PREVALENCE, RISK FACTORS AND SYMPTOMS ASSOCIATED TO INTESTINAL PARASITE INFECTIONS AMONG PATIENTS WITH GASTROINTESTINAL DISORDERS IN NAHAVAND, WESTERN IRAN

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SUMMARY

We studied the prevalence of intestinal parasites (IPs), their risk factors and associated symptoms among patients with gastrointestinal disorders. A total of 1,301 participants aged 22 days-90 years were enrolled in this study. We used a structured questionnaire to obtain socio-demographic and stool examination to investigate intestinal parasite infections. Data analysis was performed using SPSS. The overall prevalence of intestinal parasites (IPs) was 32.2% (419/1,301). Three hundred and fifty nine cases/1,301 (27.6%) were infected with a single parasite and 60/1,301 cases (4.6%) presented polyparasitism. The most common IP was Blastocystis sp. 350/1,301 (26.9%), followed by Entamoeba coli 381/1,301 (2.92%), Giardia lamblia 301/1,301 (2.3%) and Cryptosporidium spp. 171/1,301 (1.3%). Regarding the socio-demographic variables, educational status ($p = 0.001$), contact with domestic animals and soil ($p = 0.02$), age above 15 years ($p = 0.001$) and seasons ($p = 0.001$) were significantly associated to intestinal parasitic infections. Concerning clinical characteristics, the presence of IPs was significantly associated to diarrhea ($OR = 1.57; CI 95\% = 1.24-1.98; \ p < 0.001$) and dysentery ($OR = 1.94; CI 95\% = 1.03-3.66; \ p < 0.04$). Our findings suggest that IPs are one of the main causal agents of gastrointestinal disorders. Improving the knowledge on local risk factors such as poverty, low level of education, poor sanitation, contact with soil and contact with domestic animal is warranted.

KEYWORDS: Gastrointestinal disorders; Parasitic diseases; Intestinal parasites; Iran.

INTRODUCTION

Globally, intestinal parasitic infections (IPIs) are one of the main causes of human morbidity and mortality especially in developing countries in which public health standards are not as high as in developed countries. IPs are associated with climate conditions, poor sanitation, contact with soil and contact with domestic animals and cultural habits. The soil-transmitted helminths (STH) Ascaris lumbricoides, Trichuris trichiura, and hookworms are the most common causes of IPs and affect more than 2 billion people worldwide. Other common helminths in IPIs are Entamoeba histolytica (affecting 50 million people), Giardia lamblia (affecting 200 million people), and also Cryptosporidium spp., Cystoisospora belli and Microsporidia spp. in immune compromised patients.

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Gastrointestinal disorders refer to diseases involving the gastrointestinal tract such as the ones compromising the stomach, small and large intestines and rectum. They are associated to many symptoms such as diarrhea, abdominal/stomach pain, nausea and vomiting, weight loss, indigestion/dyspepsia, bloating and constipation. It has been shown that intestinal parasites have a high prevalence in patients with gastrointestinal disorders and are related to the above mentioned symptoms.

In Iran, despite a sustained improvement of the sanitary conditions that has happened during the last three decades, IPs are still highly prevalent.

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prevalent, especially among rural communities and small towns where residents survive thanks to agriculture and pastoral culture23.

The main purpose of this study was to assess the prevalence and clinical manifestations of IPIs in patients with gastrointestinal disorders in Nahavand County, Western Iran, and investigate the association of IPIs with socio-demographic factors.

MATERIAL AND METHODS

Study areas

This cross sectional study was carried out from April to September 2014 in Nahavand County, (34°14'N, 48°14'E), Western Iran. The average annual rainfall in the area is 376 mm and the average temperature ranges from -0.5 to 25.6 °C.

Population, studied variables and ethical issues

The Nahavand County has 196 villages and the population is estimated at 181,711 people in 112,297 families23. The majority of inhabitants are farmers and ranchers especially those living in rural areas and small towns around the Nahavand city.

