

Enteric Protozoal Infections among Immunocompromised and Immunocompetent People, Sana'a Town, Yemen

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Abstract

Objective: The present study conducted to assess the profile of enteric protozoal infections among immunocompromised and immunocompetent people in Sana'a Town, Yemen.

Methods: Using cross-sectional study, a total of 241 subjects were recruited. They were attended the inpatient and outpatient clinics of three general Governmental health institutions. They belonged to two groups, 102 immunocompromised patients and 139 immunocompetent controls. Relevant data were enrolled by means of questionnaire. Subjects in both groups were subjected to stool examinations by different techniques.

Results: Overall 59 cases (57.8%) of immunocompromised patients were positive for enteric protozoa, compared to 46 (33.1%) of immunocompetent controls, with statistically significant difference ($P < 0.005$). Among immunocompromised patients, *C. parvum* ranked the first (27.5%), and *E. histolytica/E. dispar* was the least (2.9%). Amongst those who immunocompetent, *G. lamblia* was the most frequently detected protozoan (15.8%), and *E. coli* was the least (1.4%). The gross rate of coccidian protozoa in immunocompromised patients were also more prevalent than immunocompetent ones, with significant association (36.3% versus 14.4% respectively), ($P < 0.001$). However, *G. lamblia* and *E. histolytica/E. dispar* among immunocompetent controls were in higher rates than immunocompromised patients (15.8% and 5.0% vs 13.7% and 2.9% respectively), in spite of being not statistically significant.

Conclusion: The prevalence rate of enteric protozoal infections among immunocompromised people was a substantial and significantly higher than immunocompetent ones.

Keywords: Enteric; Protozoal; Immunocompromised; Immunocompetent; Sana'a

Introduction

With an enormous increase in the immunocompromised people each year globally, owing to certain maladies mainly patients with human immunodeficiency virus (HIV), those with malnutrition, patients receiving a wide array of immunosuppressive therapy including corticosteroids, also who have chronic diseases, and those who with malignancy [1-3]. All are at risk of infection with opportunistic parasites chiefly coccidian protozoa [4], that may create persistent diarrhoea and severe malabsorption [5,6]. However that protozoa may not cause severe pathological changes in whichever person, as long as the immune system is functioning normally [3]. The infections

are self-limiting in immunocompetent people who readily clear the parasites [7]. Enteric protozoal infections represent one of the major challenges in the management of immunocompromised patients leading to morbidity and mortality [8]. Therefore, early and accurate diagnosis of such protozoa prevent disseminations by suitable medication. The profile of protozoal infections among immunocompetent Yemeni people was reported [9], but little concerned with infections in both immunocompromised and immunocompetent ones. The objective of this study was to assess the profile of enteric protozoal infections among immunocompromised and immunocompetent people in Sana'a Town, Yemen.

Patients and Methods

Study subjects and sampling

This cross sectional study was carried out during the period from January to May 2014 in Sana'a Town, Yemen, a total of 241 subjects were recruited. They were attended the inpatient and outpatient clinics of three general Governmental health institutions: [the national oncology centre (29 patients), and two teaching university hospitals; Al-Thawra hospital (118), and Al- Gomhoree (94)]. Subjects presenting with diarrhoea, accompanied with cramps, flatulence, low grade fever, nausea and vomiting. They belonged to two groups: the Group I comprised 102 patients who had immunodeficiency disorders; 29 cases (28.4%) with (lymphomas including non-Hodgkin and Hodgkin's disease), 28 (27.5%) with severe malnutrition, 27 (26.5%) were receiving corticosteroids, and 18 (17.6%) with chronic diseases [diabetes mellitus (10 patients), hypertension (5), heart disease (3)]. The immunodeficiency status of patients was assessed according to the percentage of lymphocytes and CD4+T-cell count from their reports. The Group II contained 139 immunocompetent subjects that selected as a control group. They were apparently healthy, who had no history of receiving immunosuppressive agents, and with age and sex matched with the Group I. Inclusion criteria in both groups were patients of both sexes, aged more than 16 year, who complained from persistent diarrhea for three days or more. Exclusion criteria were subjects not more than 16 year of age, and those who on anti-parasitic treatment a while ago or during this study. Medical sheets were filled out on each subject included; name, age, sex, history of diarrhea episode, used medications, and the medical history report including symptoms. Fresh single fecal specimen was collected from each person in a sterile labeled plastic covered cup (a portion of specimen was subjected to culture for nematode larvae) [10]. About one ml of other stool specimen portion was placed in a labeled tight-bottle containing 3 ml of 10% formalin. Each sample was processed by wet mount, iodine preparation and formalin- ether concentration for protozoan cysts, oocysts, helminthic eggs [11]. Modified Ziehl-Neelsen (MZN) staining method was applied to identify enteric coccidian protozoa [12].

