



Survey of prevalence of intestinal parasites in under hemodialysis patients referred to Sari and Zanjan hospitals, Iran

Salman Ghaffari¹, Ali Asghar Fazaeli², Roja Jafarian³, Farzane Jafarian⁴

1 Department of Medical Parasitology and Mycology, School of Medicine, Babol University of Medical Sciences, Babol, Iran

2 Department of Medical Parasitology and Mycology, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran

3 Department of General Medicine, Qeshm International School of Medicine, Qeshm International Free University, Qeshm, Iran

4 Department of Medical Parasitology and Mycology, School of Medicine, Zanjan University of Medical Sciences Zanjan AND Clinical Research Development Unit of Rouhani Hospital, Babol University of Medical Sciences, Babol, Iran

Original Article

Abstract

BACKGROUND: Patients undergoing hemodialysis are vulnerable to intestinal parasites due to a weakened immune system. This study aimed to determine the prevalence of intestinal parasites in patients undergoing hemodialysis.

METHODS: The feces of 168 under hemodialysis patients were collected from Sari and Zanjan hospitals, Iran, and the demographic and clinical information of the patients was recorded in a questionnaire. Samples were tested using wet-mount method, formalin-ether concentration, modified Ziehl-Neelsen staining for coccidia, conventional trichrome for intestinal protozoa, and modified trichrome for Microsporidia. Data were entered into SPSS software and analyzed by the chi-square test method ($P < 0.05$).

RESULTS: The prevalence of parasitic infections in patients under hemodialysis in Sari and Zanjan was 43.58% and 33.33%, respectively, with a total of 40.4%. With a prevalence of 23.28%, Giardia had the highest prevalence rate. Then, Entamoeba coli had a prevalence of 14.3% and other parasites were, respectively, Endolimax nana (6.54%), Blastocystis hominis, Entamoeba histolytica/dispar, Dientamoeba fragilis, and Cryptosporidium (each one: 1.19%), and Chilomastix mesnili (0.59%). There was no significant difference between the prevalence of intestinal parasites with age, gender, place of residence (urban or rural), and duration of hemodialysis ($P \leq 0.05$).

CONCLUSION: Pathogenic parasites such as Giardia have a high prevalence in patients undergoing hemodialysis in Sari Region. Given the potential risks of these infections, especially giardiasis, amoebiasis, and cryptosporidiosis, it is recommended that regular screening be performed to diagnose and treat parasitic diseases in this population.

KEYWORDS: Intestinal Parasites; Hemodialysis; Patients; Parasitic Infections

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Introduction

Chronic renal failure is due to the progression

Corresponding Author:

Farzane Jafarian; Department of Medical Parasitology and Mycology, School of Medicine, Zanjan University of Medical Sciences Zanjan AND Clinical Research Development Unit of Rouhani Hospital, Babol University of Medical Sciences, Babol, Iran

Email: farniag@yahoo.com

of the disease and irreversible impairment of kidney function, in which case, the human body is unable to balance electrolytes and metabolism; therefore, excretory substances such as urea are not excreted by the kidneys and cause uremia in humans. Patients undergoing hemodialysis have a weaker immune system than healthy individuals. Infections are the second leading cause of

death in hemodialysis patients, accounting for 48% of deaths in these patients;¹ therefore, they are constantly affected by infectious diseases, including parasitic and other infections.²

In recent decades, opportunistic parasitic protozoa such as *Cryptosporidium*, *Isospora*, *Blastocystis*, *Cyclospora*, *Toxoplasma*, *Microsporidia*, and intestinal pathogens such as *Entamoeba histolytica* and *Giardia* are the most important agents recognized as a danger to human health that have been identified.^{3,4} On the other hand, the growth of domestic and international travel and the rapid distribution of fresh fruits and vegetables has provided the possibility of increasing and spreading food and water-borne diseases in the world more than before.⁵ The prevalence of *Blastocystis* has been reported from 1.5% to 15% in developed countries and 30% to 50% in developing countries.⁶ In 2020, parasitic infections in hemodialysis patients were 24%.⁷

