Intestinal Parasitic Infections in Relation to Diarrhea and CD4 T-cell Count among Saudi Patients with Chronic Renal Insufficiency

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***Abstract*—**Chronic renal insufficiency (CRI) disease leads to uremia and induces a state of immunodepression. Since the immune system plays an important role in controlling parasitic diseases, so, higher frequencies of infections can be expected among CRI patients. In the present study, 33 hemodialysis diarrheic Saudi patients and 42 immunocompetent diarrheic controls were evaluated for infections with opportunistic and other associating intestinal parasites and their relevance to CD4 T- cell count and type of diarrhea. Results revealed that among hemodialysis patients, high prevalence of *Cryptosporidium*, *Isospora belli*, *Cyclospora*, microsporidia, *G. lamblia* and *Blastocystis hominis* (36.3, 93.9, 30.3, 100.0, 45.4 and 90.9% respectively) was detected. Polyparasitism was more frequent among hemodialysis diarrheic patients and was more closely associated with chronic diarrhea and lower CD_4 T cell count in comparison to control group. These findings indicate the high susceptibility of hemodialysis patients, specifically those with low CD_4 T cell count, to intestinal parasitic infections of which cryptosporidiosis is life-threatening. So, screening for such infections, should be requested from hemodialysis patients once presented with diarrhea.

***Keywords*—**Intestinal parasitic infections, hemdialysis, diarrhea, CD4 T cell count, Saudi Arabia

I. Introduction

THE immune system plays an integral part in controlling and clearing parasitic diseases. The current widespread use of immunosuppressive therapy, and the growing population of individuals with immunocompromised states have altered the pattern of some parasitic infections so as they have become a major cause of global morbidity and mortality than diseases produced by any other group of organisms (1). Increased life expectancies and the resulting aging of many human populations have increased the incidence of chronic degenerative diseases, such as chronic renal insufficiency (CRI) which leads to uremia (2) and immunodepression (3). most commonly expressed as an increase in the incidence of infections which are responsible for 48% of deaths in patients with CRI (2). Although manifestations of CRI do not exempt any organic system, diarrhea is one of its most important clinical signs (4). *Cryptosproridium*; *Cyclospora* and *Isospora* are important opportunistic emerging pathogens causing diarrheal diseases. The outcome of infection by these parasites is dependent on absolute CD4 T cell counts, with lower counts being associated with more severe, more atypical, and a greater risk of disseminated disease. Although the infections with these parasites have a serious impact on the health of hemodialysis patients, their routine diagnosis may be ignored and their prevalence among Saudi dialysis patients has not yet been studied adequately. So, the present study is meant to highlight the prevalence of the opportunistic intestinal parasites among Saudi hemodialysis patients presenting with diarrhea, and its significance in relation to CD4 T- cell count.

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II. Patients and Methods

A total of 33 adult patients (12 males and 21 females ; 16->60 years old) presenting with diarrhea and attending at the dialysis unit at Prince Salman Center for kidney diseases were included in the present study. A control group of 42 immunocompetent adults presenting with diarrhea at the Outpatient Clinic (Ambulatory Care Services) of King Khalid University Hospital with age and sex matched with dialysis patients was included. Control patients have no history of receiving specific treatment or immunosuppressive drugs and have CD4 T cell count > 500 cells⁄mm^3.

A. Methodology

All patients were subjected to: a) Full personal and medical history using structured questionnaire, present and past history of diarrhea and treatment. b) Collection of fecal samples and microscopic examination of fecal smears stained with the modified Ziehl-Neelsen staining method (Kinyoun's acid fast stain) for identification of oocycts of the coccidian species as described by Garcia (5), Trichrome staining for identification of cysts and trophozoites of intestinal protozoa (5) and Quick-microsporidian spores. c) Collection of blood samples and counting of CD_4-T-lymphocytes by flow cytometry (Becton Dickinson, Paramus, N.J., USA) as described by Dwivedi *et al*. (6).

The study protocol was approved by the ethics review board of King Saud University (King Khalid University Hospital). Informed consent from each patient was obtained. All the tests were performed after due patient consent and in accordance with the institutional ethical guide line.

B. Statistical analysis

Data analysis was done using the Student (t) test, Chi-Square test and One Way ANOVA.

III. Results and Discussion

Results are presented in tables 1-4.

TABLE I
PREVALENCE OF INTESTINAL OPPORTUNISTIC PARASITES AMONG DIARRHEIC HEMODIALYSIS PATIENTS IN RELATION TO DIARRHEIC CONTROLS

IOPs	hemodialysis patients (n =33)		Control patients (n=42)		Statistical Analysis
	No.+ve	(%)	No.+ve	(%)	
Cryptosporidium spp.	12*	(36.3)	5	(11.9)	P < 0.05
Cyclospora spp.	10**	(30.3)	6	(13.04)	P > 0.05
Isospora belli	31***	(93.93)	36	(85.7)	P > 0.05
Microsporidia	33	(100)	37	(88.09)	P < 0.05
Blastocystis	30	(90.9)	34	(80.9)	P > 0.05
G. lamblia	15	(45.4)	30	(71.4)	P < 0.05

