourished. The present study was undertaken to determine the prevalence of Cryptosporidiosis among HIV-infected patients in a tertiary care hospital in Punjab and its correlation with CD4 counts. This retrospective study was carried out in Microbiology Department at Christian Medical College and Hospital Ludhiana, from April 2012 to June 2013 among consecutively enrolled 105 HIV infected patients presenting with diarrhoea or other gastrointestinal symptoms. Stool samples were screened and examined for the presence of Cryptosporidium oocyte by Modified Ziehl-Neelsen staining method. CD4 counts of the patients were estimated by Alere Pima ^{TM} CD4 Analyser. Out of 105 stool samples, 17(16%) were positive for Cryptosporidium oocyst.CD4 count of patients positive for cryptosporidium oocyst was significantly lower (< 200/ µl). Cryptosporidium infections may be life threatening, so its early detection can be life saving. Therefore, stools of all HIV positive patients with diarrhoea or other gastrointestinal symptom should be screened for Cryptosporidium infection. Monitoring of CD4 cell count and looking for opportunistic parasitic infections are important. This is likely to optimise treatment in these patients by eradicating opportunistic pathogens and improve the quality of life of these patients.

Keywords: *Cryptosporidiosis, HIV, AIDS, CD4 Counts*

INTRODUCTION
Cryptosporidium species are intracellular, extra cytoplasmic, spore forming protozoan parasites that infect the microvillous border of the gastrointestinal and respiratory epithelium of a wide range of vertebrate animal (Chen *et al.,* 2002; Kaplan *et al.,* 2009; Goodgame, 1996). It is associated with watery diarrhoea in mammals, diarrhoea and respiratory illness in birds and gastroenteritis in reptiles and fish. Human infection is usually acquired by direct contact between persons and by ingestion of contaminated food or water.

Infection with Cryptosporidium in immunocompetent persons often results in asymptomatic or mild self-limited disease, but in HIV infected patients, particularly those with low CD4 counts, infection may result in chronic or life threatening diarrhoea, or extra-intestinal disease (Chen *et al.,* 2002; Kaplan *et al.,* 2009; Flanigan *et al.,* 1992; Frost *et al.,* 2005; Abubakar *et al.,* 2007). Hence, the present study was undertaken to determine the prevalence of Cryptosporidium among HIV infected patients in a tertiary care hospital in Punjab and its correlation with CD4 counts.

MATERIALS AND METHODS
This retrospective study was carried out in Microbiology Department at Christian Medical College and Hospital Ludhiana, from April 2012 to June 2013 among consecutively enrolled 105 HIV infected patients presenting with diarrhoea or other gastrointestinal symptom.

Study Cases
Patients who were confirmed as HIV positive cases and whose CD4 count was being evaluated were taken as study subjects. Irrespective of their signs and symptoms of gastrointestinal tract infection, each

participant was provided with standard stool collection containers labelled with participant's name and unit number. Instructions were given for the collection of stool sample. For CD4 cell count, 5 ml of whole blood sample was collected from each of the HIV infected patients by venipuncture in K_3EDTA vacutainer tubes after pre-test counselling and informed consent. To exclude the influence of circadian variations in lymphocyte subpopulations, samples were collected between 8 am to 12 pm and was analysed by Alere Pima ^{TM} CD4 Analyzer.

Parasitological examination was done in collected stools samples immediately for the presence of parasites. Conventional screening of all the faecal samples by microscopy after concentration by 10% formol ether method was carried out for detection of various ova and cysts. Duplicate smears were made from each specimen and stained with Modified Ziehl-Neelsen staining technique (Chen *et al.*, 2002; Kaplan *et al.*, 2009). Modified Ziehl-Neelsen stains positive oval to round structures with size varying from 2-6 µm, with or without the presence of retracted cytoplasm were identified as Cryptosporidium oocyst. Cryptosporidium can be differentiated from yeasts, which are similar size and shape but are not acid fast (Kaplan *et al.*, 2009) and from other protozoan parasites, such as Cystoisospora (Isospora) belli and Cyclospora species, based on size (Goodgame, 1996). Patients with mild disease may require repeat stool testing due to the high false negative rates with low organism burden (Kaplan *et al.*, 2009). CD4 counts of the patients were estimated by Alere Pima ^{TM} CD4 Analyser.

RESULTS

A total of 105 HIV positive individuals (183 total stool samples) were screened for Cryptosporidiosis during this study. CD4 enumerations for each were also performed**.** Out of 105 stool samples, 17(16%) were positive for Cryptosporidium oocysts.

TABLE 1: Age Distribution of Study Population

Age-group (years)	Total screened	Number positive for cryptosporidiosis	% Positive
< 20	1	-	-
20-29	2	-	-
30-39	22	4	18.1%
40-49	39	8	20.5%
50-59	20	2	10%
>60	21 3		14.2%

