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Original Article

Prevalence and Associated Risk Factors of Intestinal Parasitic Infections among Patients Visiting a Referral Hospital in Tehran Province, Iran

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Abstract

Background: Intestinal parasitic infections (IPIs) are still considered a public health problem of mankind, particularly in immunocompromised patients. We aimed to determine the prevalence of IPIs with an emphasis on immunocompromised patients in a referral hospital in Tehran Province, Iran.

Methods: In this cross-sectional study, 442 fecal specimens were collected randomly from patients, referred to Baqiyatallah Hospital in Tehran Province, Iran from May to September 2020. The collected specimens were examined using wet-mount, trichrome and modified Ziehl-Neelsen staining, formalin-ether concentration, and agar plate culture.

Results: The prevalence of IPIs was found 17.0% (95% CI: 13.6-20.8%). The prevalence of protozoan parasites (16.3%; 95% CI: 13.0-20.1%) was significantly higher than helminthic parasites (0.7%; 95% CI: 0.1-2.0%). *Blastocystis* spp., *Giardia lamblia*, and *Entamoeba coli* were the most common intestinal protozoan with a prevalence of 12.2%, 1.6%, and 1.4%, respectively. *E. histolytica*/*E. dispar*/*E. moshkovskii*, *Iodamoeba bütschlii*, *Cryptosporidium* spp., *Chilomastix mesnili* as protozoan species and *Hymenolepis nana*, *Dicrocoelium dendriticum*, and *Ascaris lumbricoides* as helminthic species were the other detected parasites. Multiple logistic regression revealed a significant association of IPIs infections with stool consistency and the status of immune system.

Conclusion: The prevalence of IPIs among the patients who are immunocompromised was significantly higher than immunocompetent patients ($P < 0.05$). Periodic stool examinations for screening of IPIs should be included as a part of routine medical check-up in these patients.



Introduction

Intestinal parasites infections (IPIs) are still considered a public health problem of mankind, particularly in developing countries (1). It is estimated that IPIs have affected more than 3 million individuals worldwide (2). Measures like socioeconomic, geographic, hygienic, cultural, and educational situation have resulted in an extensive range of IPIs prevalence between 4.7 to 56% in Iran (3-5).

Although a remarkable decline has occurred in the prevalence of IPIs in healthy population in recent decades, the importance of parasitic infections such as strongyloidiasis and cryptosporidiosis in immunocompromised patients is of great significance, which could result in a fatality (6-8). In addition, prior studies have proved that parasites such as *Giardia lamblia*, *Entamoeba coli*, and *Blastocystis* sp. still have a considerable prevalence in a healthy population as well as the immunocompromised patients. They could be considered as an indicator of public health and hygiene (9-11).

In a resource-limited country like Iran, with an approximate population of 80 million, identification of the prevalence and distribution pattern of IPIs is an essential step to set up a prevention and control program to enhance the health status. Hence, investigation and providing epidemiological information among vulnerable groups such as immunocompromised patients could help to design a cost-effective control program.

Therefore, this study was conducted to determine the prevalence and risk factors associated with IPIs in immunocompromised patients and immunocompetent individuals, Tehran Province, Iran.

Materials and Methods

Study design

This cross-sectional study was conducted at Baqiyatallah University of Medical Sciences,

Tehran, Iran, from May to September 2020. The samples were collected from patients visiting Baqiyatallah hospital, Tehran, Iran.

Sample size and collection

Four hundred and forty-two stool specimens were collected randomly from patients referred to Baqiyatallah hospital in Tehran province. Stool specimens were collected in the pre-labeled plastic containers and transferred to the laboratory of the Department of Medical Parasitology, School of Medicine, Baqiyatallah University of Medical Sciences for parasitological analysis.

Data collection

A pre-designed checklist including determinants of sex, age group, occupation, education level, clinical manifestations, diabetes, and immunodeficiency conditions was filled for each participant. Patients who had conditions such as cancer, chemotherapy, dialysis, and kidney transplantation were categorized as immunocompromised patients.

