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Prevalence of Intestinal Parasites and Profile of CD_4^+ Counts in HIV$^+$/AIDS People in North of Iran, 2007-2008

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Abstract: In this study 142 stool samples (64 HIV$^+$/AIDS patients and 78 non-HIV infected individuals) collected from Mazandaran province and screened for intestinal parasites, using direct wet mont, formalin-ether sedimentation concentration, modified Ziehl Neelsen and modified trichrome techniques. Each person in this study was examined for CD4$^+$ counts. Intestinal parasites were found in 11/64 (17.2%) of patients in HIV$^+$/AIDS group and in 14/78 (17.9%) of controls. Prevalence of parasites detected in HIV$^+$/AIDS individuals was as follow: *Cryptosporidium* sp. 9.4%, *Giardia lamblia* 3.1%, *Entamoeba coli* 1.6%, *E. histolytica* 1.6% and *Chilomastix mesnili* 1.6%. Prevalence of parasites in controls was as follow: *Trichostrongylus* sp. 6.4%, *G. lamblia* 3.8%, *Cryptosporidium* sp. 2.5% , *E. coli* 2.5% , *E. histolytica* 1.2% , *Hookworms* 1.2%. The mean of CD4$^+$ counts in HIV-positive group (430 cells μL^{-1}) was remarkedly less than controls (871 cells μL^{-1}) (p = 0.001). As patients usually belong to poor socio-economic backgrounds and they can hardly afford treatment, therefore, it is suggested screening and free treatment of intestinal parasites in these individuals should be taken by health centers to prevent the occurrence of these diseases in HIV$^+$/AIDS patients, as often the disease may take a fulminant form.

Key words: Intestinal parasites, profile of CD_4^+,HIV$^+$/AIDS, Iran

INTRODUCTION

Different infections in Human Immunodeficiency Virus (HIV) positive/AIDS people reduce their quality of life and life span (Kurniawan *et al*., 2009). Among them intestinal parasitic infections cause morbidity and mortality in HIV positive individuals (Mukhopadya *et al*., 1999). In these patients, progressive decline in their immunological responses make them extremely susceptible to a variety of common and opportunistic infections (Mukhopadya *et al*., 1999; Cegielski *et al*., 1999), protozoan parasites, namely *Cryptosporidium parvum*, *Isospora belli*, *Cyclospora cayetanensis*, *Microsporidia*, *E. histolytica/dispar* and *G. lamblia* account for a significant number of cases of diarrhoea in this population (Mohandas *et al*., 2002). In AIDS patients, opportunistic parasitic gut infections cause sever diarrhoea, profoundly compromise the absorptive function of the small intestine and cause significant mortality (Castello-Branco *et al*., 1996). With the impaired immunity especially in the patients with low immune level (CD_4^+<200 cells μL^{-1}) infestation with intestinal organisms resulting in diarrhoeal symptom is commonly seen (Wiwanikit, 2001). In two surveys performed in Iran, prevalences of intestinal parasites in HIV/AIDS patients have been reported 11.4 and 18.4% (Meamar *et al*., 2007; Zali *et al*., 2004). Because of important of intestinal parasites in HIV$^+$/AIDS patients and also there is only few studies regarding the prevalence of intestinal parasites and their association with CD_4^+ in HIV-infected patients are available from Iran at the present. We took up this study in North of Iran to evaluate the prevalence of such infections in HIV/AIDS patients and to emphasize the importance of stool examination for the detection of intestinal parasites.

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MATERIALS AND METHODS

This cross-sectional study was conducted in Northern Iran between September 2007 and October 2008. This study was previously assessed and approved by the Ethics Committee at the Mazandaran University of Medical Sciences.

Patients and control group selection: There were two groups, one composed of 64 HIV^+ infected patients detected by ELISA and confirmed by western blot, the other composed of 78 individuals without clinical signs of HIV infection and AIDS. In case group, 49 (76.5%) were male and 15 (23.5%) were female. In control group, 58 (74.4%) were male and 20 (25.6%) were female. There wasn't significant difference in two groups ($p>0.05$; Table 1). The mean age of HIV^+/AIDS group was 33.5 years (range: 17-58, SD: 7.44) and in the other group, the mean age of controls was 32.7 years (range: 12-62, SD: 9.48). There was no statistically significant difference by age in the HIV^+/AIDS and control groups ($p>0.05$; Table 1). All of these people gave their consent to participate in this investigation.

