

Clinical Characteristic of the HIV/AIDS Patients with Cryptosporidiosis Referring to Behavioral Diseases Consultation Center, Imam Khomeini Hospital, Tehran in 2013

Rashid Gholami¹, Shirzad Gholami², Hamid Emadi-Kouchak¹, Alireza Abdollahi³, Mona Shahriari³

1. Dept. of Infectious Diseases, Tehran University of Medical Sciences, Tehran, Iran

2. Toxoplasmosis Research Center, Mazandaran University of Medical Sciences, Sari, Iran

3. Dept. of Pathology, Imam Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran

KEY WORDS

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ABSTRACT

Background: *Cryptosporidium* is known as an opportunist disease-causing agent in man in recent decades .It causes diarrhea and intestinal disorders in the immune deficit and immune competent individuals .This study was aimed to investigate the clinical characteristics of HIV/AIDS patients with cryptosporidiosis infection.

Methods: This cross-sectional descriptive study was performed on 53 HIV/AIDS patients referred to the Behavior Disease Consultation Center of Imam Khomeini Hospital in Tehran ,Iran in .2013 First ,the patients were studied clinically and the context data were recorded in a questionnaire for parasitological examination and referred to the laboratory for eosinophil count ,and CD4 count per ml of blood.

Results : Cryptosporidiosis was observed in (7.6%) 4 of the total 53 HIV/AIDS patients .The highest prevalence of infection was observed in the age range of 30-39yr .It was observed in different sexes as 5.7% of male and 1.9% of female ,but statistically was insignificant) $P=0.163$.75% of patients had no intestinal symptom, 11.4% with acute diarrhea and 3.8% with chronic diarrhea. Cryptosporidiosis cases were observed in 5.7% of patients without intestinal symptom.

Conclusion: Practitioners in the clinical examination for the detection of the opportunistic intestinal protozoan infection should use clinical and paraclinical characteristics of the HIV/AIDS patients for the diagnostic of *Cryptosporidium* and other opportunistic parasitic diseases.

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Corresponding Information: Dr Alireza Abdollahi M.D., Department of Pathology, Imam Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran. Email: dr_p_abdollahi@yahoo.com Tel: +98-912-1220588 Fax: +98-21-88277321

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Introduction

Cryptosporidium is known as an opportunist disease-causing agent in man in recent decades. It causes diarrhea and intestinal disorders in

the immune deficit and immune competent individuals (1-5).

Prevalence of cryptosporidiosis is more in societies with immune deficient individuals and diarrheal patients. *C. parvum* is the 3rd

or 4th diarrhea causing agent in man (6-8). Cryptosporidiosis in HIV/AIDS patients appears with two clinical features: self-limited acute gastroenteritis in healthy individuals; chronic and fatal diarrhea in immunodeficient individuals. The main entry is oral and could be due to exposure to an infected person or indirectly from infected water or food staff (1, 3-9). The HIV/AIDS patients due to inefficient immune system are prone to infectious disease agents, particularly opportunistic intestinal single cell parasites such as cryptosporidiosis, isosporiasis and cyclosporiasis. These agents cause moderate, severe and consisting disorders with different clinical features in the immunocompromised patients (9-13).

Cryptosporium species, particularly of the *C. parvum*, are the main and most prevalent intestinal parasites among the HIV/AIDS patients reported worldwide (13-18). It may happen through consumption of contaminated water and foodstuff or long exposure to infected individuals (3, 10, 11). Different studies indicate the prevalence of cryptosporidiosis in the HIV/AIDS patients (9, 10, 14). On the other hand, the immune deficit HIV/AIDS patients are susceptible to cryptosporidiosis infection and the diarrhea symptom is more observed but the rate differs from 0 to 30 percent (2-6). The severity of the disease in the CD4+ < 50 mmol/m³ is high, and in the patients with CD4+ < 200 mmol/m³ is high and long lasting (9, 14-22).