Modified Ziehl-Neelsen staining technique (MZN cold method)

After concentration of stool specimen in formol-ether technique, a thin smear was prepared from one drop of each fecal sample sediment and it was fixed in absolute methanol for one minute. Smear was stained with cold carbol fuchsin for 5 minutes, the stain was rinsed off in 50% ethanol for about 3 seconds and then the stain rinsed off in clean tap water. Smear was decolorized using 1% sulfuric acid until no more color flooded from the smear. Decolorizer was rinsed off in tap water. Smear was counter stained with 0.3% methylene blue for about 1 minute. Counter stain was rinsed off in tap water and blot to dry. Smear was examined microscopically using high dry objective to identify the oocysts and oil immersion objective to see the internal morphology. In positive sample, oocysts of *Cryptosporidium parvum* and *Cyclospora cayetanensis* appeared as acid fast densely stained pink to red spherical structures measuring 4 - 6 and 8 - 10 μm in diameters respectively against a blue background [12]. Oocyst of *Isospora belli* appeared as elongated oval structure and acid fast internal mass of sporoblasts stained a bright red and outlines the oocyst wall didn't stained measuring 23 - 36 μm by 12- 17 μm [13].

Data analysis

The data in questionnaires were transcribed onto computer coding sheets, and statistically analyzed by MstatC program. The results were tested by Chi-square (X^2) test for significance between categorical variables. A value of $P < 0.05$ was considered significant.

Ethical consideration

Ethical approval was yielded from the health institutions under the study (oncology centre and hospitals). The purpose of study was explained to patients, and oral consent was obtained from each.

Results and Discussion

In the current results, overall 59 cases (57.8%) of immunocompromised patients were positive for enteric protozoa, compared to 46 (33.1%) of immunocompetent controls, with statistically significant difference ($X^2 = 6.70$, $P < 0.005$). 6.9% of immunocompromised group had more than one protozoan parasite in comparison to 4.3% of immunocompetent group. Among immunocompromised patients group, *C. parvum* ranked the first (27.5%), and *E. histolytica/E. dispar* was the least (2.9%); the prevalence rate was *G. lamblia*, *E. coli*, *C. cayetanensis* and *I. belli* 13.7%, 8.8%, 6.9% and 4.9% respectively. Amongst those who immunocompetent, *G. lamblia* was the most frequently detected protozoan (15.8%), and *E. coli* was the least (1.4%); the percentages positivity rates of *C. parvum*, *E. histolytica/E. dispar*, *C. cayetanensis* and *I. belli* were 9.4%, 5.0%, 3.6% and 2.2% respectively (Table 1). The gross rate of coccidian protozoa in immunocompromised patients was also more prevalent than immunocompetent ones, with significant association (36.3% versus 14.4% respectively), ($X^2 = 9.46$, $P < 0.001$), where the rate of *C. parvum* was significantly higher than immunocompetents (27.5% vs 9.4% respectively), ($X^2 = 8.87$, $P < 0.001$) (Table 2). Likewise, the percentages of *C. cayetanensis* and *I. belli* in immunocompromised were as well higher than immunocompetents (6.9% and 4.9% vs 3.6% and 2.2% respectively), but without significant difference (Table 2). However, *G. lamblia* and *E. histolytica/E. dispar* among immunocompetent controls were in higher rates than immunocompromised patients (15.8% and 5.0% vs 13.7% and 2.9% respectively), in spite of being not statistically significant (Table 1). Otherwise, *E. coli* in immunocompromised patients gave a significant higher rate than that of immunocompetent (8.8% vs 1.4% respectively), ($X^2 = 5.36$, $P < 0.010$) (Table 1). Concerning helminths, *Strongyloides stercoralis* larva was found in stool culture of one case of immunocompromised patients (1.0%).