The study population was composed of individuals with gastrointestinal disorders who presented one or more symptoms (abdominal pain, diarrhea, dysenteric diarrhea, crumping, nausea and vomiting, constipation) and were referred to medical laboratories in the Nahavand County. In order to calculate the sample size, we used a reference prevalence of 27% in patients with gastrointestinal disorders, a 95% level of confidence and 5% of marginal error20. The sample size required was 1,038 people, but for more accuracy 1,301 samples were collected.

A written consent was obtained from each participant, and then the participants were asked to respond to a questionnaire containing basic individual information (sex, age, residence), socio-demographic data (education, occupation), health status (water supply, contact with domestic animal and soil) and clinical symptoms such as abdominal pain (defined as a diffuse pain in the abdominal cavity), bloating (any abnormal general swelling or fullness), diarrhea (at least three loose or liquid stools per day), stomach pain (cramps or a dull abdominal ache), nausea and vomiting (the sensation or act of forcible and frequent emptying of the stomach) was filled by each participant. The study has enrolled individuals with gastrointestinal disorders that did not use any anti-parasitic drugs for one week prior to the study, and agreed to participate.

All the procedures were approved by the Ethics Committee of the Shahid Beheshti University of Medical Science (SBMU), Iran, before the beginning of the study. All the study participants were informed about the procedures and written informed consents were obtained from all of them prior to the sample collection.

Fecal sample collection and analysis

In this study, a simple random sampling technique was used. All the participants were given a clean and dry plastic collector, pre-labeled with their name and an identification number. Collected samples were sent to the medical laboratory located at the Ayatollah Alimoradian hospital, as soon as possible. All the samples were physically examined and screened for intestinal parasites by the following methods: I. Direct smear using the normal saline (0.85% NaCl solution), II. Lugol’s iodine staining, III. Formalin-ether concentration technique, IV. Modified Ziehl-Neelsen acid-fast technique for the detection of Cryptosporidium spp. oocysts, V. Trichrome staining technique for the detection of intestinal protozoa such as Giardia lamblia and Entamoeba histolytica26,27. Prepared slides were examined under light microscopy (Zeiss, Germany) with 10×, 40× and 100× magnification.

Statistical analysis

Data analysis was carried out using the Statistical Package for the Social Sciences software version 19 (SPSS, Chicago, IL, USA). For the descriptive data, frequencies and percentages were used to describe the characteristics of the participants, including the prevalence of IPIs according to the residence, education, occupation, age and gender. The Pearson’s Chi-square (Chi2) test and the Fisher’s exact test were used for comparison of proportions of IPIs between different subgroups and odds ratios (OR) and 95% confidence intervals (CI) were used to associate the variables. A p value < 0.05 was considered statistically significant. A logistic regression was used to identify a potential contribution of each of the variables for the acquisition of intestinal parasite infections.

RESULTS

Socio-demographic characteristics

A total of 1,301 participants (52.4% male, 47.6% female) aged 22 days-90 years with a mean age of 26.31 ± 22.22 were enrolled in this study. Six hundred and eighty-three subjects (52.5%) lived in rural areas and 618 subjects (47.5%) lived in urban areas. Six hundred and eighteen (47.5%) had access to clean treated potable water and 683 (52.5%) used untreated water (river, well, rain). Four hundred and eighty-five (37.3%) reported a frequent contact with domestic animals or soil. The percentage of participants categorized as government employees, farmer, student, house wife, other and children < 17 years old were 7.5%, 11.4%, 8.2%, 20.1%, 12.8% and 40%, respectively.

Prevalence and distribution of intestinal parasitic infections

Overall, 32.2% (419/1,301) of the participants were infected with at least one intestinal parasite species. The prevalence rate among males (231/682 cases; 33.9%) was slightly higher than in females (188/619 cases; 30.4%). The most common IP was Blastocystis sp. (350 participants, 26.9%), followed by Entamoeba coli (38 participants, 2.92%), Giardia lamblia (30 participants, 2.3%) and Cryptosporidium spp. (17 participants, 1.3%). The prevalence of other IPs is presented in Table 1.