IOPs: intestinal opportunistic parasites. - No. +ve: positive number.
* mixed infection with *Cyclospora* spp. and *Isospora belli*.
** mixed infection with *Cryptosporidium* spp.
*** mixed infection with *Cryptosporidium* spp. and *Cyclospora* spp.
**** ANOVA test

TABLE II
CD4 T-CELL COUNT AMONG HEMODIALYSIS INFECTED PATIENTS IN RELATION TO INFECTED CONTROLS

IOPs	CD4 cell count/mm^3 (Mean ± S.D)		Statistical analysis*
	hemodialysis patients	controls	
Cryptosporidium spp.	995.4+ 530.8	1793.2 ± 650.6	P < 0.05
Cyclospora spp.	938.1+ 504.03	1351.3 ± 757.5	P > 0.05
Isospora belli	922.6 + 516.9	1862.7 ± 1147.2	P < 0.001
Microsporidia	947.41 + 536.3	1815.5 + 1133.4	P < 0.0002
Blastocystis	926.6 + 523.3	1805.5 + 940.3	P< 0.0001
G. lamblia	958.6 + 647.3	1830.5 + 1009	P < 0.01

IOPs: intestinal opportunistic parasites. * Chi square test

TABLE III
RELATIONSHIP BETWEEN CD4 T-CELL COUNT AMONG INFECTED HEMODIALYSIS PATIENTS AND INFECTED CONTROLS

IOCs \ patients*	CD4 T-cell count < 500/mm^3 n=6	CD4 T-cell count ≥ 500/mm^3 n=27	** Statistical analysis
	***No+ve (%)	No+ve (%)	
Cryptosporidium spp.	4(66.7)	8(29.6)	P < 0.05
Cyclospora	2(33.3)	8(29.6)	P > 0.05
Isospora spp.	6(100)	25(92.5)	P > 0.05
Microsporidia	6 (100)	27 (100)	—
Blastocystis	6 (100)	24 (88.8)	P > 0.05
G. lamblia	4 (66.6)	11 (40.4)	P > 0.05

* hemodialysis patients ** Chi Square test *** All have mixed infection

TABLE IV
INTESTINAL PARASITIC INFECTIONS AND CD4 CELL COUNT IN RELATION TO TYPE OF DIARRHEA AMONG HEMODIALYSIS PATIENTS

IOPs \ Patients	Acute diarrhea (N=2) No+ve (%)	Acute diarrhea (N=2) CD4 count Mean ± SD	Chronic diarrhea (N=31) No +ve (%)	Chronic diarrhea (N=31) CD4 ccount Mean ± SD	Statistical analysis
Cryptosporidium	1 (50)	668.78+0.00	11(35.4)	1100.78±511.4	* p< 0.05 ** P>0.05
Cyclospora spp.	0 (0)	0.0	10(32.2)	938.1± 504.03	p< 0.05 ** P>0.05
Isospora belli	1(50)	668.78±0.00	30 (96.7)	922.6± 516.9	p < 0.05 ** P>0.05
Microsporidia	2(100)	690.5±172.19	31(100)	957.5± 551.5	* p< 0.05 ** P>0.05
Blastocystis	1(50)	912.3±0.00	29(93.5)	927.1±534.64	p< 0.05 ** P>0.05
G. lamblia	0 (0)	0.0	15 (48.3)	985.3± 647.3	

IOPs: intestinal opportunistic parasites. No. ex.: examined number. - No. +ve : positive number. *Chi square test to compare between % of infection
**Student "t" test to compare between mean CD4 count

Immunosuppression has different consequences for the host depending on its magnitude, and will alter the range of pathogens to which they are susceptible (7). Parasites can cause serious infections in immunocompromised hosts, especially in patients with impaired cellular immunity (8). Patients undergoing hemodialysis are at significant risk of infection with opportunistic parasites (9).

Results of the present work showed that *Cryptosporidium*, *Isospora belli*, *Cyclospora*, microsporidia, *G. lamblia* and *Blastocystis* were highly prevalent among dialysis patients, and still prevalent among diarrheic controls. This emphasizes that these parasites are causative agents of diarrhea in immunocompetent patients in addition of being opportunistic parasites. The difference here is that urgent detection and management are required for the dialysis patients because one of these infections, i.e. cryptosporidiosis is life-threatening. This is in accordance with Goetz *et al.* (10) who reported microsporida infection in renal transplant recipients undergoing immunosuppressive therapy. Similarly, Massry *et al.* (11), Descomps and Chatenoud (12) and Chonchol (13) stated that hemodialysis patients are susceptible to opportunistic infections as a result of leukocyte dysfunction and impaired immunologic response. Brenner (14) added that parasitic infections are the second cause of mortality among dialysis patients. Ocak and Eskiocak (15) mentioned that chronic renal insufficiency is usually associated with uremia that impairs antigen presentation, T-cell activation, and causes impaired antibody production. Hazarati *et al.* (16) assumed that increased time of uremic status might weaken immune system progressively with resultant increased risk of infectious disease.