Studies performed on hemodialysis patients in other parts of the world indicate parasitic infections in this group of patients. Studies in this field have been conducted in some countries, each with different results.⁸⁻¹⁰ In a study on 74 hemodialysis patients in Turkey, the rate of *Cryptosporidium* infection was 15.74%.⁸ In a study on hemodialysis patients in Saudi Arabia, 43.6% of them had parasitic infections. *Blastocystis* had the highest prevalence (23.9%).⁹ Moreover, in a study conducted in Egypt, the prevalence of parasitic infections in patients undergoing hemodialysis was 40%.¹⁰ In Iran, limited studies have been conducted on the prevalence of parasitic infections among patients undergoing hemodialysis. There are different reports on the prevalence of parasites in renal patients undergoing hemodialysis in some areas.^{11,12} Although these studies are limited, they show the prevalence and importance of these infections in hemodialysis patients. The rate of infection with intestinal parasites in high-risk and vulnerable groups is basically a function

of the overall infection status of these parasites in each region. Due to its location in the northern part of Iran, Mazandaran Province, has special climatic conditions that provide the basis for the spread of some intestinal parasites. Zanzan Region, Iran, in terms of the extent of agriculture and animal husbandry and the size of rural areas, has provided relatively good conditions for the spread of parasitic infections.

However, the climate is different from the northern region of Iran, and at least, some parasitic infections are expected to be less prevalent. Adopting a parasite control and prevention program in vulnerable groups is important and requires completing the information in different geographical areas. Therefore, this study was performed to determine the prevalence of intestinal parasites in patients undergoing hemodialysis in Sari and Zanzan hospitals.

Methods

The target population in this study was patients with renal failure undergoing hemodialysis in Sari and Zanzan hospitals. From October to February 2013, a total of 168 patients, including 117 patients from Hazrat Fatemeh Zahra and Imam Khomeini Hospitals in Sari and 51 patients from Vali-e Asr Hospital in Zanzan (Zanzan Dialysis Center) were examined (dissertation registration code: A-12-245-1).

The method of sample selection was convenience sampling. The inclusion criterion included patients who had been on dialysis for at least one month and underwent hemodialysis two to three times a week.

People with other illnesses were excluded from the study, including cancer, diabetes, and any other disease that results in immunodeficiency, were excluded from the study. In addition, stool samples were taken from patients who had taken barium, bismuth, oil, or antibiotics one to two weeks before.

Before taking the sample, a consent form was obtained from them.

All information such as age, gender, place of residence, and duration of dialysis was collected in a questionnaire. Fresh stool samples were collected from patients three times in a maximum of ten days (for example, every one to three days, up to ten days) and transferred to the Department of Parasitology at Babol (Iran) and Zanzan Universities of Medical Sciences. While recording the sample information, first microscopic direct detection method was performed on fecal samples using physiological serum, Lugel (Padtan Teb Co., Iran), and preparation of wet spread and the results were recorded. Then the formalin-ether concentration method was performed. Besides, permanent staining by modified Ziehl-Neelsen methods was used to detect coccidia, conventional trichrome to detect intestinal protozoa, and modified trichrome to detect microsporidia.

The obtained information was entered into SPSS software (version 16, SPSS Inc., Chicago, IL, USA). Chi-square test was used to examine the presence or absence of a relationship between variables ($P \geq 0.05$).^{13,14}

Results

Of 168 patients, 89 were men (52.97%), and 79 were women (47.02%). The mean age of patients was 55 years. The prevalence of

parasitic infection in all patients undergoing hemodialysis was 40.47% (68 cases), of which 51 (43.58%) were related to patients undergoing hemodialysis from Sari and 17 (33.33%) were related to patients undergoing hemodialysis from Zanzan.