Data collection and stool and blood examinations: All individuals in this study were interviewed by using a questionnaire to collect sociodemographic and clinical data. Stool samples were collected in 10% buffered formalin and SAF (sodium acetate acetic formaldehyde) in two sterile plastic containers and were transported to research laboratory of Mazandaran University of Medical Sciences. Stool speciments were collected and examined microscopically following direct and formalin-ether concentration methods (Garcia and Bruckner, 1993). In brief, stool samples were collected in labeled, leak-proof and clean plastic stool cups and brought to the laboratory. Direct microscopy of smears was performed for the detection of ova, larvae, trophozoites and cysts of intestinal parasites. Furthermore, a concentration procedure was employed that involved mixing the stool samples with formalin, treating with ether and centrifuging. The layers of ether, formalin and debris were discarded and the residues were used to investigate for the presence of intestinal parasites (Garcia and Bruckner, 1993). Detection of *Cryptosporidium* oocysts in the concentrated stool was done using the modified cold Ziehl Neelsen staining technique (Smith, 1995).

Table 1: Age and sex distribution of HIV+/AIDS and control individuals

	HIV^+/AIDS		Control	
Age groups (year)	Male	Female	Male	Female
10-19	0 (0)	2 (3.1)	5 (6.4)	4 (5.1)
20-29	13 (20.3)	3 (4.6)	12 (15.4)	3 (3.9)
30-39	29 (45.3)	5 (7.8)	26 (33.3)	8 (10.2)
40-49	7 (10.9)	3 (4.7)	13 (16.6)	5 (6.4)
50<	0 (0)	2 (3.1)	2 (2.6)	0 (0)
Total	49 (76.5)	15 (23.5)	58 (74.4)	20 (25.6)

Values in brackets are percentage

Briefly, a concentrated smear of the stool was made on a clean grease- free slide and fixed in methanol for 3 min. The slide was immersed in cold carbol fuchsin and stained for 15 min. It was then thoroughly rinsed in tap water and decolorized in 1% HCL in methanol for 10-15 min. After rising again in tap water, the slide was counter stained with 0.4% malachite green for 30 sec. The slide was then air-dried and observed under the compound light microscope using 40× objective lens for the presence of *Cryptosporidium* oocysts, which was confirmed under the oil- immersion objectives, as small pink to red spherules on pale green background (Yemisi *et al*., 2007). Modified trichrome stain was used for detection of Microsporidia spores (Garcia and Bruckner, 1993). Five milliliters of EDTA from each subjects was collected to count WBC, Eosinophil and CD_4^+. Determination of $CD4^+$ counts was performed by flow cytometry that include of whole blood samples collected in EDTA from 78 normal and 64 HIV^+/AIDS individuals. Immunophenotyping of the lymphocyte subsets was done by using three color flow cytometric methods (partech, Germany). The blood samples were stained by using FITC conjugated mouse anti- human CD_4 (clone MT 310) and mouse anti-human CD_3 (clone UCHTI) conjugated with RPE.CYS monoclonal antibodies. All, reagents were purchased from Dakocytomation (Denmark).

Statistical analysis: Comparisons were made between the HIV^+/AIDS and control group. Differences were compared by χ^2, fisher exact tests and student t-test by using SPSS. 15.00. The $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

A total of 142 of individuals were enrolled in the study. Sixty four HIV^+/AIDS patients (case group) and 78 without clinical signs (control group) were examined for intestinal parasitic infections. Among the 64 HIV^+/AIDS patients, intestinal parasites were detected in 11 cases (17.2%); *Cryptosporidium* sp. (9.4%) and *G. lamblia* (3.1%) were the most common parasites. Among 78 controls analyzed, *Trichostrongylus* sp. (6.4%), *G. lamblia* (3.8%), *Cryptosporidium* sp. (2.5%) and *E. coli* (2.5%) were the most common parasites. Intestinal helminthes have been only detected in control group. Prevalence of intestinal parasites detected was shown in Table 2.