The intestinal parasitic infections are one of the main infectious agents causing disorder, inability and morbidity in the HIV/AIDS individuals with definite clinical manifestation (16, 18, 20). Diarrhea is the main clinical symptom in the HIV/AIDS patients and is observed in more than 40% of the patients in the African and American countries and in Iran (10). The onset of chronic diarrhea is main indicator in the HIV/AIDS patients, particularly those with CD4 < 200 mmol/m³ (18, 22).

Therefore, identification of the etiologic

agents in every 5-10 yr period in the high risk groups is needed and hence laboratory diagnosis, proper treatment, control and prevention is very important (10, 11, 23-25). Study of these data could be useful in clinical medicine and parasitology and studying infectious diseases in Iran would be conducive to the proper management of infection in the HIV/AIDS patients (17, 22).

This study was aimed to investigate the clinical characteristic of the HIV/AIDS patients with Cryptosporidiosis infection, referred to the Behavior Disease Consultation Center of Imam Khomeini Hospital, Tehran, in 2013.

Materials and Methods

This cross-sectional descriptive study was performed on 53 HIV/AIDS patients referring to the Behavior Disease Consultation Center of Imam Khomeini Hospital, Tehran, Iran. First, the patients were studied clinically and the context data were recorded in questionnaires for parasitological examination and referred to the laboratory for eosinophil count, and CD4 count per ml of blood. Sampling was performed from the HIV/AIDS patients having referring to the hospital in 2013. Consent was obtained from the patients. Data of each sample were recorded by clinical evaluation.

The variables under study were as follows: age, gender, marital status, occupation, socioeconomic conditions (income), level of education, living place, clinical symptoms like diarrhea, abdominal cramp, vomiting, nausea, fever, type of diarrhea (less than two weeks) and chronic (more than two weeks), number of CD4 lymphocytes and history of antibiotic therapy.

Parasitological examination

The stool samples of HIV/AIDS patients after referring to the laboratory were collected in three separate sessions (three times). There was certain limitation of finding patients, but we did our best

to find more cases. First, the direct wet mount of stool sample was prepared. The concentration process was done by formalin ether method. The sedimentation of the sample was preserved in 5% formalin and 70% alcohol and sent to the parasitology laboratory of the health faculty of Tehran University of Medical Sciences, and parasitology laboratory of Sari Medical College, Mazandaran University of Medical Sciences, Iran. The oocyst of the parasite was observed by direct wet mount and concentration method (formalin-ether concentration and modified acid fast stain) by light microscope (objective lens 100 x) (12, 19, 26).

Smear from stool samples were prepared from the sediment of the concentrated specimen and stained by kinyoun (cold) acid-fast procedure kinyoun (cold) acid-fast procedure. Oocysts of *Cryptosporidium* are seen as round pink-red objects on a pale green background by the method of Kinyoun acid fast staining, containing sporozoites (19, 20, 27- 30).

Patients' data were considered confidential, no extra cost was constrained and no intervention was performed in our study. The study design,

protocols, procedures and informed consent form were approved by the Medical Ethics Committee of Tehran University of Medical Sciences.

The obtained data were entered in SPSS software, and analyzed by chi- square test. The level of $P < 0.05$ was considered significant.

Results

Cryptosporidiosis was observed in 4 (7.6%) of the total 53 HIV/AIDS patients. The patients had the clinical features and the blood testing for eosinophil and CD4+count was positive.