Type of protozoan	Protozoal infections				Chi-Square X ² (P. value)
	Immunocompromised (n = 102)		Immunocompetent (n = 139)		
	No.	%	No.	%	
<i>Cryptosporidium parvum</i>	28	27.5	13	9.4	8.87 (<0.001)♦
<i>Cyclospora cayetanensis</i>	7	6.9	5	3.6	1.03 (<0.200)
<i>Isospora belli</i>	5	4.9	3	2.2	1.02 (<0.200)
<i>Giardia lamblia</i>	14	13.7	22	15.8	0.14 (<0.500)
<i>Entamoeba histolytica/E. dispar</i>	3	2.9	7	5.0	0.56 (<0.200)
<i>Entamoeba coli</i>	9	8.8	2	1.4	5.36 (<0.010)♦
Overall prevalence	59*	57.8	46#	33.1	6.70 (<0.005)♦

Table 1: Enteric protozoa among immunocompromised and immunocompetent Yemeni people.

♦ $P < 0.05$, *7 patients (6.9%) had more than one type of protozoa.

#6 patients (4.3%) had more than one type of protozoa.

N.B.: Only one(1.0%) of immunocompromised patients had larva of *Strongyloides stercoralis*.

Type of protozoan	Coccidian Protozoal infections				X ² (P. value)
	Immunocompromised (n = 102)		Immunocompetent (n = 139)		
	No.	%	No.	%	
<i>C. parvum</i>	28	27.5	13	9.4	8.87 (<0.001)♦
<i>C. cayetanensis</i>	7	6.9	5	3.6	1.03 (<0.200)
<i>I. belli</i>	5	4.9	3	2.2	1.02 (<0.200)
Total	37*	36.3	20#	14.4	9.46 (<0.001)♦

Table 2: Enteric coccidian protozoa among immunocompromised and immunocompetent Yemeni people.

♦ $P < 0.05$, *3 patients had more than one type of coccidia.

#1 patient had more than one type of coccidia.

Components of the immune system can be functionally or genetically abnormal as a result of either acquired mainly caused by (HIV infection, lymphoma, high-dose steroids and other immunosuppressive medications) or congenital immunodeficiency illnesses. All may affect humoral immunity or compromise T-cell function. Persons with immunosuppression are at risk of infection with enteric protozoa. The outcome of infection is dependent on absolute CD4+ cell counts, with lower counts being associated with more severe, atypical disease, and a greater risk of disseminated illness [3,14]. Enteric protozoa still remain the most complexity of immunocompromised patients [15].

In the present study, the overall prevalence of enteric protozoal infections among immunocompromised patients was significantly higher than immunocompetent controls, 57.8% compared to 33.1% respectively. This goes in accordance with a study conducted in Saudi Arabia that reported a significant higher rate of infections among patients who immunosuppressed than that of immunocompetent, 43.2% vs 21.5% respectively [16]. Also a previous study carried out in Egypt revealed a high rate of parasitic infection amounted to 94% in immunosuppressed vs 60% in immunocompetent subjects [17]. Furthermore, the rate of enteric parasitic infections in immunocompetent Egyptian patients in comparison to immunosuppressed ones was 41% vs 50.6% respectively [18]. On the other hand, the rates of infection among immunosuppressed patients and immunocompetent hosts in Ethiopia were similar [19]. The rates of infection may have differed according to multiple factors; geographical distribution, socioeconomic, sanitary and environmental conditions, type of the study, hospital or community based, sample size, different selection methods of cases. In addition to these, the parasitological techniques applied in each study could influence the outcome of results [20,21].

In our outcomes, a higher rate of multiple protozoal infections among patients who immunocompromised (6.9%) than immunocompetent (4.3%), may attributable to low level of resistance in immunocompromised, in whom exposure to multiple parasites is increased. However, immunocompetents might have a high level of resistance in whom the exposure is prevented to some extent [22].

