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# Parasitology Research

## Intestinal protozoan and helminthic infections among hemodialysis and cancer patients --Manuscript Draft--

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Abstract:	<p>Intestinal parasitic infections (IPIs) can be a severe threat to immunocompromised patients. This is particularly true for those undergoing chemotherapy and hemodialysis. The present research is aimed at identifying intestinal parasites that might be present in immunocompromised patients. In this cross-sectional study 1040 stool samples were collected (279 samples from hemodialysis patients, 362 samples from chemotherapy patients and 399 samples from the control group) from March to September 2017. The samples were tested by direct, formalin-ether methods for protozoa and ova of intestinal parasites and Ziehl-Neelsen staining methods for coccidian parasites such as <i>Cryptosporidium</i> species. The overall parasitic infection rate was higher (15%) in hemodialysis patients and 11.3% in chemotherapy patients whereas the lowest rate was observed (7.3%) in the control group. The infectivity rates were statistically significant (<math>P = 0.008</math>) when compared with the control group. The most prevalent parasites present were <i>Blastocystis hominis</i> (8.9% of the cases), <i>Entamoeba coli</i> (1.6%), <i>Iodamoeba butschlii</i> (0.8%), <i>Endolimax nana</i> (0.6%), <i>Chilomastix mesnili</i> (0.5%), <i>Strongyloides stercoralis</i> (0.5%) and <i>Taenia</i> species (0.15%), whereas <i>Giardia lamblia</i> was found present only in the control group. Statistical analyses revealed a significant correlation between gastrointestinal symptoms (such as some abdominal pain, diarrhea) and the ratio of chemotherapy frequency to scheduled frequency and parasitic infections (<math>P = 0.001</math>). There was not a correlation between prevalence of parasites with age or education levels of the infected individuals. Results of the present study suggest that a periodic stool examinations in special parasitological laboratories should be included as part of routine and general medical care.</p>
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## Intestinal protozoan and helminthic infections among hemodialysis and cancer patients

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## ABSTRACT

Intestinal parasitic infections (IPIs) can be a severe threat to immunocompromised patients. This is particularly true for those undergoing chemotherapy and hemodialysis. The present research is aimed at identifying intestinal parasites that might be present in immunocompromised patients. In this cross-sectional study 1040 stool samples were collected (279 samples from hemodialysis patients, 362 samples from chemotherapy patients and 399 samples from the control group) from March to September 2017. The samples were tested by direct, formalin-ether methods for protozoa and ova of intestinal parasites and Ziehl-Neelsen staining methods for coccidian parasites such as *Cryptosporidium* species. The overall parasitic infection rate was higher (15%) in hemodialysis patients and 11.3% in chemotherapy patients whereas the lowest rate was observed (7.3%) in the control group. The infectivity rates were statistically significant ( $P = 0.008$ ) when compared with the control group. The most prevalent parasites present were *Blastocystis hominis* (8.9% of the cases), *Entamoeba coli* (1.6%), *Iodamoeba butschlii* (0.8%), *Endolimax nana* (0.6%), *Chilomastix mesnili* (0.5%), *Strongyloides stercoralis* (0.5%) and *Taenia* species (0.15%), whereas *Giardia lamblia* was found present only in the control group. Statistical analyses revealed a significant correlation between gastrointestinal symptoms (such as some abdominal pain, diarrhea) and the ratio of chemotherapy frequency to scheduled frequency and parasitic infections ( $P = 0.001$ ). There was not a correlation between prevalence of parasites with age or education levels of the infected individuals. Results of the present study suggest that a periodic stool examinations in special parasitological laboratories should be included as part of routine and general medical care.

**Keywords:** Parasites, dialysis, chemotherapy, prevalence

## Introduction

Intestinal parasitic infestations cause a variety of clinical conditions, the majority of which are related to the gastrointestinal (GI) tract. Intestinal parasites are among the most common human infections distributed worldwide (Bora 2016). One fourth of all known diseases are caused by parasites (Cleaveland Laurenson and Taylor 2001).