The rate of infection in terms of age is depicted in Table 1. From the viewpoint of infection with intestinal protozoan parasite from the genus *Cryptosporidium*, the highest prevalence of infection was observed in the age range of 30-39 yr (Table 1). Cryptosporidiosis was observed in different sexes as 5.7% in male and 1.9% in female, which statistically was insignificant (Table 2). (The prevalence of infection with opportunist parasite in the businessman was 5.7% and 1.9% housewife (Table 3). Statistically, there was insignificant relationship between

Table 1

Frequency distribution and percentage of age groups and infection with *Cryptosporidium* in the HIV/AIDS patients, having referred to the Behavior Disease Consultation Center of Imam Khomeini Hospital, Tehran, 2013

Age group	Number and %	Infection with <i>Cryptosporidium</i> , number and %
10-19 yr	2 (3.8)	-
20-29 yr	6 (11.4)	1 (1.9)
30-39 yr	28 (52.8)	2 (3.8)
40-49 yr	12 (22.8)	1 (1.9)
50-59 yr	5 (9.5)	-
Total	53 (100)	4 (7.6)

Table 2

Frequency distribution and percentage of sex groups with *Cryptosporidium* in the HIV/AIDS patients having referred to the Behavior Disease Consultation Center of Imam Khomeini Hospital, Tehran, 2013

Sex groups	Number and %	Cryptosporidiosis number and %
Male	35 (66)	3 (5.7)
Female	18 (34)	1 (1.9)
Total	53 (100)	4 (7.6)

Table 2

Frequency distribution and infection with *cryptosporidium* in the HIV/AIDS having referred to the Behavior Disease Consultation Center of Imam Khomeini Hospital, Tehran, 2013

Occupation	Number %	Cryptosporidiosis number %
Employed	3 (5.7)	-
Businessman	27 (60.4)	3 (5.7)
Housewife	12 (22.8)	1 (1.9)
Jobless	12 (22.8)	-
Total	53 (100)	4 (7.6)

occupation and parasitic infection.

The majority of the HIV/AIDS patients (60.4%) had under 12- standard education and 1.9% with university education. From the viewpoint of cryptosporidiosis, all of the cases had under 12- standard education (7.6%). Statistically, there was significant relationship between the level of education and cryptosporidiosis ($P < 0.05$).

From the viewpoint of clinical symptoms, and frequency distribution in the HIV/AIDS patients, 75% had no intestinal symptom, 11.4% with acute diarrhea, and 3.8% with chronic diarrhea. Cryptosporidiosis cases were observed in 5.7% of patients without intestinal symptom. Statistically, an insignificant relationship was observed between clinical symptom and parasite infection.

Frequency distribution and percentage of marital status in the HIV/AIDS patients indicated 79.2% as married and 11.4% as unmarried. Cryptosporidiosis was observed in 3.8% of the unmarried subjects. A statistically significant relationship was observed between marital status and parasite infection ($P < 0.05$).

Eosinophils count in 60% and 32% of the HIV/AIDS patients was 1-5% and 5-10%, respectively. Prevalence of *Cryptosporidiosis* in the patients with 1-5% eosinophil count was observed in 3.8%. In all, infection with parasite in the patients with 5-10% eosinophil count indicated statistically significant relationship between eosinophil count and parasite infection ($P < 0.05$).

It was noticed that, 67.7% and 15.2% of the HIV/AIDS positive subjects had CD4+ count $200 >$ and $100 <$ ml, respectively. In all of the *Cryptosporidium* infected HIV/AIDS patients (7.6%) had $CD4+ < 200$ ml, the prevalence of the *Cryptosporidium* was observed 5.7% in the $CD4+$ count 100-199/ml. Statistically, there is a significant relationship between cryptosporidiosis and $CD4+$ number ($P < 0.05$).

86.8% of the total 53 subjects were under regiment therapy (AZT, 3TC, EFV) and cryptosporidiosis observed in 3.8% of this group. Statistically, an insignificant relationship was found between the type of ART and parasite infection. 69.8% of the study subjects used PCP, TB and/or PCP and TB medication for prevention, and 30.2% of them did not use any prevention medication. Cryptosporidiosis was observed in 3.8% of TB group, and 1.9% of PCP group. A statistically insignificant relationship has been found between using prevention therapy and infection with cryptosporidiosis in the study subjects. 86.7% of the study subjects received ART. Cryptosporidiosis was observed in 3.8% of subjects receiving ART. Cryptosporidiosis had an insignificant relationship with the study subjects regarding benefiting or not benefiting from ART. Moreover 13 (24.5%) of study subjects changed ART, the cryptosporidiosis was observed in 1 (1.9%) with ART positive and 3 (5.7%) with ART negative. In the ART negative, infection was three times more than the ART positive subjects, statistically of significant difference ($P < 0.05$).