The difference between the total infection ratio in Sari patients and the total infection in Zanzan patients was not significant ($P = 0.11$). Among intestinal parasites, *Giardia* pathogenic parasite with a prevalence of 23.21% ($n = 39$) had the highest prevalence of infection in patients undergoing hemodialysis, followed by total *Entamoeba* with a prevalence of 14.28% ($n = 24$). Other parasites, including *Endolimax nana* (6.5%) ($n = 11$), *Blastocystis hominis*, *Entamoeba histolytica/dispar*, *Dientamoeba fragilis*, and *Cryptosporidium* were detected at 1.19% (2 cases each one). The lowest infection rate was related to *Chilomastix mesnili*, with a prevalence of 0.59% (1 person). No worm infestations were observed in any patients' fecal samples (Figure 1).

The age range of participants in this study was from 12 to 86 years, and the highest percentage of infection was observed in the age groups of 31 to 68 years (63.69%). The pathogenic parasite *Entamoeba histolytica/dispar* was also observed in this age range; however, no significant difference was observed between the prevalence of intestinal parasites and age groups ($P \geq 0.05$) (Table 1).

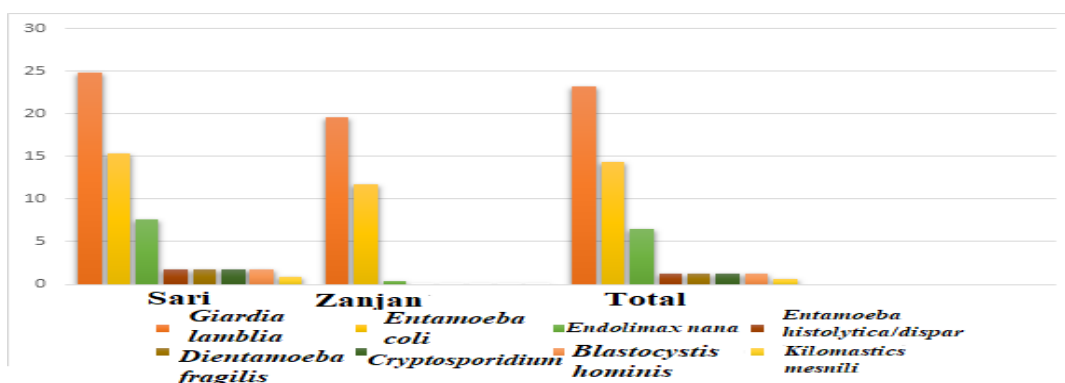


Figure 1. Distribution of intestinal parasites in hemodialysis patients from Sari and Zanzan Regions, Iran

Table 1. Distribution of intestinal parasites in hemodialysis patients in Sari and Zanjan, Iran, by age

Parasites' names	12-30 years	31-49 years	50-68 years	69-86 years	Total (n = 168)	P
	old (n = 5)	old (n = 44)	old (n = 63)	old (n = 56)		
Giardia lamblia	1 (20.00)	10 (22.72)	17 (26.98)	11 (19.64)	39 (23.21)	0.81
Entamoeba coli	1 (20.00)	7 (15.90)	8 (12.69)	18 (14.28)	24 (14.28)	0.94
Endolimax nana	0 (0)	2 (4.54)	5 (7.93)	4 (7.14)	11 (6.54)	0.83
Entamoeba histolytica/dispar	0 (0)	1 (2.27)	1 (1.58)	0 (0)	2 (1.19)	0.73
Dientamoeba fragilis	0 (0)	0 (0)	1 (1.58)	1 (1.78)	2 (1.19)	0.25
Cryptosporidium	0 (0)	2 (4.54)	0 (0)	0 (0)	2 (1.19)	0.12
Blastocystis hominis	0 (0)	0 (0)	1 (1.58)	1 (1.78)	2 (1.19)	0.83
Chilomastix mesnili	0 (0)	1 (2.27)	0 (0)	0 (0)	1 (0.59)	0.41

Data are presented as number and percentage

Comparison of the rate of infection in each of the detected parasites did not show a significant difference between the male and female groups ($P \geq 0.05$, in all cases). The results of this study showed that the prevalence of parasitic infection in urban and rural areas was 36.30% ($n = 61$) and 63.69% ($n = 107$), respectively (Table 2).

