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Prevalence of Parasitic Infections in Cancer Patients and Healthy Individuals in Cancer Patients and Healthy Individuals in Isfahan, Iran during a 5-Year Investigation

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Abstract

Purpose: The objective of this study is to evaluate the prevalence of parasitic infections in cancer patients and control group in Isfahan, Iran (2014-2019).

Methods: Three Stool samples from 187 cancer patients and 144 healthy individual collected. Direct smear using the normal saline, Lugol's iodine staining, Formalin-ether concentration method, Modified Ziehl-Neelsen acid-fast method, and modified Trichrome staining technique performed for each sample.

Results: Generally, the prevalence of parasitic infection in cancer patients was 39%. The rate of infection in control group was 28%. *Blastocystis hominis* was the most prevalent parasite in both cancer patient and control group, 18.7% and 13.2%, respectively. Other parasitic infection were as follows: *Entamoeba coli* (10.2%), *Endolimax nana* (6.4%), and *Giardia lamblia* (4.8%).

Conclusions: Prevalence rate of parasitic infections particularly helminthic cases declined during the past decades. However, protozoan parasitic infection are still considerable.

Keywords: Parasitic infection; Iran; Cancer patients; Helminth infection; Protozoan infection

Introduction

Cancer accounting for about 13% of all deaths in 2008, globally, with a predicted eleven million deaths in 2030. Viral, bacterial, and protozoan infections are the most substantial causes of death due to cancer which are preventable [1]. Parasitic infections are still important in all over the world especially in developing countries and further in immune-suppressed patients [2]. Parasitic infections are often self-limiting in healthy Individuals, but it may cause morbidity and even mortality in immunocompromised patients [3]. Parasitic infections are among of infections which can occur in cancer patients [4]. Based on available information, 3.5 billion people involved, and 450 million people are ill as a consequence of infection with parasites [5]. Cancer patients are at risk for opportunistic parasitic infections with *Cryptosporidium*, *Cyclospora* and *Isospora*, which considered as emerging agents causing diarrhea and even death in immunodeficient patients [6]. In other words, when the host's immune system is weakened, opportunistic infections usually become pathogenic [7]. Today, as a consequence of the increasing rates of immune-suppress therapy and widespread use of chemical drugs particularly in cancer patients, opportunistic parasitic infections become more prevalent in immune-suppressed patients and considered as one of the most significant causes of morbidity and mortality in immune-suppressed patients including cancer patients [8]. Since the majority of studies carried out only in protozoan infections in immunocompromised patients in Iran and worldwide [9-11], we aimed to investigate the prevalence rate of both protozoan and helminth infections in cancer patients which carried out only by few studies [12,13]. Due to the importance of parasitic infections in cancer patients, careful considerations must carried out in order to decrease such infections in high-risk groups like cancer patients; in this study, the prevalence of parasitic infections in cancer patients during a five-year survey (2014-2019), investigated. Finally, in current study, the prevalence rate of parasitic infection in cancer patient and control group compared to

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know current epidemiological status of parasitic infection in Isfahan, Iran in both cancer and healthy individuals.

Subjects and Methods

Study population

The whole study approved by the relevant ethics committee of the Isfahan University of Medical Science, Iran, (IR.MUI.REC.1392.005).

This cross sectional study conducted on 187 cancer patients at Oncology Department of Seyed-al-Shohada Hospital, Isfahan University of Medical Sciences and some health care centers and 144 healthy individual without cancer as control group during five years (2014-2019).

A written and verbal informed consent obtained from all participants in the study including participants above 16 years old and legal guardians of children who participated in the study.

A questionnaire filled by all the participants containing information about gender, age, residence, education, occupation, contact with animals, clinical symptoms. Furthermore, before starting the study, consent obtained from all the participants.

Stool examination

Three stool sample collected from all the individuals. In order to examine the stool samples, the collected samples sent to Department of Parasitology of Medicine in Isfahan University of Medical Sciences.

First, samples checked macroscopically (color, form of the stool). Then, microscopic examination carried out for each sample according to National Committee for Clinical Laboratory Standards (NCCLS) guideline as follows:

1. Direct smear using the normal saline,
2. Lugol's iodine staining,
3. Formalin-ether concentration method,
4. Modified Ziehl-Neelsen acid-fast method,
5. Modified Trichrome staining technique

All slides were examined under light microscopy with 10×, 40× and 100× magnification.

Statistic analysis

Data analysis performed using SPSS software version 24. For the descriptive data, the prevalence of parasites according to gender, age characterized with frequencies. The Pearson's Chi-square (Chi²) test used for statistic relationship. A *p* value <0.05 considered statistically significant.

Results

Totally, 187 cancer patients and 144 healthy individual enrolled in the current study. Table 1 completely shows the Socio-demographic characteristics of both cancer patients and control group. All the participants were between 10-81 years old, in cancer patient group, the majority were more than 40 years old while the majority in control group were under 40 years old. Most of the patients and controls were female, married and were lived in urban areas (Table 1).