Sanad and Al-Malki (17) found that the rate of *Cryptospondium* infection among kidney transplant patients under immunosuppressive therapy was 84% which is much higher than that detected in the present study in dialysis patients. The difference between the level of immunosuppression induced by the aggressive chemotherapy protocols, usually followed up, in kidney transplant patients (18, 19) and that induced in dialysis patients is most probably expected to be the cause of this discrepancy. This can be confirmed by the mean CD4 count obtained among dialysis patients in the present study (995.4+530.8/mm^3). Many authors reported that *Cryptosporidium* infection is expected among HIV patients at lower CD4 counts, mostly below 200/mm^3 (20, 21).

In agreement with our results, Ferreira (22) reported that among the most important protozoa that were incriminated for causing severe diarrhea in the immunosuppressed patients were *Cryptosporidium parvum*, *Isospora belli*, *Cyclospora cayetanensis* and microsporidia. Similarly, Santana *et al.* (23) stated that *Cyclospora cayetanensis* is a cause of clinical disease in immunosuppressed hosts and added its relevance with prolonged, severe and highly recurrent diarrhea. Certad *et al.* (24) found that *I. belli* accounted for up to 20% of cases of diarrhea in AIDS patients. Zali *et al.* (25) stated that *Blastocystis hominis* has to be considered as an opportunistic

parasite and added that it was the second most prevalent in HIV-infected patients in Iran, with most of the cases were seen in patients with diarrhea. Seyrafian *et al*. (26) found that 11.5% dialysis patients were infected with *Cryptosporidium* and were significantly higher than in control groups. They added that cryptosporidiosis in the immunocompromised individuals is usually associated with chronic diarrhea and can be life threatening. Dwivedi *et al*. (6) identified enteric opportunistic parasites among 62.7% of HIV infected individuals, of which *Cryptosporidium*, *Giardia lamblia*, microsporidia and *Isospora belli* were significantly associated with diarrhea. Evering and Weiss (8) found that *Cryptosporidium* was the most common protozoon to be encountered in immunocompromised patients. Domenech *et al*. (27) recognized *Cryptosporidium* as a cause of diarrhea associated with a high mortality in immunocompromised patients.

In the present study, all of the dialysis patients were infected with more than one parasite, a finding indicates the high susceptibility of such patients to parasitic infections and necessitates requests for intestinal parasites once presented with diarrhea. However, our reference control group showed also a high percentage of multiparasitic infections which indicates that any diarrheic patient, even non-immunosuppressed, has to be requested for intestinal parasites. These results are in agreement with Graczyk *et al*. (28) who stated that the presence of parasites in the gastrointestinal tract modulates the immune response, predisposing to infection with other enteropathogens and favoring multi-parasitism. Dwivedi *et al*. (6) found that chronically infected diarrheal patients had polyparasitic infections.

It is noticed that the infection rates of intestinal opportunistic parasites in dialysis patients worldwide are contradictory in comparison to each other and to our results. This may be related to socioeconomic state, geographical locality and population general health or may be a consequence of the quality of drinking water and food hygiene.

Among patients with chronic renal insufficiency , the rate of infection with *Cryptosporidium* species was significantly higher among patients with CD4- T cell count < 500/ mm^3 in comparison to patients with CD4 T-cell count > 500/ mm^3. The rates of infection with *Cyclospora*, *Isospora* and *G. lamblia* (60%, 30%, 70% respectively) were insignificantly ($P > 0.05$) higher among patients with CD 4 T-cell count < 500/mm^3 in comparison to patients with CD4 T-cell count > 500/mm^3 (47.05, 23.5, 58.8% respectively). On the other hand, the rate of infection with microsporidia spp. was 100% among all CRI patients with CD4 T-cell count < 500/mm^3 or > 500/mm^3 (100%).

It is apparent, from results of the present study, that low CD4 T cell count is associated with infections with opportunistic intestinal parasites. Therefore, has to be continually checked for, in people who are at risk.

Among patients with chronic renal insufficiency, 2/31 patients had acute diarrhea. One of these two was infected with *Cryptosporidium* and *Blastocystis* the other had infection with *Isospora belli*, both were infected with microsporidia . Out of 33 with CRI, 31 had chronic diarrhea.

In agreement with our results, Tuli *et al*. (29) found that *Cryptosporidium* spp. was the most commonly acquired protozoa causing chronic diarrhea among immunocompromised patients in India, followed by microsporidia. They added that the isolation rates decreased with the increase in the CD4 cell counts. The CD4 levels were inversely proportional to the duration of diarrhea and patients with chronic diarrhea had lower CD4 counts than those who had acute diarrhea.

In **c**onclusion, the present study clarified that enteric infections with opportunistic parasites are highly prevalent among diarrheic hemodialysis patients and polyparasitism is frequent. The outcome of infection is dependent on absolute CD4 T cell counts, with lower counts being associated with more severe disease. So, optimizing the immunologic status of individuals at risk may help to reduce acquisition of such opportunistic parasitic infections and the likelihood of developing life-threatening diarrhea.

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