



## Frequency of Intestinal Parasites in Patients with Malignancy in Ardabil Province, Northwest Iran

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### ABSTRACT

**Background:** Despite continued and comprehensive planning of the world health organization (WHO), intestinal parasitic infections are a serious public problem in developing countries. Due to the high prevalence of cancers in Ardabil province and subsequently the high possibility of intestinal parasitic infections among the people, the aim of this study was to assess the frequency of intestinal parasites in patients with malignancy in this area.

**Methods:** In this cross-sectional study, 100 fecal samples were collected from patients with cancer during February to September 2015. The specimens were examined for intestinal parasites using direct smear, formal-ethyl acetate concentration, agar plate culture and Ziel-Neelsen staining technique.

**Results:** The overall frequency of intestinal parasitic infections in studied cancer patients was 10%. The infection rates of detected intestinal parasites were *Cryptosporidium* spp. oocyst 4%, *Blastocystis hominis* 3%, *Giardia lamblia* 2% and *Taenia* spp. 1%.

**Conclusion:** Despite the low frequency of intestinal parasites, there is a need to screen cancer patients for some important parasitic infections such as *Cryptosporidium* spp. and *Strongiloides stercoralis* because of irreparable effects of those parasites on the patients and to increase awareness among clinicians regarding the occurrence of parasitic infections in these patients.

## 1. Introduction

Parasitic diseases cause major morbidity and mortality throughout the world. Among them intestinal parasites are widely prevalent and cause significant medical and public health problems.

World Health Organization reported that about 3.5 billion people are affected worldwide and 450 million are ill as a result of intestinal parasitic infections [1, 2]. The prevalence of these parasites

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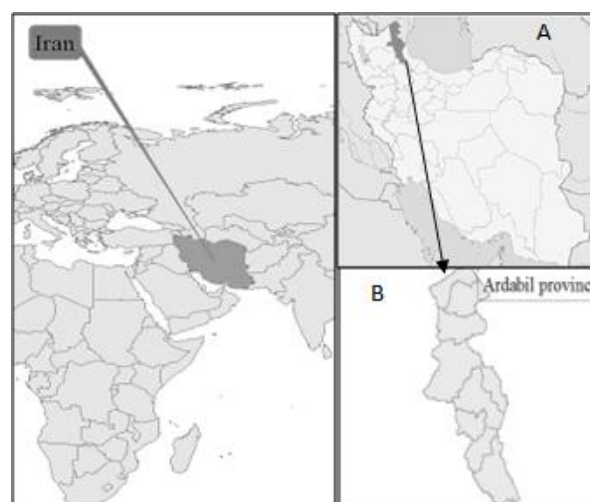
in developing countries varies depending on the environmental, economic, regional, political, cultural and social factors. In such countries, lack of access to healthcare, inadequate sanitation and malnutrition increase susceptibility to infection [3]. The common transmission ways of these infections are contaminated food or drinking water, and they may also spread from person to person by fecal-oral contact [4]. Furthermore, the role of immune system in acquisition of these infections is inevitable. Immune system plays an important role in modifying the establishment of infection, controlling disease, limiting the severity and dissemination of the disease, and assisting in clearance or control of the parasite [5]. However, in cancer and AIDS patients the severity and rate of parasitic diseases progression are compounded. During the past decades major advances in medicine have enabled patients with cancer to live prolonged productive lives though the result of these achievements is a main deficiency of the host -defense mechanisms that puts the patients at the risk of parasitic infections. Cancer individuals are more susceptible to acquire infection, and after exposure they show more severe disease once the infection is established. Infection disseminates rather than being localized and patients are unable to clear parasites leading to greater morbidity and mortality in these patients [6]. In recent years, with increasing the number of susceptible hosts and use of novel immunosuppressive drugs the frequency of parasitic infections has increased in such patients [7]. About 340 parasitic species infect more than three billion people worldwide.

Some of the common parasites found in immunosuppressed patients are *Giardia lamblia*, *Entamoeba histolytica*, *Strongyloides stercoralis*, *Cryptosporidium parvum*, *Cyclospora cayatanensis*, *Isospora belli*, and *Microsporidia* spp. In spite of recent increases in people with various cancers in Iran, there are only very few studies about the prevalence of parasitic infections in this population [8-10]. This study was carried out in order to investigate the frequency of intestinal parasites in fecal samples of cancer patients in Ardabil province, northwest Iran.

## 2. Material and Methods

### 2.1. Study area

Ardabil province, a mountainous land, is located in the north west of Iran and has an area of 18,011 km<sup>2</sup> with a population of 1.6 million people, where 46% of people live in urban areas. Its coordinates are 38°15'05"N and 48°17'50"E. This province is divided into two geographical parts, mainly mountainous and 1/3 as plateau (Fig. 1).