Three hundred and fifty nine participants (27.6%) were infected with a single parasite and in 60 cases (4.6%) polyparasitism was observed. The prevalence of intestinal helminths in this study (5 cases, 0.38%) was significantly lower than intestinal protozoa (416 cases, 32%) (p < 0.001).

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Risk factors for intestinal parasitic infections (IPIs)

The results of the univariate analysis to evaluate the risk factors associated to IPIs and socio-demographic, environmental and personal hygiene features are presented in Table 2. Among the selected socio-demographic variables, some were significantly associated to IPIs: educational status ($p = 0.001$), contact with domestic animals and soil ($p = 0.02$), age above 15 years ($p = 0.001$) and seasons ($p = 0.001$). However, regarding other socio-demographic characteristics such as sex, residence, water supply, and occupation, they did not show any association with the finding of parasites (Table 2). Moreover, according to the period of the year (season), it was observed that the prevalence of IPIs in July, August and September was significantly higher than in April, May and June ($p = 0.001$).

Using a binary logistic regression analysis, variables such as age, sex, residence, educational level, occupation, contact with domestic animals and soil, as well as the water supply status were added to the model. Then, we used a forward logistic regression to perform the analysis. Among the added variables, only the age (OR = 2.07; CI $95\% = 1.4-2.8$; $p < 0.001$) and the seasons (OR = 1.16; CI $95\% = 1.28-2.13$; $p < 0.001$) were identified as major socio-demographic determinants of IPIs.

Clinical features associated to IPIs

Concerning clinical characteristics, the presence of IPIs was significantly associated to diarrhea (OR = 1.57; CI $95\% = 1.24-1.98$; $p < 0.001$), dysentery (OR = 1.94; CI $95\% = 1.03-3.6$; $p < 0.04$) and constipation (OR = 0.298; CI $95\% = 0.141-0.633$; $p < 0.001$). The frequencies of other clinical characteristics including the presence of abdominal pain, nausea and vomiting, stomach pain and bloating did not show significant differences between parasite-infected and non-infected participants (Table 3). According to these data diarrheic disease may result from IPIs in patients with gastrointestinal disorders.

DISCUSSION

The results of the present study have indicated that approximately one third of patients with gastrointestinal disorders were infected with one or more IPIs. This rate is slightly higher than the results of a previous study conducted in similar populations in Zahedan, where the prevalence of IPIs was 27.3%. The differences of results can be attributed to peculiarities of the studied regions. Zahedan and Nahavand have poorer hygiene conditions and lower socio-economic status compared to the areas studied by Zebardast et al. It is well established that providing a basic infrastructure and suitable education are effective tools to decrease the prevalence of IPIs. In Iran, some areas located at the western, southern and eastern regions of the country are less developed in comparison with areas close to the capital and people live in traditional-built houses, although using untreated water from wells or rivers.

The findings of our study showed that protozoan infections (32%)}
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In recent years, there has been a decrease in the incidence of intestinal helminth infections such as ascariasis, strongyloidiasis and hookworm infections in Iran\textsuperscript{20,30}. This decline is probably due to general improvements in sanitation in Iran. However, some helminth parasites with direct fecal-oral transmission, such as \textit{Hymenolepis nana} and \textit{Enterobius vermicularis} remain common in many parts of the country\textsuperscript{30}. In addition, among the five cases of helminth infections that were found in this study, \textit{Hymenolepis nana} and \textit{Enterobius vermicularis} were observed in one and three cases, respectively. Moreover, other authors in Iran and other countries have also found higher frequencies of protozoan compared to helminth infections\textsuperscript{31-33}.