There is evidence that some parasitic infections are associated with cancer development. The level of evidence of this association varies from high to low; in any case, a long time interval is mandatory for the development of cancer. *Opisthorchis viverrini* and *Clonorchis sinensis* are associated with cholangiocarcinoma, *Schistosoma hematobium* with bladder cancer (Samaras et al. 2010). According to the World Health Organisation (2019) certain viruses, bacteria, and parasites can act as biological carcinogens. It is suggested that in low- and middle-income countries as many as 15% of cancers diagnosed in 2012 could be attributed to carcinogenic infections; Martel et al. (2020) estimated for 2018, around 2.2 million infection-attributable cancers were diagnosed worldwide. According to Cancer Research UK (2020) bile duct cancer and bladder cancer can be directly attributed to parasites.

Changes in social and cultural context have influenced the human-parasite relations during the past century. Currently, parasitic diseases are more prevalent in underdeveloped countries (Bloom and Murray 1992). Patients undergoing chemotherapy or hemodialysis are considered to be immunocompromised and specific steps in healthcare are followed to reduce the risk of infection (Ferreira and Borges 2002). It has been observed that healthy individuals could also be affected by intestinal parasites. They often express self-limiting symptoms, but they can increase the risk of infecting immunocompromised individuals (Bora 2016).

Infections increase the mortality rates and it is considered as the second highest cause of mortality for hemodialysis patients (Tonelli et al. 2006). There is also evidence that there is a high rate of parasitic infections in cancer patients, especially those that undergo chemotherapy (Zabolinejad et al. 2013; Barazesh et al. 2015). Directing the awareness of physicians towards the early diagnosis, and rapid and proper treatment of such pathogens could reduce the morbidity and mortality rate as well as reducing the medical costs.

The present study was conducted to investigate the prevalence of IPIs in two groups of patients (hemodialysis patients and chemotherapy patients), compared with the control (patients not diagnosed with those conditions) group.

## **Material and Methods**

### *2.1. Ethics statement*

The cross-sectional study was approved by the Ethics Committee of Guilan University of Medical Sciences with ethics number of IR.GUMS.REC.1396.141.

### *2.2. Study area and sample collection*

The patients who were eligible for the study, were informed about the research plan and their consent was taken before recruitment. Participants were referred from Razi Hospital, Guilan Oncology Center, Kianmehr Hemodialysis Center, and Caspian Hemodialysis Center in Rasht, Guilan province. During this period, fresh stool samples were collected from 362 cancer patients, 279 hemodialysis patients and 399 individuals (control group) who were without any signs and symptoms of malignancy. During the first stage, demographic data were collected (age, gender, residence and education). Further self-reported information about their health was obtained. For

the participants with cancer, the type of cancer, number of completed chemotherapy sessions undergone, the date of the first chemotherapy session was recorded. For the hemodialysis patients, the following parameters were self-reported: the number of sessions, the date of their first dialysis and the frequency of treatment was recorded. Participants that took anti-parasitic drugs, antibiotics, mineral oils, barium, bismuth and non-absorbable anti-diarrhea drugs two weeks prior were excluded from the study. The inclusion criteria for the control group were:

1. Patients without chronic infections
2. Cancer patients receiving at least one course of chemotherapy
3. Hemodialysis patients at least three months post their first dialysis were included in the study.

### *2.3. Stool sample examination*

Fecal samples were collected in specimen containers from each individual and were transferred to the Department of Parasitology, School of Medicine, Guilan University of Medical Sciences. Macroscopic investigation of stool samples was performed to detect mucus and blood. The microscopic screening included direct wet smear (saline preparation and iodine preparation) and formalin-ether concentration to detect intestinal amoeba and flagellate (Bora et al.2016; Garcia 2007). The oocysts of coccidian parasites (*Cryptosporidium* spp., *Isospora belli*), were examined with a modified acid-fast technique using light microscopy with a magnification of 100× according to Garcia et al. (1983).

### *2.4. Statistical analysis*



The data were statistically analyzed using SPSS software (SPSS version 16). Differences between variable groups were analyzed by Chi-Squared test, and test significance level was  $p < 0.05$ .