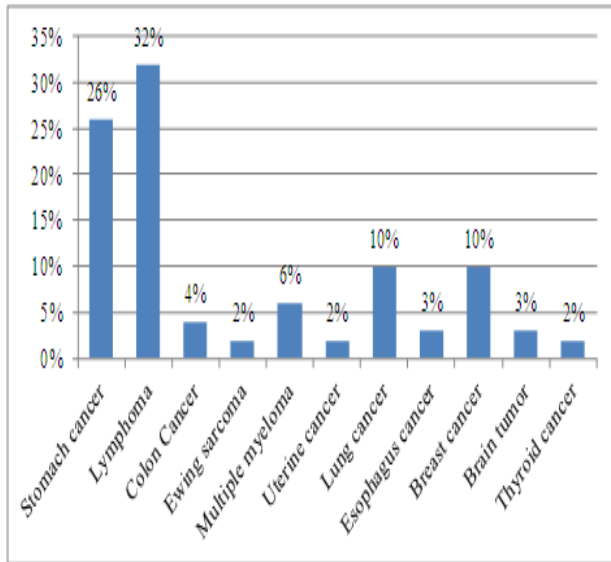
**Fig. 1:** Geographic location. (A) Location of Ardabil province in Iran map (B) Map of Ardabil province and its cities.

### 2.2. Sample collection

The current cross-sectional study was conducted from February to September 2015. During the study period a total of 100 samples were collected from cancer individuals (Fig. 2). Patients were first requested to complete a questionnaire. The questionnaire included the following information: age, sex, location, level of education, occupation, kind of disease, history of contact with animals, history of anti-parasitic drugs and drinking water sources. All patients had weakened immune system due to chemotherapy.

The fecal samples were collected freshly in stool specimen containers, which were properly labeled.

The samples were transferred to the Department of Parasitology, School of Medicine, Ardabil University of Medical Sciences, and were stored at 4°C for further microscopic examination.



**Fig. 2:** Distribution of the different types of cancer in patients participated in the study.

### 2.3. Parasitological procedures

All specimens were microscopically examined with saline and iodine wet mounts for detection of protozoan oocysts, cysts, helminthic eggs and larvae before concentration using sieving and flotation. To diagnose *Strongyloides stercoralis* larvae in stool samples nutrient agar culture was performed. In the agar plate method, 2 grams of specimens were placed in the center of an agar medium in a 9cm plastic Petri dish, then covered and sealed round the edge with adhesive tape, and incubated at room temperature (about 28°C) for 48h. Thereafter, plates were examined under a compound microscope with a green filter at 40 magnification. Fecal samples were collected in 10% formaldehyde and processed by Formalin-Ethyl Acetate sedimentation concentration methods. First, fecal suspension was passed through the gauze placed over a 15ml conical centrifuge tube. Second, 3ml of ethyl acetate was added and shaken vigorously in an inverted position for 30 seconds and then centrifuged at

2000 rpm for 1 minute. Finally debris from the top of the tube was removed and several drops of concentrated specimen were transferred to the slide. Each sample was microscopically examined. Identification of parasites was carried out by morphological characteristics. In addition, samples were screened by means of a modified Ziehl-Neelsen method. A thin smear was made of the pellet obtained from the Sedimentation concentration methods and stained by the modified Ziehl-Neelsen technique.

After staining, the smear was examined at 100X magnification for detection of *Cryptosporidium* spp. oocysts in stool samples [11]. A sample was recorded as positive if at least one parasitic form was observed.

### 2.4. Statistical analyses

Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) software program for Windows version 16.0. The differences between the groups of categorical variables were analyzed by chi-square test.

The level of statistical significance was set at  $p < 0.05$ .

## 3. Results and Discussion

The results confirmed the overall frequency rate of parasitic infections as 10% (10 patients).

Stool cultures on nutrient agar plates for detection of *Strongyloides stercoralis* larvae showed no infection. The highest rate of infection was related to *Cryptosporidium* spp. oocysts 4% (4 patients) and frequencies of other parasites were as follows: *Blastocystis hominis* 3% (3 patients), *Giardia lamblia* 2% (2 patients) and *Taenia* spp 1% (1 patient) respectively (Table 1).

Among the patients who participated in the study, 41% were female and 59% male and the study showed that there was no significant association between sex and parasite infection ( $p = 0/839$ ). Age distribution of the patients showed that the majority of patients belonged to the age group of 40-60 and no significant association was observed with parasite infection ( $p = 0/751$ ). In

case of educational status, most patients were uneducated (44 %) and the results did not exhibit any significant relation ( $p = 0/637$ ).