The findings of the present study showed that \textit{Blastocystis} sp. was the most common IP. This finding is in agreement with a previous study carried out in a similar population\textsuperscript{22}. The pathogenicity of \textit{Blastocystis} is still under debate. Some studies have strongly suggested that it is pathogenic and can be associated to several disorders\textsuperscript{34,35}, whereas other studies have reported that it is a commensal organism rather than a pathogenic one, and it is probably not responsible for clinical symptoms\textsuperscript{36}. The Centers for Disease Control and Prevention (CDC) suggests that \textit{Blastocystis} treatment is necessary when there are no other causes for the occurrence of diarrhea or gastrointestinal disorders\textsuperscript{37}. Previous clinical studies have shown that abdominal pain

### Table 2

Univariate analysis of risk factors associated with intestinal parasitic infection among individuals with gastrointestinal disorders in Nahavand County, western Iran (n = 1301)

| Variables                              | Positive n (%) | Negative n (%) | OR               | CI\textsubscript{95%} Lower | CI\textsubscript{95%} Upper | p-value |
|----------------------------------------|----------------|----------------|------------------|-----------------------------|-----------------------------|---------|
| Gender                                 |                |                |                  |                             |                             | 0.177   |
| Male                                   | 231 (33.9)     | 451 (66.1)     | 1.174            | 0.93                        | 1.483                       |         |
| Female                                 | 188 (30.4)     | 431 (69.6)     | Reference        |                             |                             |         |
| Age (Year)                             |                |                |                  |                             |                             | <0.001  |
| ≤15                                    | 120 (22.7)     | 408 (77.3)     | Reference        |                             |                             |         |
| 16-30                                  | 99 (38.5)      | 158 (61.7)     | 2.13             | 1.542                       | 2.944                       | <0.001  |
| 31-45                                  | 85 (37.9)      | 139 (62.1)     | 2.079            | 1.483                       | 2.915                       | <0.001  |
| 46-60                                  | 74 (39.2)      | 115 (60.8)     | 2.188            | 1.532                       | 3.123                       | <0.001  |
| >60                                    | 41 (39.8)      | 62 (60.2)      | 2.248            | 1.442                       | 3.505                       | <0.001  |
| Educational Status                     |                |                |                  |                             |                             | <0.001  |
| Pre school                             | 87 (21.2)      | 316 (78.4)     | Reference        |                             |                             |         |
| Illiterate                             | 54 (38.8)      | 85 (61.2)      | 2.354            | 1.552                       | 3.572                       | <0.001  |
| Primary school                         | 101 (35.1)     | 187 (64.9)     | 2.002            | 1.424                       | 2.814                       | <0.001  |
| Secondary school                       | 111 (36.9)     | 190 (63.1)     | 2.165            | 1.549                       | 3.027                       | <0.001  |
| College and above                      | 66 (38.8)      | 104 (61.2)     | 2.352            | 1.591                       | 3.475                       | <0.001  |
| Residence                              |                |                |                  |                             |                             | 0.474   |
| Rural                                  | 225 (32.9)     | 458 (67.1)     | Reference        |                             |                             |         |
| urban                                  | 194 (31.4)     | 424 (68.6)     | 0.918            | 0.727                       | 1.159                       |         |
| Occupation (≥17 years old)             |                |                |                  |                             |                             | 0.791   |
| Gov’t employer                         | 33 (34)        | 64 (66)        | Reference        |                             |                             |         |
| Farmer & shepherd                      | 62 (41.9)      | 86 (58.1)      | 1.398            | 0.821                       | 2.38                        | 0.217   |
| Student                                | 41 (38.3)      | 66 (61.7)      | 1.205            | 0.679                       | 2.137                       | 0.524   |
| House wife                             | 103 (39.3)     | 159 (60.7)     | 1.256            | 0.771                       | 2.046                       | 0.359   |
| Other                                  | 62 (37.3)      | 104 (62.7)     | 1.156            | 0.684                       | 1.954                       | 0.588   |
| Contact with domestic animal & soil    |                |                |                  |                             |                             | 0.021   |
| yes                                    | 175 (36.1)     | 310 (63.9)     | 1.323            | 1.043                       | 1.68                        |         |
| No                                     | 244 (29.9)     | 572 (70.1)     | Reference        |                             |                             |         |
| Water supply status                    |                |                |                  |                             |                             | 0.590   |
| Untreated (river, well, rain water)    | 225 (32.9)     | 458 (67.1)     | 1.067            | 0.842                       | 1.352                       |         |
| Treated pipe water                     | 194 (31.4)     | 424 (68.6)     | Reference        |                             |                             |         |
| Seasons                                |                |                |                  |                             |                             | <0.001  |
| spring                                 | 139 (25.8)     | 399 (74.2)     | Reference        |                             |                             |         |
| summer                                 | 280 (36.7)     | 483 (63.3)     | 1.664            | 1.305                       | 2.121                       |         |