### 3. Results

This study compares the prevalence of intestinal parasites between 641 immunocompromised patients (including 362 chemotherapy and 279 hemodialysis patients), and 399 'healthy' subjects as defined above. 49.9% of the participants were female and 50.1% male and their age range was 60-79 years old (Table 1). For all groups the infection rates 13% (see Table 1). The IPI rates for the individual groups were 11.3%, 15% and 7.3% for chemotherapy patients, hemodialysis patients and the control group, respectively. The IPI rates were significantly different between the three groups ( $P = 0.008$ ). *Blastocystis hominis* was the most prevalent (8.9%) parasite observed in patients followed by *Entamoeba coli* (1.6%), *Iodamoeba butschlii* (0.8%), *Endolimax nana* (0.6%), *Chilomastix mesnili* (0.5%), *Strongyloides stercoralis* (0.5%) and *Taenia* species (0.15%). In the present study, helminthes, namely *S. stercoralis* and *Taenia* species were the only pathogenic parasites detected in the patients. Similar to the patients, *B. hominis* was the most prevalent (8.9%) parasite observed in the control group, followed by *G. lamblia* (2%), *I. butschlii* (0.5%), *E. coli* (0.25%), *E. nana* (0.25%), and *C. mesnili* (0.25%). The pathogenic protozoa, *G. lamblia*, was the only pathogenic parasite detected in the 'control group (Table 2). Multiple infections were detected in five participants (four in the hemodialysis and one in the cancer group). In all multiple infection cases, *B. hominis* was a constant finding. The association among the prevalence of IPIs, demographics (age, gender, education, residence) and clinical symptoms is

presented in Table 3. The rate of IPI was significantly higher in rural, compared with urban residence ( $P = 0.001$ ). However, demographic characteristics were not associated with the prevalence of IPIs (see Table 3). For the hemodialysis group it appears that there is no significant correlation between the prevalence of IPIs and the length of time since diagnosis. The effect of the chemotherapy sessions on the IPI was also investigated. The cancer patients were grouped according to the planned chemotherapy sessions completed. Group one included participants that completed less than half of the planned session; the second group included participants that had completed half of the planned sessions and the third included participants that had completed more than half of the sessions. The rate of parasitic infection was 9.8%, 22.2% and 8.2% in the first, second and third group, respectively. The difference was statistically significant ( $p = 0.02$ ). The rate of parasitic infection observed in patients with uterine and ovarian cancer (13.8%), gastrointestinal cancer (12.4%) and lung cancer (4.8%) was not statistically significant.

#### 4. Discussion

The prevalence of intestinal parasites in chemotherapy patients, hemodialysis patients and the control group was 11.3%, 15%, and 7.3%, respectively. Rasti et al. (2017) reported IPI of 7.6% in cancer patients and 11.9% of IPIs in hemodialysis patients of Kashan, Iran. The main reason for the lower incidence in cancer patients compared with its prevalence in the hemodialysis patients may be because of the short-term effects of the drugs used in chemotherapy (Barazesh et al. 2015). Hemodialysis patients sometimes spend up to 20 years undergoing hemodialysis. Such long-term care increases the risk of contaminations, from parasitic infections including from the health care services (Karadag Tamer and Dervisoglu 2013; Alter et al. 2011). Individuals, who are undergoing