The prevalence of parasitic infection in patients undergoing hemodialysis did not show a statistical relationship with increasing the duration of dialysis ($P \geq 0.05$) (Table 3).

Discussion

In this study, parasitic diseases had a relatively high prevalence in patients undergoing hemodialysis, so that out of a total of 168 stool samples, 40.47% of all patients had one or more species of pathogenic or non-pathogenic parasites. In a study conducted by Kulik et al., 86 healthy dialysis patients in Brazil were

considered using 10% formalin maintenance and Kinyoun method. The prevalence of parasitic infection in these patients was 45.1% and in healthy individuals 25.7%.⁶

In a study conducted by Naeini et al. in Iran, using the modified Ziehl-Neelsen staining method, the prevalence of parasitic infections in kidney transplant patients and healthy individuals was 33.3% and 20%, respectively.¹⁵

In a study conducted by Nasiri et al. in Iran, 13915 stool samples were received from non-dialysis patients. Modified formalin-ethyl acetate deposition method showed that the rate of parasitic infection was 4.7%;¹⁶ this rate is much lower than that reported in studies of patients with kidney problems. These results are probably due to the greater susceptibility of these individuals as a group at risk for infections due to weakened immune systems.¹⁷

Table 2. Distribution of intestinal parasites in hemodialysis patients in Sari and Zanjan, Iran, by gender and location

Parasites' names	Gender			P	Residence			P
	Men (n = 89)	Women (n = 79)	Total (n = 168)		Urban (n = 61)	Rural (n = 107)	Total (n = 168)	
Giardia lamblia	22 (27.71)	15 (21.51)	39 (23.21)	0.18	13 (21.31)	26 (24.29)	39 (23.21)	0.65
Entamoeba coli	13 (14.60)	11 (13.99)	24 (13.69)	0.44	13 (21.31)	11 (10.28)	24 (14.28)	0.14
Endolimax nana	8 (8.98)	3 (6.79)	11 (54.6)	0.07	3 (4.91)	8 (7.47)	11 (6.54)	0.51
Entamoeba histolytica/dispar	0 (0)	2 (2.53)	2 (1.19)	0.18	1 (1.63)	1 (0.93)	2 (1.19)	0.68
Cryptosporidium	2 (2.24)	0 (0)	2 (1.19)	0.13	0 (0)	2 (1.86)	2 (1.19)	0.28
Chilomastix mesnili	0 (0)	1 (1.26)	1 (0.59)	0.34	1 (1.63)	0 (0)	1 (0.59)	0.18
Dientamoeba fragilis	0 (0)	2 (2.53)	2 (1.19)	0.18	0 (0)	2 (1.86)	2 (1.19)	0.28
Blastocystis hominis	1 (1.12)	1 (1.26)	2 (1.19)	0.93	1 (1.63)	1 (0.93)	2 (1.19)	0.68

Data are presented as number and percentage

Table 3. Distribution of intestinal parasites in hemodialysis patients in Sari and Zanjan, Iran, by duration of dialysis