Discussion

Study on the intestinal parasite infection, particularly on the *Cryptosporidium* infection in the HIV/AIDS patients with symptom or symptomless diarrhea based on the causing agent prevalence rate, considering the time and place of occurrence of the disease the study was performed at the best condition. Clinically, and from a diagnostic viewpoint, study of the common infectious disease such as the opportunistic and emerging intestinal parasites at different time; particularly on the HIV patients referred to the healthcare setting is very important. The results of relevant studies conducted on the HIV patients in recent years in Iran and the other countries are clinically and parasitological different (22-26), because, in the HIV patients cryptosporidiosis is one of the main and serious opportunistic intestinal parasite (27-30). Prevalence of cryptosporidiosis indifferent studies in Iran in the gastroenteritis and HIV patients, and other groups varies from less than 1% to 20% (20, 28, 30 - 36).

Considering the limitation and problem in finding the HIV patients and availability of specialized medical diagnostic laboratory in the hospitals of Iran and need for new tests in the identification of the opportunistic emerging single cell such as *Cryptosporidium* and *Microsporidium* and evaluation of the clinical traits of patients infected with the opportunistic single cell parasite and referring to modern diagnostic laboratories could have main role in the on time treatment and prevention of the complications, and severe disorder caused by infections (28,30-31). For this reason, in the present study, the clinical traits and individual characteristics of the HIV patients infected with *Cryptosporidium* were evaluated. The prevalence of intestinal parasite in the HIV patients were reported having referred to health settings of Abijan) Nigeria (in 85 HIV patients, 24.7% of these patients had parasite infections (37) The presence of *Cryptosporidium* and *Microsporidium* by parasitological method was

investigated in 71 stool samples of HIV patients with chronic diarrhea. The obtained data showed the prevalence rate in 9 (12.67%) which is higher than the rate of infection in all of the HIV patients studied in the present study in one year (30).

Guks et al. studied 105 HIV patients having referred to the National College Affiliated Hospital of Seoul (Korea) by parasitological method and rate of cryptosporidiosis was found 10.5% (26).

The difference in the type of the study, availability of the subject under study, place and equipment of the laboratory are the reasons of different findings on the prevalence of cryptosporidiosis. Therefore, medical staff, particularly the physicians in addition to calling for the paramedical tests findings, should evaluate the disease clinically and refer the patients to specialized laboratory for stool examination. The stool sample should be tested by Acid Fast, auramine-rhodamine and molecular method (PCR) especially in the immune deficit HIV patients (8, 14, 19, 30, 37). In the present study we found that, 67.7% of the study subjects had CD4 count higher than 200/ml and 15.2% lower than 100/ml. In all of the 7.6% cases, the CD4 count was 100-199. Most frequency distribution of HIV patient with CD4<200/ml was observed in the age group of 30-39 yr.

In this study, in order to determine the relation between *Cryptosporidium* infection and onset of clinical symptoms in the HIV patients with CD4 < 200/ml, a control group (HIV negative) was also under study. Sampling in the both groups included the stool and blood. The stool sample was stained by Acid Fast method for the presence of *Cryptosporidium*. Of 82 case subjects, 56.1% with diarrhea, 68.4% infected with *Cryptosporidium* had diarrhea, which differed in comparison with the non-diarrhea group. Most frequency distribution of HIV patients with CD4 < 200/ ml was observed in the age group of 20-40 yr (16). Therefore, in the HIV patients with CD4 < 200/ml, the chance of infection

increases with the opportunistic parasite such as *Cryptosporidium* and *Isospora*. This matter was shown in the study of Girma et al. (12). Diarrhea (acute and chronic) is the main health problem in the HIV patients with CD4 < 200/ml.