There was no significant difference in prevalence of intestinal parasite infections between HIV$^+$/AIDS and control groups ($p>0.05$). Prevalence of intestinal parasites detected in different ages has been shown in Table 3. There was no significant difference between intestinal infection in different age groups between HIV$^+$/AIDS and control groups ($p>0.05$). CD_4^+ T- cell counts were done in all HIV-positives and controls. The CD_4^+ counts were remarkedly depressed in all HIV-positive patients. The mean of CD_4^+ counts in HIV$^+$/AIDS was 430 cells μL^{-1} and in control group was 871 cells μL^{-1} ($p = 0.001$). Although, some other studies evaluate association between AIDS (with diarrhea) and CD_4 in patients, in our study most of patients suffering from constipation due to drug abusing, with the exception of the most individuals infected with *Cryptosporidium* had diarrhoea and the CD_4^+ counts in these patients were significantly lower than control group ($p>0.05$). The mean of CD_4^+ counts for each parasite, in HIV$^+$/AIDS patients and control individuals infected with intestinal parasite have been shown in Table 4.

Opportunistic infections constitute a major health problem in patients infected with HIV. Among these, intestinal parasitic diseases are the commonest and a major cause of morbidity and mortality in HIV positive individuals worldwide (Chaisson *et al*., 1998). The coccidian parasites (*Cryptosporidium* sp., *Isospora belli*, *Cyclospora* sp. and *Microsporidium* sp.) are foremost among the enteric parasites in these patients (Smith *et al*., 1988). Almost all of the HIV-positive patients enrolled in this study were adults, a predominance of them were male (76.5%) and most of them were intravenous drug abusers.

Table 2: Prevalence of intestinal parasite infections diagnosed by stool examination from 64 HIV-positive and 78 control individuals in Mazandaran

Parasite species	HIV$^+$/AIDS (n = 64)	Control (n = 78)
Helminths		
Trichostrongylus sp.	0 (0)	5 (6.4)
Hookworm	0 (0)	1 (1.2)
Protozoa		
Cryptosporidium sp.	6 (9.4)	2 (2.5)
Giardia lamblia	2 (3.1)	3 (3.8)
Entamoeba coli	1 (1.6)	2 (2.5)
Entamoeba histolytica	1 (1.6)	1 (1.2)
Chilomastix mesnili	1 (1.6)	0 (0)
Total	11 (17.2)	14 (17.9)

Differences between two groups were not significant. Values in brackets are percentage

In our study, prevalence of intestinal parasites in HIV$^+$/AIDS patients was 17.2%. In Iran, Zali *et al*. (2004) also have been showed same prevalence rate, 18.4%. Of course Meamar *et al*. (2007) have been reported prevalence rate of 11.4 and 11.6% in HIV$^+$/AIDS and healthy people, respectively. In other countries, its prevalence rates have been reported 30-84.3% (Mohandas *et al*., 2002; Cimerman *et al*., 1999; Wiwanikit, 2001; Ibrahim *et al*., 2007; Kurniawan *et al*., 2009). Different results show that there is no special pattern of occurance of parasites among HIV$^+$/AIDS patients in different countries, even in different regions in the same country.

Such as our survey, Meamar *et al*. (2007) have been showed that there was no significant difference in prevalence of intestinal parasites between HIV$^+$/AIDS and control groups. The similarity in frequencies of extra cellular intestinal parasites in both groups, could be explained by the fact that control Th_2 CD_4^+ lymphocytes, which are highly necessary to protect the host against such parasites, remain less affected than Th_1 in HIV seropositive patients (ZU *et al*., 1992).

In a study performed in Iran, prevalence of *Cryptosporidium* was 0.9% in HIV$^+$ individuals (Meamar *et al*., 2007) and in other previous study, its prevalence in HIV-positive patients was 1.5% (Zali *et al*., 2004). Other studies show that in Brazil 7% (Cimerman *et al*., 1999), Nigeria 16.83% (Ibrahim *et al*., 2007) and in other study in HIV$^+$ individuals with diarrhoea in the same country 52.7% (Yemisi *et al*., 2007) infected with *Cryptosporidium*. Prevalence of *Cryptosporidium* in HIV$^+$ patients in Indonesia, Cuba and India has been reported 4.9, 11.9 and 23.6%, respectively (Kurniawan *et al*., 2009; Escobedo and Nunez, 1999; Gupta *et al*., 2008). In other study in India, in 39.8% of HIV positive cases *Cryptosporidium* was detected (Tuli *et al*., 2008). In this study although the prevalence of *Cryptosporidium* sp. in HIV$^+$/AIDS (9.4%) was more than control group (2.5%), there was no significant