Parasites' names	Duration of dialysis			Total (n = 168)	P
	1 to 12 months (n = 11)	13 to 36 months (n = 70)	More than 36 months (n = 87)		
Giardia lamblia	3 (27.27)	12 (17.14)	24 (27.58)	39 (23.21)	0.28
Entamoeba coli	1 (9.09)	6 (8.57)	24 (19.54)	24 (14.28)	0.13
Endolimax nana	0 (0)	6 (8.57)	5 (5.74)	11(6.54)	0.51
Entamoeba histolytica/dispar	0 (0)	2 (2.58)	0 (0)	2 (1.19)	0.24
Dientamoeba fragilis	0 (0)	1 (1.42)	1 (1.42)	2 (1.19)	0.92
Chilomastix mesnili	0 (0)	1 (1.42)	0 (0)	1 (0.59)	0.49
Cryptosporidium	0 (0)	0 (0)	2 (2.29)	2 (1.19)	0.03
Blastocystis hominis	0 (0)	1 (1.42)	1 (1.41)	2 (1.19)	0.92

Data are presented as number and percentage

A study by Seyrafian et al. was performed in Iran using modified acid-fast staining; stool samples from 104 outpatients with chronic hemodialysis, 91 healthy family members, and 140 healthy individuals were examined for *Cryptosporidium* oocysts. 11.5% of dialysis patients, 4.4% of healthy family members, and 3.6% of healthy individuals were infected with *Cryptosporidium*. The prevalence of *Cryptosporidium* infection was not related to gender, age, duration of dialysis, history of kidney transplantation, or history of use of immunosuppressive drugs. The prevalence of *Cryptosporidium* infection in our study was 1.19%, which was lower than the study of Seyrafian et al. In our study, the prevalence of parasites had no significant relationship with gender, age, and duration of dialysis. However, since hemodialysis patients are candidates for kidney transplantation, general preventive measures against *Cryptosporidium* infection should be considered.¹²

In another study conducted by Seyrafian et al. in Iran, the rate of parasitic infection in dialysis patients was 43.9%. In this study, which was performed by maintenance in 10% formalin, formalin-ethyl acetate precipitation, and trichrome staining, the highest percentage of infection (8%) belonged to *Blastocystis*; then *Entamoeba coli* (5.6%) and *Endolimax nana* (4.2%) had a lower infection ratio.¹⁸ The results of our study showed that *Giardia* intestinal parasites (23.21%) had the highest and

Chilomastix mesnili (0.59%) had the lowest prevalence of parasitic infection in hemodialysis patients. Besides, *Entamoeba* with a prevalence of 14.28%, *Endolimax nana* with a prevalence of 6.54%, and *Blastocystis hominis* with a prevalence of 1.19% were identified in the present study.

Shehata et al. examined the stool and blood samples of 120 hemodialysis patients and 100 healthy patients for parasitic infections in Egypt. The prevalence of parasitic intestinal infection in hemodialysis patients was 12% to 52.5% compared to healthy individuals. Moreover, worm-related infections were not observed among patients and healthy individuals. *Cryptosporidium* (35.5%), *Blastocystis hominis* (2.24%), and *Microsporidia* (11.7%) were the most common parasites among hemodialysis patients. The prevalence of *Cryptosporidium* was lower in our study (1.2%), but the prevalence of *Blastocystis hominis* (1.19%) was almost close to this study. As in Shehata et al. study, in our study, no worm infestation was observed in the patients' samples, and all of them were protozoan infestations.¹⁹

Blastocystis hominis is an anaerobic and protozoan parasite found in the human large intestine and many other vertebrates. In the new class, Blastocysts belong to the monophyllite group or the natural group of Stramenopiles. The parasite spreads globally and is transmitted directly to various hosts

through cysts and contaminated water and food. The prevalence of this parasite is 100% in developing countries and more than 56% in developed countries. Blastocystis-related infections range from gastrointestinal (GI) disorders such as diarrhea and bloating to irritable bowel syndrome (IBS) and skin lesions such as urticaria. The parasite is transmitted through the skin and oral feces. Occurrence and prevalence of Blastocystis varies due to lack of resistance in patients, differences in health behaviors and habits, type of food, inadequate and unsanitary drinking water, wastewater use, lack of access to sanitation, type of weather, seasons, geographical conditions, relationship with animals, the patient's age, and poor socioeconomic conditions.²⁰⁻²⁴