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4 dialysis treatments have a high risk of acquiring infections through contact with nursing staff,  
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6 equipment and materials, on surfaces or from hands (Kulik et al. 2008). The prevalence of  
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8 intestinal parasites in the present study is lower than the-prevalence that has been reported among  
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10 immunocompromised and cancer patients in Turkey (43.7%), and Egypt (18%) (Baiomy et al.  
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12 2010; Thom Kleinberg and Roghmann 2013), and hemodialysis patients in Brazil (45.1%) (Kulik  
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14 et al. 2008). The prevalence of parasitic infestation in patients with cancers in India was found to  
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16 be 80%. However, there was not a control group in that study) (Bora et al. 2016). The prevalence  
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18 of IPIs was higher (10–63%) among the patients with different immunosuppressed status  
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20 compared with the control group (Bora et al. 2016; Kulik et al. 2008; Al-Qobati Al-Maktari and  
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22 Derhim 2012; Togeh et al. 2000; Al-Megrin 2010; Ashrafi Tahbaz and Rahmati 2010). The wide  
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24 variation of prevalence may be attributed to the differences in geographical distribution of  
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26 parasites, sanitary practices, and different selection criteria of cases (Bora et al. 2016). In the  
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28 present study, non-pathogenic intestinal protozoan, *B. hominis* was the most prevalent (8.9%)  
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30 parasite observed in immunocompromised patients followed by *E. coli* (1.6%), *I. butschlii* (0.8%),  
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32 *E. nana* (0.6%), *C. mesnili* (0.5%) (Table 2). *B. hominis* was found to be the most common  
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34 intestinal parasite in all studied groups; *G. lamblia* was found once in the control group (Table 2).  
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36 Similar findings have been reported in other studies (Bora et al. 2016; Kulik et al. 2008; Al-Qobati  
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38 Al-Maktari and Derhim 2012; Togeh et al. 2000; Al-Megrin 2010; Ashrafi Tahbaz and Rahmati  
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40 2010; Kazemi et al. 2013). *B. hominis* may act as a pathogen in immunocompromised subjects and  
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42 lead to various types of infections (with or without symptoms). Water, pets, and vegetables are  
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44 probably the main sources of infections. Research indicates that *B. hominis* may be the source of  
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46 such infections (Rao et al.2003; Motta and Silva 2002; Stensvold et al.2007; Abdel-Hameed and  
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48 Hassanin 2011; Iguchi et al. 2009). *B. hominis* and four non-pathogenic protozoans, namely *E.*  
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4 *coli*, *I. butschlii*, *E. nana*, and *C. mesnili*, had the highest prevalence among the patients and control  
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6 groups (Table 2). *Giardia* and *Cryptosporidium* are two important waterborne parasites  
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8 (Mahmoudi et al. 2013; 2017). In previous studies, *Cryptosporidium* and *Giardia* (oo)cysts were  
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10 detected in surface water samples in Guilan (Mahmoudi et al. 2013; 2015 2017). But, examination  
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12 of stool specimens with formalin ether and Ziehl – Neelsen staining revealed no positive  
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14 *Cryptosporidium* oocyst in either of the three groups (Table 1). Infections by this protozoan have  
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16 been related to poor-quality drinking water (Mahmoudi et al. 2017; Seyrafian et al. 2006; Botero  
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18 et al. 2003; Chieffi et al. 1998), but it cannot be discounted that these patients acquired the parasites  
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20 from contaminated water, despite the fact that in Guilan, water used by nearly 100% of the  
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22 population is subject to quality control. The frequency of *Cryptosporidium* spp. in adult  
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24 hemodialysis patients was reported 4.6% in Brazil (Kulik et al. 2008), 11.5% in Iran (Seyrafian et  
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26 al. 2006), and 20.27% in Turkey (Turkcapar et al. 2002). In addition, in the present study, two  
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28 pathogenic helminths, *S. stercoralis* (0.5%) and *Taenia* species (0.15%) were the only pathogenic  
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30 parasitic helminthes detected in the examined patients. Strongyloidiasis is a soil transmitted  
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32 endemic disease, and most immunocompromised patients are exposed to the infection and its side  
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34 effects, such as diarrhea (Ashrafi Tahbaz and Rahmati 2010).  
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43 We observed three cases (0.3%) of infection with this parasite in one hemodialysis and two cancer  
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45 patients. The number of cases observed are lower than the number of cases reported in another  
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47 study in Iran (9.7%) (Sharifdini et al. 2018). Undoubtedly, certain measures, such as not using  
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49 human feces as fertilizer on farms, and health education for farmers could be recommendations  
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51 for the reduction of worm infections and protection of public health. Most cases of infections were  
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53 observed in males rather than females in all three groups, however, they were not statistically  
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55 significant (Table 1). Several studies have suggested that the prevalence of IPIs in males differ  
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from females (Al-Qobati Al-Maktari and Derhim 2012; Kia et al. 2008; Sayyari et al. 2005). The IPIs were significantly different in relation to the age, where the prevalence rates of IPIs (12.5%) in the age group 60–79 years was higher than other age groups. In some similar studies, the highest prevalence was reported in the age group above 50 years (Barazesh et al. 2015; Monsef et al. 2008). No significant relationship was observed between the prevalence of IPIs and gastrointestinal symptoms (such as some abdominal pain, diarrhea) (Table 1) and similar observations were reported by Naeini et al. (2012). The present research demonstrated no significant association between education level and IPIs prevalence (Table 3), which is in line with the finding of Barazesh et al. (2015). Nevertheless, several studies observed a significant relationship between education level and IPIs prevalence (Naeini et al. 2012; Choy et al. 2014; Daryani et al. 2012). The current study demonstrates a significant association between IPIs prevalence and residence (9.6% among 438 patients living in cities and 18.7% among 203 patients living in rural areas). Omrai et al. (2015) found a recognizable relationship between IPIs prevalence and residence but Naeini et al. (2012) and Barazesh et al. (2015) found no significant difference between these two variables. The differences may be the result of agriculture activities and other effective factors on growth, reproduction and transmission of parasites in rural areas as compared with cities (Barazesh et al. 2015; Naeini et al. 2012; Omrani et al. 2015). We separated hemodialysis participants according to length of time receiving the treatment into three categories: 1 – 12 months, 13 – 24 months and  $\geq 25$  months. No significant correlation was observed between IPIs prevalence and hemodialysis duration. The association between PDSS (proportion of done sessions of chemotherapy to scheduled session) and IPIs prevalence was assessed by allocating the PDSS into three categories according to the chemotherapy cycles received: less, intermediate and more than half of chemotherapy cycles to scheduled session. Using this classification, we demonstrated that IPIs are