**Table 1:** The number of parasites found in cancer individuals who participated in the study.

Detected parasite	No. of parasites in patients with malignancy	Proportion (%)
<i>Taenia spp</i>	1	1%
<i>Blastocystis hominis</i>	3	3%
<i>Giardia lamblia</i>	2	2%
<i>Cryptosporidium spp</i>	4	4%
Total	10	10%

In this study the majority of patients (32%) suffered from lymphoma, but a significant association between positive cases and type of cancer was not observed ( $p = 0/637$ ). In addition, the results of the study showed that infection with intestinal parasites is not statistically significant related to residence ( $p < 0.288$ ) and drinking water source ( $p = 0/094$ ) (Table 2). Healthy immune system protects the body against infections especially parasitic infections but in cancer patients, suppressed immune system by chemotherapy predisposes them to protozoan and helminthic infections which are responsible for considerable morbidity and mortality. These infections have been variably reported among cancer individuals from various parts of Iran; however, data in Ardabil province has been limited. In this study, we evaluated the frequency of intestinal parasites in cancer individuals.

Intestinal parasitic infections showed low prevalence in our population with an overall rate of 10%. This frequency is lower than those previously reported in other studies conducted in Nepal (36.9%) and in India (53.02%) [6, 12].

This discrepancy could be explained by health condition of study area and the sample size.

According to our findings, 4% of patients tested positive for *Cryptosporidium* spp. oocysts. Our results were similar to the studies conducted in Saudi Arabia (8%), Colombia (3.6%) and Turkey (5.2 %) [13-15]. Cryptosporidiosis represents a major public health problem. This infection has been reported worldwide, and its prevalence varies in different regions [16]. In developing countries, Cryptosporidiosis remains a clinically significant opportunistic infection in immunodeficiency patients, causing potentially life-threatening diarrhea [17]. Previous studies in Iran demonstrated that 0.3- 3% of immunodeficiency patients were infected with this pathogen [18]. Despite Ziehl-Neelsen staining is gold standard detection method, it seems that the new methods such as ELISA technique could be used as a complementary test to yield better detection results for this pathogen [19]. Many studies have been carried out on the frequency of intestinal parasites among patients with malignancies in Iran and the results revealed *Giardia lamblia* and *Blastocystis hominis* frequencies between 1 to 8% and 0.4- 5.1% respectively [8, 10]. In this study our results were in line with studies discussed and frequency rates of *Blastocystis hominis* and *Giardia lamblia* were 3% and 2% respectively [20, 21]. The results of stool cultures on nutrient agar plates showed no infection with *Strongyloides stercoralis* larvae. *Strongyloides stercoralis* is an intestinal nematode of humans with a worldwide distribution, affecting about 100 million humans [22, 23]. Although most infected individuals are asymptomatic all patients are at risk of developing severe complicated *Strongyloidiasis*, particularly if they are immunosuppressed by chemotherapy like cancer patients. In our study, *Taenia* spp were isolated from 1 % of patients. This result is in accordance with other studies conducted in Nigeria and India [24, 25]. To our knowledge, this is the first study of parasitic intestinal infections in cancer individuals in Ardabil and it shows the importance of intestinal pathogens for the screening process for some opportunistic parasites in these populations.

#### 4. Conclusion

Regarding the fact that enteric parasites can cause serious complications and interfere with disease control, the screening policy for intestinal parasites should continue. In spite of the low frequency of parasitic intestinal infection in our study, a prospective case control study with more participants would perhaps help us to present important epidemiological information between

cancer individuals and intestinal parasites in this region. We recommend larger and well-designed studies to evaluate the association of parasitic infections with other immune com promising infections. Stool parasite examination is recommended before chemotherapy for cancer patients suffering from parasitic diseases.

**Table 2:** Sociodemographic distribution of cancer individuals participating in the study.

Risk factors for parasitic infection	Patients (%)	p-value
Gender		
Male	59	
Female	41	p=0/839
Age (year)		
0-20	1	
20-40	21	
40-60	42	p=0/751
>60	36	
Educational status		
Uneducated	44	
Read and write	17	
Elementary school	14	p=0/637
High school and above	25	
Location		
Urban	64	
Rural	36	p=0/288
Contacted with animals		
Yes	27	
No	73	p=0/540
Anti-parasitic drugs usage		
Yes	5	
No	95	p=0/41
Drinking water source		
Piped water	77	
Bore holes	15	p=0/094
River	8	

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