The frequency distribution and percentage of recorded clinical symptom was different with the data given earlier (12, 16). Difference was observed between the parasite infections in the HIV patients with CD4 < 200/ml compared to the control group. Generally, the data of the study indicate the effect of *Cryptosporidium* on the onset of diarrhea in the immunocompetent individuals having gastroenteritis symptom and the immune deficit individuals. This effect is observed in the HIV positive patients with CD4 < 200/ml (9, 12, 16, 22, 26).

One of the effective factors in reducing the severity of viral infection is treatment with ART that leads to the increase response of CD4 + of immune system. In the absence of vaccination and antiviral drugs, particularly in case of HIV patients in the developing countries, the chance of contracting opportunistic infections increases (1, 6, 12, 31, 34). In our study, 24.5% of the study cases, had ART change, in that, cryptosporidiosis in 1.9% of subjects with positive ART change and 5.7% with negative ART was observed. That is, in the group with negative change ART, the infection is three times more than the group with positive change ART. Therefore, use of ART increases recovery of the patients, as shown earlier in (11, 12).

The highest rate of infection to cryptosporidiosis infection was observed in the age group of 30-39 yr (3.8%). All age groups are susceptible to infection with this parasite. In this study, prevalence of infection with *Cryptosporidium* in men was three times more than in women (in men 5.7% and in women 1.9%). Other relevant studies somewhat agree with our findings (17, 21, 23, 29).

Since the parasites enter the body through consumption of contaminated food and water,

training of HIV patients on the hygiene in order to prevent this disease is needed (2, 3, 37). In the present study, cryptosporidiosis in businessmen and housewives was 5.7% and 1.9% respectively. Infection with cryptosporidiosis in the subjects with high school education occurred in 7.6%, which comprises 73.6% of the study subjects. 79.2% of the study HIV/AIDS patients were married and 11.4% unmarried, but most of the cryptosporidiosis was observed in the 3.8% unmarried patients, indicating the difference of infection between married and unmarried cases. Therefore, in the clinical investigation of cryptosporidiosis and the other opportunistic parasites, role of occupation and marital status as predisposing factors must be considered.

Eosinophil count in the patients with parasite infection is a diagnostic tool in complement clinical trial, particularly stool examination repeat. Therefore, after clinical evaluation of HIV patients referring to the health care setting with sophisticated equipment is necessary.

In our study, we found that 69.8% of the examined patients used PCP, TB and/or PCP and TB medication for prevention, and 30.2% did not use preventive medicine. The highest rate of cryptosporidiosis (3.8%) was observed in the group receiving TB, 1.9% in PCP group, and the group not receiving preventive medicine. Therefore, probably benefiting from preventive medicine could help reduce cryptosporidiosis, which should be clarified in the further studies. The opportunistic intestinal protozoan infection by parasitological and the advanced molecular method in the next studies in all of health care setting should be focused upon by medical universities.

The consultant physicians while treating the HIV/AIDS patients with gastroenteritis in the other countries is somewhat more than Iran. The other studies in Iran shows different rate of prevalence by studying the stool samples of the patients, therefore the complementary method of parasitological for accurate diagnosis is necessary

(4, 5, 19, 23, 28, 30). Therefore introduction of this parasite to the physicians, laboratory staff and conducting of workshop and training sessions in order to encourage the laboratories to use the modified Zeal Nelson method and Acid Fast Thrichome stains which are cheap and easy to perform.

Conclusion

Practitioner of clinical examination for the detection of the opportunistic intestinal protozoan infection should use clinical and paraclinical characteristics of HIV/AIDS patients for the diagnosis of *Cryptosporidium* and other opportunistic parasitic diseases.

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Conflict of interest

The authors declare that there is no conflict of interests.

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