The coccidian protozoa among immunocompromised patients were more prevalent than immunocompetent subjects in the current work. The rates of *C. parvum*, *C. cayetanensis* and *I. belli* among immunocompromised patients were higher than immunocompetent controls. Our result is supported by previous findings in Egypt and India that stated higher rates of *Cryptosporidium*, *Isospora*, and *Cyclospora* among those who immunosuppressed than that of immunocompetent [17,23].

In the present findings, *C. parvum* was the commonest parasite encountered in the immunocompromised (27.5%), and in a significant higher rate than immunocompetents (9.4%). This in line with discrete studies executed in Yemen that have reported a higher rate of *C. parvum* among lymphomas immunosuppressed patients than immunocompetents (30.1% vs 11.3% respectively) [24,25]. As well a study from Egypt have detected a higher proportion of cryptosporidiosis among those who immunosuppressed (60.2%) than that of immunocompetent (42.2%) [17]. The higher rate of cryptosporidiosis among patients who immunocompromised than that of immunocompetent in the current results, may be ascribable to the effect of more suppression of cellular immunity as most factor protecting against the parasite. Impairment of local and cell-mediated immunity against enteric parasites may facilitate the enabling of some pathogens causing severe, acute clinical manifestations including extravagant, prolonged aqueous diarrhea [26]. In our work, majority of immunocompromised patients suffer from severe extended watery diarrhea. This goes in concurrence with other study that deduced, cryptosporidiosis is severe and protracted, and creates profuse watery diarrhea in immunocompromised patients [27].

Cyclospora was the second coccidian detected in the current findings and in a high rate amounted to 6.9% in patients who immunocompromised vs 3.6% in immunocompetent that more or less agreed with 5.3% vs 4.6% in immunosuppressed and immunocompetent Yemeni subjects respectively [24,25]. *Cyclospora* was found in 5.9% of Saudi Arabian immunocompromised patients [28]. However, lower rate was in Indian patients (1.7%) [29]. Also in our results, *I. belli* was found in a higher rate among those who immunocompromised than that of immunocompetent (4.9% vs 2.2% respectively). This is propped by preceding study that detected a higher proportion of infection among patients who immunosuppressed (9.1%) than that of immunocompetent (6.3%) [17]. Moreover, *E. coli* infection among immunocompromised patients gave a significant higher rate than that of immunocompetent (8.8% vs 1.4% respectively).

In general, the higher levels of aforesaid protozoa among those who immunocompromised in the current study may reinforce the theory of immunodeficiency as a determinant for opportunistic infections [15,26].

To the contrary, *G. lamblia* and *E. histolytica*/*E. dispar* among immunocompetent controls were more prevalent than immunocompromised patients (15.8% and 5.0% vs 13.7% and 2.9% respectively). In a prior study carried out in Egypt reported a higher rates of *G. lamblia* and *E. histolytica* among immunocompetent group than those who immunosuppressed, 17.6% and 24.6% vs 4.8% and 6% respectively [17]. The bowel of immunocompromised patients may be submitted to somewhat alterations and fluctuations that possibly become improper environment for the growth of *G. lamblia* and *E. histolytica* to some extent [30].

In the current work, only 1.0% of immunocompromised patients had *Strongyloidiasis stercoralis*. This is consolidated by another study that reported the opportunistic infection of *Strongyloides* in immunosuppressed patients [31,32].

As a rule, infectious maladies are dissimilarity distributed all over the world that depend on many factors including environmental, climatic, life style, occupation and other causes. The limitation of immune system in different groups of immunosuppressed people creates them more susceptible to many illnesses [1,21].

Conclusion

It can be concluded that, prevalence rate of enteric protozoal infections among immunocompromised people in Sana'a Town was a substantial and significantly higher than immunocompetent ones. This was due to mainly coccidian protozoa and symptomatic people represented a small part of infection. Undoubtedly, more studies should be directed to the asymptomatic infections. The immunocompromised patients should be examined periodically for the parasitic infections and the specific treatment is definitely recommend to avert the complications of disseminated parasites.

This is among the infrequent reports on gastrointestinal protozoal infections in immunocompromised and immunocompetent Yemeni people.

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