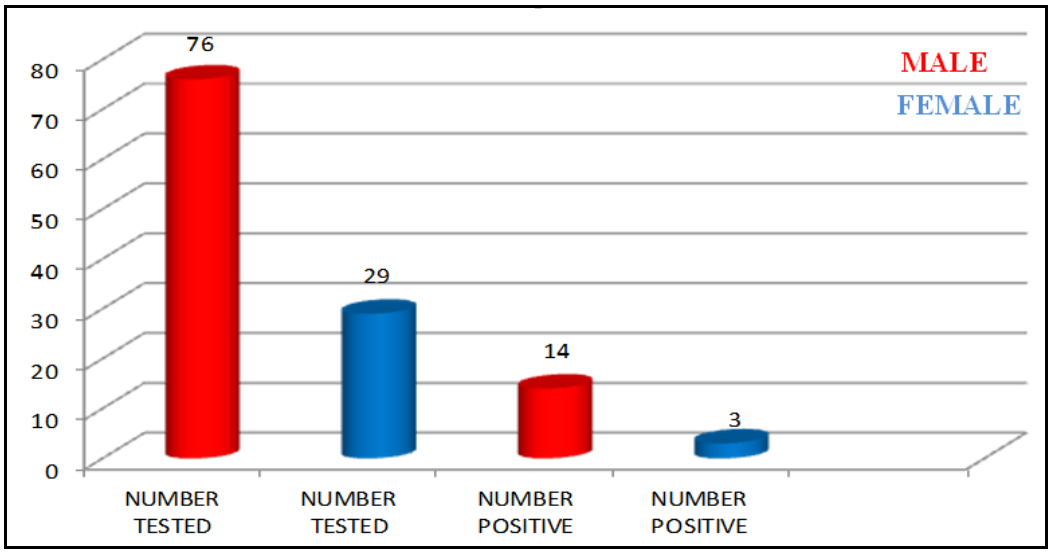

Figure 1: Gender-related distribution of Cryptosporidium infection among HIV infected

The highest prevalence was observed in participants in the 40-49 years age group (20.5%) followed by the 30-39 years age group (18.1%) as shown in table1. The study population was made up of 76 males and 29 females aged 0-70 years. Figure1 shows the gender-related distribution of Cryptosporidiosis among HIV-infected subjects. Of the 105 stool samples examined 17(16%) was positive for Cryptosporidium oocysts. Prevalence was significantly higher among males 14/76(18.4%) compared to females 3/29(10.3%), among subjects with diarrhoea and among subjects with CD4 lymphocyte count <200 cells/µl. The study population consisted of 1 patients with CD4 > 500/µL, 2 patients with CD4 = 200 - 499/µL, and 14 patients with CD4 < 200/µL.

Table 2: Association between CD4 counts and Cryptosporidium spp

CD4 count	Number of Cryptosporidium spp
>500/µL	1
200 – 499/µL	2
< 200/µL	14

DISCUSSION

In the HIV era, the infections by opportunistic agents are on the rise. In case of HIV positive individuals worldwide, opportunistic infections of the gastrointestinal tract are one of the major causes of morbidity and mortality (Chaisson *et al.,* 1998).

This retrospective study tried to determine the prevalence of Cryptosporidium parasites among the HIV positive patients.

In this study of HIV-infected patients in Punjab, the overall prevalence of Cryptosporidium oocysts was 16%. In HIV/AIDS patients' presenting with diarrhoea Cryptosporidium infection was found in clustered**.** This prevalence is consistent with findings from previous studies in different parts of the world among patients with HIV (Gatei *et al.,* 2008; Rao *et al.,* 2007; Dwivedi *et al.,* 2007; Andualem *et al.,* 2007) but however at variance with previous reports by Kamisky *et al.,* in Honduras (Kamissky *et al.,* 2004) and Nwokediuko *et al.,* in Nigeria (Nwokediuko *et al.,* 2002) who did not find Cryptosporidium species among 133 and 161 HIV infected Hondurans and Nigerians respectively. The possible explanation for these findings could be that the study subjects could have been on specific chemotherapy prior to the study or may not have presented with low CD4 counts as observed in our study.

There seems to be a correlation between HIV and Cryptosporidial infection. Studies from other parts of the world on HIV/AIDS-associated diarrhoea have implicated Cryptosporidium; Taiwan (0.5%) (Hung *et al.*, 2007), India (33%) (Dwivedi *et al.,* 2007) and Brazil (8.1%) (Tatiana *et al.,* 2008). The first reports of human cases of Cryptosporidiosis were in 1976 (Meisel *et al.,* 1976) followed over the next four years by a handful of reports largely of disease in immunosuppressed host. However, the Centres for disease control (CDC) in the USA recognized the importance of Cryptosporidium as a major human pathogen in 1982 when outbreak of Cryptosporidial diarrhoea in 21 patients with advanced HIV disease (MMWR, 1982). Chronic Cryptosporidiosis is a qualifying diagnosis for CDC-defined AIDS. In the United States 3-4% of persons have Cryptosporidiosis at the time of CDC-defined AIDS diagnosis and an estimated 10-15% develops Cryptosporidiosis during the course of HIV disease (Soave, 1988). In Sydney Australia, 5% of patients with advanced HIV disease undergoing gastrointestinal evaluation have Cryptosporidium enteritis (Colebunders *et al.*, 1988).

The prevalence of Cryptosporidiosis was significantly higher among HIV/AIDS-infected patients with CD4 lymphocyte count of <200cells/µl. This finding is consistent with previous reports in Cameroon (Sarfati *et al.,* 2006) by Sarfati *et al.,* who observed a significantly higher prevalence of opportunistic protozoa (32%) among patients with CD4 cell counts less than 50cells/µl and Dwivedi *et al.,* (Dwivedi *et al.,* 2007) in India who found enteric coccidian parasites causing diarrhoea in those with lower CD4 cell count.

Infection with HIV causes a gradual decline in the peripheral CD4 helper lymphocyte. These lymphocytes are part of the body's immune system and play a key role in cell mediated immunity. But as HIV destroys these lymphocytes, HIV-infected patients becomes predisposed to opportunistic infections. As their CD4 lymphocyte count falls below 200 cells/µl, they become prone to a wide range of opportunistic infection including cryptosporidiosis.

Conclusion

This study shows that routine examination of stool sample for coccidian parasites must be incorporated in the follow up of patients with HIV/AIDS as this is likely to optimise treatments in these patients by eradicating opportunistic pathogens and improve the quality of life of these patients.

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