n: number; OR: odds ratio; Reference: The subgroup is considered as baseline

were more common compared to helminthes (0.38%). In recent years, there has been a decrease in the incidence of intestinal helminthes infections such as ascarasis, strongyloidiasis and hookworm infections in Iran\textsuperscript{30,31}. This decline is probably due to general improvements in sanitation in Iran. However, some helminthes parasites with direct fecal-oral transmission, such as \textit{Hymenolepis nana} and \textit{Enterobius vermicularis} remain common in many parts of the country\textsuperscript{30}. In addition, among the five cases of helminthes infections that were found in this study, \textit{Hymenolepis nana} and \textit{Enterobius vermicularis} were observed in one and three cases, respectively. Moreover, other authors in Iran and other countries have also found higher frequencies of protozoan compared to helminthes infections\textsuperscript{31-33}.
and diarrhea are two of the main symptoms among *Blastocystis*-positive patients\(^{22,24}\). Excepting for *Blastocystis*, *Giardia* lamblia (2.3%) and *Cryptosporidium* spp. (1.3%) were the most pathogenic protozoan parasites. In a recently published study that has been carried out in Iran, 4.73%, 2.30% and 0.06% of the studied population had *Blastocystis*, *Giardia* lamblia and *Cryptosporidium* spp., respectively\(^{19,20}\). These zoonotic protozoa are a great threat to public health in Iran. They can be transmitted through contaminated water and food. Animal wastes and farming practices are important sources of water and food contamination. Thus it is necessary to keep animals away from water and food sources that are used by humans.

Considering the symptoms, the results of our study have shown that there was a significant association between the presence of IPs and the finding of diarrheal stools. Diarrhea is one of the most reported symptoms due to IPs. *Entamoeba histolytica* can be the cause of severe dysentery, colitis, constipation, tenesmus and extra-intestinal complications\(^8\). *Giardia lamblia* is responsible for acute diarrhea, steatorrhea and malabsorption\(^9\). *Cryptosporidium* spp. infections has been increasingly recognized as a cause of diarrhea in both immune competent and immune compromised patients, as well as in children\(^{19,20}\). Although in our study, most of referred participant were infected with nonpathogenic protozoan parasites.

In this study, the described clinical symptoms are common complications induced by IPs as well as by bacterial, viral or fungal agents. All the enrolled participants were examined by a physician, but we did not have access to their previous laboratory and medical records that would allow a more accurate interpretation on whether the symptoms were due to IPs or to bacterial infections and/or malignancies.

Worldwide, previous studies have shown that there are several risk factors associated to the high prevalence and incidence of IPs including untreated water supply, age, poor sanitation, low family income, low level of education, low level of parental education, eating unpeeled/unwashed vegetables and fruits, contact with livestock or pets and poor personal hygiene\(^{40,44}\). In our study, variables such as age above 15 years, low level of education, low level of parental education, eating unpeeled/unwashed vegetables and fruits, contact with livestock or pets and poor personal hygiene were considered as baseline risk factors of IPs. Results of the current study have shown that adult patients (aged \(\geq 15\) years) are almost twice as likely to be infected with IPs. Outdoor activities practiced by adults and a higher exposure to sources of IPs may explain the significantly higher prevalence of IPs among these participants. Moreover, another reason for this finding is the higher prevalence of *Blastocystis* infections according to the increasing age\(^{23,24}\). In our study, the prevalence of IPs was slightly higher in males (33.9%) than in females (30.4%), although there was no statistically significant difference \((p = 0.197)\). This result is in agreement with previous studies in Iran\(^{23,44}\). Our findings have indicated that the season is a significant predictor of IPs. The Nahavand County is located in a mountainous