In a polymerase chain reaction (PCR)-sequencing study conducted by Izadi *et al.*, in Iran, on 346 patients with immunodeficiency, including patients undergoing hemodialysis, the prevalence of patients infected with *Cryptosporidium* was 3.46%.²⁵ Infection with this parasite is made through contaminated water, food, liquids, personal contact, or animal-to-human contact.²⁶ Transmission is more common in places with poor health and in people living in groups. Symptoms of the disease are more common in people with defective immune systems and include weakness and disability, diarrhea, severe abdominal pain, weight loss, anorexia, and systemic infection.^{27,28}

In another study conducted by El-Kady *et al.* in Egypt, using microscopic, concentrated, and acid-fast modified staining (to identify *Cryptosporidium*), 66% of 150 dialysis patients with kidney transplantation had parasitic infections. Parasitic agents included *Cryptosporidium* (60%), *Entamoeba histolytica* (21%), and *Giardia lamblia* (12%).²⁹

In Iran, Mahmoudi *et al.* examined 330 fecal samples of patients with chronic renal failure undergoing hemodialysis by direct microscopic

observation and formalin-ether for intestinal parasites. The prevalence of intestinal parasites in patients undergoing hemodialysis was 23.9%. *Endolimax nana* was the most common parasitic species in dialysis patients with 6.4%. The highest parasite infection rate was observed in the age group of 51-65 years (29.7%). There was a statistically significant relationship between age and prevalence of parasitic intestinal infection, but there was no significant relationship between sex and the prevalence of intestinal parasitic infections. In addition, there was a statistically significant relationship between the prevalence of parasitic intestinal infections with the duration of dialysis and physical health status. However, there was no significant relationship with the place of residence.³⁰

In a study conducted by Mahmoudi *et al.* in Iran, using direct microscopic observation, formalin ether, and Ziehl-Neelsen staining, 279 stool samples from hemodialysis patients, 362 samples from chemotherapy patients, and 399 samples from the control group were tested. The overall rate of parasitic infection was 15% in hemodialysis patients, 11.3% in chemotherapy patients, and 7.3% in the control group. The parasites found in this study included *Blastocystis hominis* (8.9%), *Entamoeba coli* (1.6%), *Iodamoeba buetschlii* (8.8%), *Endolimax nana* (6.6%), *Strongyloides stercoralis* (0.5%), and *Taenia* (0.15%). *Giardia lamblia* was observed only in the control group. The present study also identified *Blastocystis hominis*, *Entamoeba coli*, *Endolimax nana*, and *Giardia*. These studies indicate that periodic stool examinations in specific parasitic laboratories should be part of the routine and general medical care.³¹ Intestinal parasitic infections can lead to severe and long-term illness in patients with kidney problems. Diagnostic delays due to clinical suspicions of intestinal parasites and limitations of standard diagnosis can worsen outcomes in these patients in different parts of

the world. Especially, since these patients may travel to different areas, physicians everywhere should be aware of the risk of various intestinal parasitic infections in patients with kidney problems.

Studies have shown that different species of parasites including *Cryptosporidium*, *Isospora belli*, *Giardia*, *Blastocystis*, *Balantidium*, *Entamoeba histolytica*, and *Diantamba fragillis* with several intestinal worms including *Capillaria* species and *Ascaris lumbricoides* cause infections with *Trichuris trichiura* worms. *Giardia lamblia* is also a cause of recurrent diarrhea among mammals and humans worldwide.³² In this study, 43.58% and 33.33% of hemodialysis patients in Sari and Zanjan were infected with parasitic infections, respectively. The high prevalence of parasite infection in Mazandaran can be due to climatic conditions (humid and temperate), which cause more survival of parasitic agents and their spread and transmission. This difference may be due to climatic differences, although the number of samples in Zanjan was relatively small, which may be an influential factor.¹³