more prevalent in members of the second category (more than two other categories) and the difference is statistically significant. Chandramathi et al. (2012) used the same categorization and demonstrated the higher prevalence of *B. hominis* and *Microsporidium* species in cancer patients during the intermediate chemotherapy cycles (Chandramathi et al. 2012). The poisonous effect of chemotherapy medicine on the immune system reduces the immune function (Koivusalo and Hietanen 2004; Solomayer et al. 2003), thus causing augmentation for the parasitic infection. At the end of the therapy course, the anti-oxidant system of patients reacted to the poisonous effects of chemotherapy drugs, which may boost their immune system and eventually overcome the parasite (Chandramathi et al. 2012). We found 11.9% prevalence rate in cancer patients with different types of leukemia with the highest and the lowest rates of IPIs occurring in patients with uterine and ovarian cancer (13.8%) and lung cancer (4.8%), respectively (statistically insignificant difference). In India, Rudraparta et al. (1997) reported a 16.5% prevalence rate for IPIs in patients with gastrointestinal cancer. A prevalence rate of 35.9% in children with leukemia has been reported by Zaboli Nejad et al. (2013). The difference may be attributed to methods (direct and ELISA) used to diagnose parasitic infection, while we only used the direct method. This study revealed the prevalence of parasitic infections was higher in two immunocompromised groups (undergoing chemotherapy and hemodialysis) compared with the control group in the study area. Detection of intestinal protozoan could be used as indicators of poor hygiene practices (Chieffi et al. 1998).

## 5. Conclusion

Most of the observed protozoans in the present study were non-pathogenic. Intestinal pathogenic protozoans are transmitted in a similar way to non-pathogenic parasites, infection with pathogenic

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4 parasites is always possible in this study area. Given the fact that some endemic parasitic infections  
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6 such as *S. stercoralis* may be lethal for immunocompromised patients, periodic parasitology  
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8 examinations are highly recommended for hemodialysis and chemotherapy patients.  
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#### 14 **Conflicts of interest**

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16 We have no conflict of interest regarding this study.  
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**Table1:** Demographic characteristics of Immunocompromised patients and Control group.