Laboratory analysis

The consistency, color, presence of blood, and adult helminths were examined macroscopically for each sample. In addition, a wet-mount slide was prepared and examined microscopically under low-power objective (10×) and high dry objective (40×) to identify trophozoites, cysts and oocyst of protozoa parasites and helminths ova. A formalin-ether concentration technique was performed to identify ova, cysts, or oocysts of parasites. In addition, a trichrome staining procedure was conducted for each sample for identification of trophozoites and cysts of protozoan parasites. Besides, slim slides were prepared and fixed with absolute methanol for 3 min and slides were stained using modified Ziehl-Neelsen method for detection of oocysts. All stained slides were examined microscopically

with high magnification (1000×). Finally, a part of each collected specimen (approximately 2 g) was cultured on agar plates in order to identify parasitic larvae (12). All microscopic evaluations and identification were made by an observer and positive slides were confirmed by a second observer. Specimens were considered positive if any helminth eggs, larvae, cysts, oocyst, and trophozoites of protozoans were detected by at least one of the mentioned methods.

Statistical analysis

The frequency was calculated for qualitative and categorical variables. The associations between IPIs and determinant were assessed using univariate and multiple logistic regressions. Statistical significance was accepted at p -values <0.05. Statistical analysis was done using IBM® SPSS® Statistics version 25 (SPSS Inc., Chicago, IL, USA).

Ethics approval

This study was reviewed and approved by the ethics committee of Baqiyatallah University of Medical Sciences with the code number: IR.BMSU.REC.1399.485. In accordance with Helsinki declaration and guidelines, a written consent from each patient was obtained for publication of their personal and clinical details.

Results

Of 442 stool specimens examined, 241 (54.5%) and 201 (45.5%) were collected from males and females, respectively. Children, made up only 4.5% of the studied population while the majority of the participants were middle-aged people (40-60 years of age; 37.3%) (Age range: 1-86, mean 44.9 ± 18.6). Only 5% of the participants had a postgradu-

ate degree, while 38% of them had a high school education. About 35.9 % of participants were categorized as immunocompromised patients because of cancer, chemotherapy, dialysis, and kidney transplantation. Besides, 18 (4.1%) of patients had diarrheic stool.

At least one species of the IPIs was found in 75 (17.0%; 95% CI: 13.6-20.8%) of the participants in the current study. The prevalence of protozoan parasites 16.3% (95% CI: 13.0-20.1%) was significantly higher than helminthic parasites 0.7% (95% CI: 0.1-2.0%). *Blastocystis* spp., *G. lamblia*, and *E. coli* were the most common intestinal protozoan with a prevalence of 12.2% (95% CI: 9.3-15.6%), 1.6% (95% CI: 0.6-3.2%), and 1.4% (95% CI: 0.5-2.9%), respectively. *Entamoeba histolytica*/*E. dispar*/*E. moshkovskii*, *Iodamoeba bütschlii*, *Cryptosporidium* spp., and *Chilomastix mesnili* were the other detected protozoan parasites. In the case of helminthic parasites, one case (0.2%; 95% CI: 0.0-1.3%) of each of *Hymenolepis nana*, *Dicrocoelium dendriticum*, and *Ascaris lumbricoides* was detected (Table 1). However, the further following-up revealed that the *D. dendriticum* case was a spurious infection due to consumption of contaminated livestock liver. In addition, multiple infections with 2 or 3 parasitic agents constituted 8% of 75 infected cases.

The results of univariate and multiple logistic regression analyses of the risk factors associated with IPIs among the participants embedded in Table 2. Among possible risk factors investigated in this study, the stool consistency and the status of immune system were found to have a significant association with intestinal parasitic infections ($P < 0.05$). There was no association between the infection and sex, age group, occupation, education level, and diabetes.

Table 1: Prevalence of IPIs among the participants (n = 442)

<i>Parasite</i>	<i>Frequency</i>	<i>Prevalence% (95%CI)^a</i>
Protozoa		
<i>Blastocystis</i> sp.	54	12.2 (9.3-15.6)
<i>Giardia lamblia</i>	7	1.6 (0.6-3.2)
<i>Entamoeba coli</i>	6	1.4 (0.5-2.9)
<i>Iodamoeba bütschli</i>	5	1.1 (0.4-2.6)
<i>Cryptosporidium</i> sp.	3	0.7 (0.1-2)
<i>Chilomastix mesnili</i>	2	0.5 (0.1-1.6)
<i>Entamoeba histolytica/E. dispar/E. moshkovskii</i>	2	0.5 (0.0-1.6)
Total ^b	72	16.3 (13.0-20.1)
Helminths		
<i>Hymenolepis nana</i>	1	0.2 (0.0-1.3)
<i>Dicrocoelium dendriticum</i>	1	0.2 (0.0-1.3)
<i>Ascaris lumbricoides</i>	1	0.2 (0.0-1.3)
Total	3	0.7 (0.1-2.0)
Total ^b	75	17.0 (13.6-20.8)