Parasitic diseases are often associated with non-specific signs and symptoms, and physicians cannot diagnose the disease by physical examination alone. Paraclinical and laboratory tests are necessary to determine parasitic infection, genus, and parasite species. Should be reviewed and used. According to studies conducted in different regions of Iran and the world and limited laboratory methods with less sensitivity than standard methods, the overall prevalence of infection has been different. It is possible that by performing simultaneous diagnostic methods of direct spread, formaldehyde-concentration, and trichrome staining on a sample, the chances of increasing cases of GI parasites in the study population are higher than the results obtained.^{32,13}

Deoxyribonucleic acid (DNA) extraction

and PCR are required to determine the amoeba. Contaminated water is the most common way of transmitting these parasites. However, the most common areas affected by this infection are poor water and food hygiene.^{33,34} Among intestinal parasites, *Giardia* pathogenic parasites with a prevalence of 23.21% had the highest prevalence of infection in patients undergoing hemodialysis, and then *Entamoeba* with 14.28%. Other parasites included *Endolimax nana* with 6.54%, *Blastocystis hominis*, *Entamoeba histolytica/dispar*, *Diantamba fragilis*, and *Cryptosporidium*, each detected at 1.19%. The lowest level of infection was related to *Chilomastix mesnili* (0.59%). No worm infections were observed in any of the patients' fecal samples. *Giardia*, *Entamoeba histolytica/dispar*, and *Diantamba fragilis* were identified as pathogenic parasites, *Cryptosporidium* and *Blastocystis hominis* as opportunistic parasites, and *Entamoeba coli*, *Endolimax nana*, and *Chilomastix mesnili* as non-pathogenic parasites.

Although non-pathogenic parasites are not important in pathogenicity, they indicate environmental pollution, lack of hygiene, and unsanitary contact of people with the infected environment.^{35,36} No parasitic microspore infection was observed in this study. Laboratory diagnosis of *Microsporidia* is difficult by non-molecular methods. Modified trichrome staining and molecular PCR are standard and reliable methods for *Microsporidia*.³⁷ Many *Microsporidia* infections are transmitted to humans through water contaminated with animal waste. These infections cause prolonged diarrhea with abdominal pain and weight loss in patients, especially those with defective immune systems. The study of geographical location shows the prevalence of *Microsporidia* in different regions of southern and central Iran (due to hot and humid climate), as well as Tehran Province (due to high population and

low level of health in some areas).³⁸

The results also showed that parasite infection was higher in rural patients (63.69%), but there was no significant relationship between patients' location (village or city) and the prevalence of parasitic infections. Different ways and sources of parasite transmission in different regions significantly impact the rate and difference in the prevalence of parasitic infections. A wide range of worms and protozoa can cause infections. These microbes are colonized in the digestive tract of animals and humans. The transmission and distribution of parasites occurs through human-to-human, animal-to-human, oral-fecal route, parasitic contaminated hands, contaminated food, drinking or non-drinking water, insects, especially flies and beetles, rodents, sewage mice, and cats are also dust.³⁹⁻⁴² Because Iran is a very diverse country in terms of climate and is composed of different socio-cultural patterns, there are different patterns of parasitic distribution in this country. Therefore, knowing the environmental status can indicate the appropriate habitat of parasites, based on which, human infections can be detected. In addition, based on the distribution and biological patterns of parasite distribution, we can initiate appropriate prevention and treatment programs.⁴³

Conclusion

Parasitic infections were observed in hemodialysis patients in Sari and Zanjan. Opportunistic parasitic infections are considered critical in this group of patients with weakened immune systems. Therefore, it is suggested that the necessary training be given to these patients to prevent, control, and treat parasitic infections. These patients should be examined and periodically cared for. In addition, appropriate medications should be prescribed for their treatment if the infection is diagnosed.

Conflict of Interests

Authors have no conflict of interests.

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