### Table 3

| Clinical features | Examined individuals n | Infected with intestinal parasites n (%) | OR | CI\(_{95}\%) | p-value* |
|-------------------|------------------------|----------------------------------------|----|------------|---------|
|                   |                        |                                        |    | Lower      | Upper   |
| Abdominal pain    |                        |                                        |    |            |         |
| Yes               | 980                    | 322 (32.9)                            | 1.130 | 0.860 | 1.485 | 0.409 |
| No                | 321                    | 97 (30.2)                             | Reference |          |          |         |
| Nausea or vomiting|                        |                                        |    |            |         |
| Yes               | 58                     | 23 (39.7)                             | 1.406 | 0.820 | 2.411 | 0.250 |
| No                | 1243                   | 396 (31.9)                            | Reference |          |          |         |
| Stomach pain      |                        |                                        |    |            |         |
| Yes               | 522                    | 165 (31.6)                            | 0.959 | 0.756 | 1.217 | 0.752 |
| No                | 779                    | 253 (32.6)                            | Reference |          |          |         |
| Diarrhea          |                        |                                        |    |            |         |
| Yes               | 585                    | 221 (37.8%)                           | 1.573 | 1.245 | 1.988 | <0.001 |
| No                | 716                    | 198 (27.7%)                           | Reference |          |          |         |
| Dysentery         |                        |                                        |    |            |         |
| Yes               | 40                     | 19 (47.5%)                            | 1.948 | 1.035 | 3.663 | 0.04 |
| No                | 1261                   | 400 (31.7%)                           | Reference |          |          |         |
| Constipation      |                        |                                        |    |            |         |
| Yes               | 62                     | 8 (12.9%)                             | 0.298 | 0.141 | 0.633 | <0.001 |
| No                | 1239                   | 411 (33.2%)                           | Reference |          |          |         |

OR: odds ratio; n: Number; Reference: The subgroup is considered as baseline; *Chi 2 test was used.
area (far from the sea) and its weather in summer (July, August and September) is little to moderately warm (20-35 °C), whereas in spring (April, May and June) the weather is colder (10-18 °C). Another explanation to the higher prevalence of IPs during the summer could be the existence of more agricultural practices. This finding is in agreement with previous studies that have indicated that endemic regions have seasonal variations in the prevalence of IPs.46-47.

There were some limitations in this study. Firstly, stool samples were collected only once, so that it is likely that the analysis of three consecutive stool samples could have increased the diagnostic sensitivity. Secondly, there is a possibility that some of the participants have decided to take antiparasitic drugs prior to the stool sampling, considering that metronidazole, mebendazole and albendazole can be purchased without prescription in Iran. Thirdly, due to the lack of facilities and financial funding we could not use all the laboratory available techniques, such as the Baerman technique that is used to find larvae of worms including strongyloides or adhesive tape to detect Enterobius vermicularis. The lack of these procedures might explain the absence of helminth infections in this study. In addition, it is likely that the prevalence of some protozoa such as Entamoeba histolytica and Blastocystis could be even higher if culture techniques had been used.

In conclusion, the findings of this study have indicated that IPs are important causes of gastrointestinal disorders. Moreover, despite a significant reduction in the prevalence of intestinal helminths, the prevalence and incidence of intestinal protozoa are still a major public health concern in Iran. Thus, effective control programs to reduce the prevalence and incidence of IPs should be considered in public health policies. Considering that the incidence of IPs are significantly associated to poverty, lower levels of education, poor environmental sanitation, contact with soil and animals, there is an urgent need that these factors are effectively addressed.

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CONFLICT OF INTEREST

The authors do not have any conflict of interest to report regarding this manuscript.

AUTHORS’ CONTRIBUTION

H. Kiani and A. Haghighi designed the study, performed the research, analyzed data and, evaluated clinical records. They also collaborated to the manuscript writing. A. Rostami analyzed data and collaborated to the manuscript writing and revision. S. J. Seyyed Tabaei and N. Zebarast performed the laboratory assays and evaluated the clinical records. A. Solgi helped to collect samples and clinical data. All the statistical analysis was performed by E. Azargashb.

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