^a CI: confidence interval
^c There were also some cases of multiple infection with two or three species

Table 2: Association between socio-demographic determinants of participants and prevalence of IPIs (n=442)

<i>Determinants</i>	<i>Frequency</i>	<i>%</i>	<i>No. of positive</i>	<i>prevalence% (95%CI)</i>	<i>OR (95% CI)</i>
Sex					
Male	241	54.5	44	18.3 (13.6-23.7)	1
Female	201	45.5	31	15.4 (10.7-21.2)	1.22 (0.74-2.02)
Age group (yr)					
≤10	20	4.5	2	10.0 (1.2-31.7)	1
11-20	25	5.7	7	28.0 (12.1-49.4)	0.44 (0.03-6.18)
21-30	64	14.5	12	18.8 (10.1-30.5)	1.55 (0.14-16.45)
31-40	71	16.1	18	25.4 (15.8-37.1)	0.92 (0.09-9.02)
41-50	59	13.3	11	18.6 (9.7-30.9)	1.35 (0.14-12.96)
51-60	106	24.0	13	12.3 (6.7-20.1)	0.91 (0.09-9.02)
61-70	72	16.3	11	15.3 (7.9-25.7)	0.55 (0.05-5.39)
71-80	20	4.5	0	0.0	0.72 (0.07-7.07)
80<	5	1.1	1	20.0 (0.5-71.6)	0.00
Occupation					
Government employee	177	40.0	31	17.5 (12.2-23.9)	1
Self-employed	257	58.1	43	16.7 (12.4-21.9)	1.48 (0.17-12.51)
Unemployed	8	1.8	1	12.5 (0.3-52.7)	1.40 (0.16-11.72)
Educational level					
High school	168	38.0	31	18.5 (12.9-25.2)	1
Diploma	114	25.8	27	23.7 (16.2-32.6)	2.26 (0.50-10.19)
Undergraduate	138	31.2	15	10.9 (6.2-17.3)	3.10 (0.68-14.13)
Postgraduate	22	5.0	2	9.1 (1.1-29.2)	1.22 (0.25-5.74)
Diabetes					
Yes	83	18.8	11	13.3 (6.8-22.5)	1
No	359	81.2	64	17.8 (14.0-22.2)	0.31 (0.35-1.40)
Stool consistency					
Diarrheic	18	4.1	8	44.4 (21.5-69.2)	1
Normal	424	95.9	67	15.8 (12.5-19.6)	4.26 (1.62-11.19)*
Immune system					
Immunocompetent	283	64.1	2	0.7 (0.1-2.6)	1
Immunocompromised ^a	159	35.9	73	45.9 (38-54.0)	0.00 (0.00-0.03)*

^a Cancer, chemotherapy, dialysis, and kidney transplant, OR: odds ratio, * Statistically significant, Statistical test: univariate and multiple logistic regressions

Discussion

Regular assessment of the prevalence of IPIs, especially in high-risk segments of the population such as immunocompromised patients is vital to design a targeted, cost-effective and appropriate control plan. The present study was conducted among the patients in a referral hospital, Tehran.

The overall prevalence of IPIs among the present study participants was 17.0%. This finding was consistent with a similar other study which was conducted in Tehran which the prevalence of IPIs was reported as 21.2% among patients with gastrointestinal disorders (13). However, a higher prevalence of 29.75% infection was also reported in patients referred to Kashani hospital, Tehran (14). The lower prevalence in our study might have resulted from the development of public health infrastructures in Tehran during recent years. In another study, which was conducted in Rudehen, near Tehran, the prevalence of IPIs was 32.7% in the general population (15). The prevalence of IPIs in a healthy population in different cities and rural areas of Iran fluctuates vastly from 4.7 to 56% (4, 5). In a study conducted among inhabitants of Rudbar-e Jonub County, southeast of Kerman province, the overall prevalence of IPIs was reported 34.2% (16). In addition, the higher remarkable prevalence of 35.1%, 48.8%, and 56% were reported in rural population and nomadic tribes of Hamadan, Hormozgan and Chaharmahal and Bakhtiari provinces, respectively (5, 17, 18).