	Hemodialys	Cancer	Control	Total
	N=279	N=362	N=399	N=1040
Variable	N (%)	N (%)	N (%)	N (%)
Age group				
≤30	12 (4.3%)	17 (4.7%)	19 (4.8%)	48 (4.6%)
31-59	53 (19%)	108 (29.8%)	110 (27.6%)	271 (26.05%)
60-79	164 (58.8%)	188 (51.9%)	202 (50.6%)	554 (53.3%)
≥80	50 (17.9%)	49 (13.5%)	68 (17%)	167 (16.05%)
Sex				
male	160 (57.3%)	171 (47.2%)	190 (47.6%)	521 (50.1%)
female	119 (42.7%)	191 (52.8%)	209 (52.4%)	519 (49.9%)
Educational level				
Primary school	214 (76.7%)	277 (76.5%)	235 (58.9%)	726 (69.8%)
High school	48 (17.2%)	61 (16.9%)	129 (32.33%)	238 (22.9%)
University	17 (6.1%)	24 (6.6%)	35 (8.77%)	76 (3.36%)
Location				
city	207 (74.2%)	131 (36.2%)	248 (62.1%)	586 (56.3%)
village	72 (25.8%)	231 (63.8%)	151 (37.8%)	454 (43.6%)
Symptoms				
No	235 (84.2%)	284 (78.5%)	358 (89.7%)	877 (84.3%)
Yes	44 (15.8%)	78 (21.5%)	41 (10.3%)	163 (15.7%)

**Table 2:** Prevalence of Intestinal parasitic infections and infection rate among immunocompromised and Control groups.

Parasite	Hemodialysis N (%)	Chemotherapy N (%)	Total of immunocompromised N (%)	Control N (%)	<i>P</i> value
<i>Blastocystis hominis</i>	28 (10.1%)	29 (8%)	57 (8.9%)	16 (4%)	
<i>Entamoeba coli</i>	7 (2.5%)	3 (0.8%)	10 (1.6%)	1 (0.25%)	
<i>Endolimax nana</i>	2 (0.7%)	2 (0.6%)	4 (0.6%)	1 (0.25%)	
<i>Iodamoeba butschlii</i>	2 (0.7%)	3 (0.8%)	5 (0.8%)	2 (0.5%)	
<i>Chilomastix mesnili</i>	2 (0.7%)	1 (0.3%)	3 (0.5%)	1 (0.25%)	
<i>Giardia lamblia</i>	0 (0%)	0 (0%)	0 (0%)	8 (2%)	
<i>Cryptosporidium</i> <i>spp.</i>	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
<i>Strongyloides stercoralis</i>	1 (0.36%)	2 (0.55%)	3 (0.5%)	0 (0%)	
<i>Taenia spp.</i>	0 (0%)	1 (0.28%)	1 (0.15)	0 (0%)	
Infected	42 (15%)	41 (11.3%)	83 (13%)	29 (7.3%)	0.008
Non-infected	237 (85%)	321 (88.7%)	558 (87%)	370 (92.7%)	



**Table 3:** Demographic characteristics according to the existence or absence of parasites in immunocompromised patients.

		Parasitized (108) N (%)	Non-parasitized (932) N (%)	Total (1040) N (%)	<i>P</i> value
Variable					
Age group	≤30	6 (12.5%)	42 (87.5%)	48 (100%)	0.43
	31-59	26 (9.6%)	245 (90.4%)	271 (100%)	
	60-79	64 (11.6%)	490 (88.4)	554 (100%)	
	≥80	12 (7.2%)	155 (92.8%)	167 (100%)	
Sex	male	56 (10.7%)	465 (89.3%)	521 (100%)	0.78
	female	52 (10.2%)	467 (89.8%)	519 (100%)	
Educational level					
	Primary school	81 (11.1%)	645 (88.9%)	726 (100%)	0.87
	High school	18 (7.6%)	220 (92.4%)	238 (100%)	
	University	9 (11.8%)	67 (88.2%)	76 (100%)	
Location					
	urban	45 (7.7%)	541 (92.3%)	586 (100%)	0.001
	rural	63 (13.9%)	391 (86.1%)	454 (100%)	
Symptoms					
	No	76 (8.7%)	801 (91.3%)	877 (100%)	0.001
	Yes	32 (19.6%)	131 (80.4%)	163 (100%)	