Table 3: Prevalence of intestinal parasites in HIV$^+$/AIDS and control individuals by age and sex

	HIV$^+$/AIDS			Control			
Age groups (year)	Male	Female	Total	Male	Female	Total	p-value
10-19	0 (0)	2 (3.1)	2 (3.1)	0 (0)	2 (2.6)	2 (2.6)	0.60
20-29	3 (4.7)	1 (1.6)	4 (6.3)	2 (2.6)	0 (0)	2 (2.6)	0.666
30-39	2 (3.1)	2 (3.1)	4 (6.2)	3 (3.8)	1 (1.3)	4 (5.1)	0.49
40-49	0 (0)	0 (0)	0 (0)	2 (2.6)	3 (3.8)	5 (6.4)	0.50
50<	0 (0)	1 (1.6)	1 (1.6)	0 (0)	0 (0)	0 (0)	0.66
Total	5 (7.8)	6 (9.4)	11 (17.2)	7 (9)	6 (7.7)	13 (16.7)	N.S

Values in brackets are percantage. NS: Not significant

Table 4: Prevalence of intestinal parasites in HIV$^+$/AIDS and control individuals associated with CD_4^+ counts

	HIV$^+$/AIDS		Control	
Parasites	n	CD_4^+ (Mean±SD)	n	CD_4^+ (Mean±SD)
Trichostrongylus sp.	0	-	5	840±140.95
Hookworm	0	-	1	731
Cryptosporidium sp.*	6	325.5±170.21	2	818±123.03
Giardia lamblia	2	318[1]	3	1016±150.49
Entamoeba coli	1	354	2	933.5±28.99
Entamoeba histolytica	1	342	1	435
Chilomastix mesnili	1	272	0	-
Total**	11	336.57±116.41	14	810.09±169.46

[1]CD_4^+ count has been done on only one person's blood. *p<0.05 **p<0.001

difference between them. Some other studies have been shown *Cryptosporidium* as the predominant pathogen with significant association to diarrhoea cases (Tarimo *et al*., 1996). Since, most of HIV$^+$/AIDS individuals in our survey were without diarrhoea, therefore these carrier hosts is not diagnosed and cured and this parasite may be a cofactor for improvement of HIV to AIDS.

Giardia lamblia, in comparison with intestinal coccidian is not considered as an opportunistic agent and less frequently observed to cause sever illness in HIV$^+$/AIDS patients (Gupta *et al*., 2008). In the present study, *G. lamblia* was the second most prevalent parasite detected in both groups. Meamar *et al*. (2007) also have been reported similar finding in HIV$^+$ and HIV$^-$ individuals in Iran. In other study in Iran, this rate in HIV$^+$ patients has been reported between 1-7.3% (Zali *et al*., 2004). Prevalence of *Giardia* in HIV-positive patients in other countries was as follow: 1.9% in Indonesia (Kurniawan *et al*., 2009), 1.98% in Nigeria (Ibrahim *et al*., 2007), 3.8% in Ethiopia (Hailmeriam *et al*., 2004), 6% in Cuba (Escobedo and Nunez, 1999), 11.8% in India (Gupta *et al*., 2008) and 16% in Brazil (Cimerman *et al*., 1999). Our findings in this study show giardiasis doesn't occur in greater prevalence in HIV-positive than control group individuals.

No case of *Microsporidium* was found in our study. This may attribute to the difficulty these organisms in fecal specimens by conventional staining techniques due to their small size (1-3 μL) (Brandonoisio *et al*., 1999) and partly due to different risk factors for HIV infection among this Iranian population, which included more intravenous drug abusers than homosexuals (Zali *et al*., 2004).

Although, according to CDC the coccidian parasites are considered as opportunistic pathogens in AIDS patients, due to the patients usually belong to poor socio-economic backgrounds and they can hardly afford treatment, therefore, it is suggested screening and free treatment of intestinal parasites in these individuals should be taken by health centers to prevent the occurrence of these diseases in HIV$^+$/AIDS patients, as often the disease may take a fulminant form.

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