The results of the mentioned studies indicate the fact that the IPIs especially, protozoan parasites still have considerable prevalence in rural areas and more sanitary infrastructures and controlling programs are required.

In our study, specific diagnostic methods such as trichrome and Ziehl-Neelsen staining as well as stool culture were administrated to enhance the accurate diagnosis. In addition, direct wet-mount slides in diarrhetic specimens

makes the diagnosis of motile trophozoites trustworthy (19). However, in most studies, specific diagnostic methods such as acid-fast staining and stool culture have not been administrated for diagnosis of coccidiosis and strongyloidiasis. The importance of this issue will become perceptible regarding immunocompromised patients as untreated infections lead to fatality (20). The prevalence of *Cryptosporidium* spp. in our study is consistent with a similar study conducted in Tehran, in which *Cryptosporidium* and *Isospora* oocyst in immunocompromised patients reported 0.9% and 1.1%, respectively (21). Hemodialysis and cancer patients due to impaired immune system are prone to various enteric infections and parasites such as *Cryptosporidium* spp. and *Blastocystis* spp. and in our study, the mentioned protozoans were reported as the most prevalent agents of intestinal complications (22, 23).

The most predominant infection in our study was due to *Blastocystis* spp. and *G. lamblia* like the most of aforementioned studies, and as most of the infected individuals are asymptomatic patients who carry parasites and contaminate water and food sources widely. Besides, the viability of cysts in the environment improves their chance of infecting a new host (24, 25).

In the current study, two samples contained *E. histolytica*/*E. dispar*/*E. moshkovskii* protozoans. Although one of the specimens was dysenteric and hematophagous trophozoites were observed in both direct and trichrome stained slides, as long as molecular identification has not been conducted for them, it was impossible to distinguish them at the species level. In addition, other protozoa such as *E. coli*, *I. bütschlii*, and *C. mesnili* were identified in our study which their presence indicates the fecal-oral transmission in the host, and is a suitable indicator for the general assessment of the hygiene status, although they cause no intestinal diseases.

Among possible risk factors investigated in this study, the stool consistency and the status

of immune system were found to have a significant association with intestinal parasitic infections ($P < 0.05$). There was no association between the infection and sex, age group, occupation, education level, and diabetes. Regarding the educational levels of patients, although no significant association was found between the prevalence of IPIs and level of education, the results of this study indicate that as the level of literacy increases, the rate of IPIs decreases. Educated people are more aware of the transmission of IPIs and they may apply the necessary measurements to avoid the infection.

In terms of helminthiasis, only three samples were contained parasitic ova of *A. lumbricoides*, *H. nana* and, *D. dendriticum*. It is undeniable that in recent decades the prevalence of helminthic infections in Iran has dramatically plunged but those with direct fecal-oral transmission such as *H. nana* are common in some areas (26). Although the prevalence of *H. nana* was reported merely 0.2% in the current study, the zoonotic aspects of *H. nana* and the role of synanthropic rodents in spreading the parasite in rustic areas are inevitable (27). Furthermore, the reported *D. dendriticum* infection in this study was revealed a spurious infection but human cases of dicrocoeliasis in northern areas of Iran have been recorded (28).

In terms of limitations, stool specimens were collected once from each patient whereas for standard diagnosis of intestinal parasites, at least three samples in three alternate days are necessary. Additionally, because of financial limitations and limited amenities, molecular methods for identification of *Entamoeba histolytica*/*E. dispar*/*E. moshkovskii* complex were not done. As the specimens of this study collected in a hospital, the results could not be generalized to the whole society.

Conclusion

The present study revealed considerable IPIs among patients referred to in a referral hospi-

tal in Tehran province, Iran. Protozoan infections prevalence was remarkably higher than helminth infections and the intestinal parasite *Blastocystis* spp. was recognized as one of the most significant causes of infection especially in immunocompromised patients. IPIs infection could cause serious clinical complications in immunocompromised patients. Our findings showed a considerable prevalence of IPIs among immunocompromised patients. Besides taking into account the presented epidemiological information, health authorities should adopt a new concept, including raising public awareness about IPIs transmission routes, health education, and treatment of infected individuals. Also, periodic stool examinations should be recommended as a part of a routine medical check-up in immunocompromised patients.

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Conflict of interest

The authors assert that they have no competing interest.

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