Macroscopic form of stool of 95.2% patients and 97.2% control group were normal (soft or formed), while stool of 4.8% of patients had a watery form, 2.8% stool of control group was watery and difference between macroscopic form of stool in patients and control

Table 1: Sociodemographic characteristics of cancer patient and control group in Isfahan, Iran (2014-2019).

Variable		Cancer patients= 187 N %	Control group=144 N %
Gender	Male	52 (27.8)	68 (47.2)
	Female	135 (72.2)	76 (52.8)
Age group	≤40 years old	48 (25.7)	76 (52.8)
	>40 years old	139 (74.3)	68 (47.2)
Residency	Rural	18 (9.6)	6 (4.2)
	Urban	169 (90.4)	138 (95.8)
Marriage status	Married	176 (94.1)	59 (41)
	Single	11 (5.9)	85 (59)
Educational level	Diploma and lower	156 (83.4)	117 (81.3)
	Upper diploma	31 (16.6)	27 (18.8)

Table 2: Clinical manifestation of parasitic infection in cancer patients and control group in Isfahan, Iran (2014-2019).

Clinical presentation	Cancer patients=187 N (%)	Control group=144 N (%)	P value
Abdominal pain	-	-	< 0.001
Yes	41 (21.9)	5 (3.5)	-
Diarrhea	-	-	0.04
Yes	13 (7)	3 (2.1)	-
Constipation	-	-	0.37
Yes	1 (0.5)	0	-

group was statically significant (*p* = 0.006).

75.4% of patients did not have any contact with animals, in recent months. In control group, 72.9% had no contact with animals, significant difference did not reveal between contact with animal and parasitic infection (*p* = 0.6).

Clinical symptoms in cancer patients and control group presented in Table 2. Clinical presentation including abdominal pain, diarrhea and constipation presented in patients and control group. In the case of abdominal pain and diarrhea, difference in patient group and control group was significant (*p* value = 0.04).

The most prevalent parasite in cancer patients was *Blastocystis hominis* (35 participants, 18.7%), followed by *Entamoeba coli* (19 participants, 10.2%), *Endolimax nana* (6.4%), *Giardia lamblia* (4.8%). In control group, the most common parasite was *Blastocystis hominis* (19 participants, 13.2%), followed by *Entamoeba coli* (6.9%), *Giardia lamblia* (2.8%) and *Chilomastix mesnili* (2.8%). The prevalence rate of *Blastocystis hominis* in cancer patient (18.7%) and healthy individual (13.2%) did not have a meaningful significant. The prevalence rate of other parasites presented in Table 3. No infection observed with isospora and cyclospora in all of the participants.

From seventy-three (39%) of infected patients, 39 patient (20.9%) infected with a single parasite, 27 patient (14.4%) infected with two parasites and seven patient (3.7%) infected with three parasites. In control group among forty one individual (28%), 21%, 5.6% and 1.4% infected with one, two and three parasites, respectively.

Discussion

Results of present study showed that 39% (30 patients) and 103 of control group (28%) infected with parasitic infection. No significant difference in parasitic infection observed between patients and

Table 3: Prevalence of parasitic infections in cancer patients and control group in Isfahan, Iran (2014-2019).

Type of parasites	Cancer patient=187 N %	Control group=144 N %	P value
Protozoa			
<i>Blastocystis hominis</i>	35 (18.7)	19 (13.2)	0.2
<i>Entamoeba coli</i>	19 (10.2)	10 (6.9)	0.30
<i>Endolimax nana</i>	12 (6.4)	3 (2.1)	0.06
<i>Giardia lamblia</i>	9 (4.8)	4 (2.8)	0.34
<i>Chilomastix mesnili</i>	8 (4.3)	4 (2.8)	0.46
<i>Cryptosporidium spp.</i>	8 (4.3)	1 (0.7)	0.08
<i>Dientamoeba fragilis</i>	3 (1.6)	1 (0.7)	0.45
<i>Iodamoebabucheli</i>	2 (1.1)	1 (0.7)	0.7
<i>Microsporidia</i>	2 (1.1)	0	0.21
<i>Entamoeba histolytica/dispar</i>	1 (0.5)	0	0.37
Total protozoan infection	63 (33.7)	32 (22.2)	0.02
Helminth			
<i>Enterobius vermicularis</i>	9 (4.8)	7 (4.9)	0.98
<i>Hymenolepis nana</i>	5 (2.7)	2 (1.4)	0.42
<i>Ascaris lumbricoides</i>	1 (0.5)	0	0.37
Total helminth infection	12 (6.4)	6 (4.2)	0.37

control group, ($p>0.05$). This rate of infection is higher than previous study [12]. In a similar study carried out in Tehran, Iran, in 2018, eighty five cancer patients evaluated for parasitic infection, the results showed that the prevalence of parasites in cancer patients was 25.9% which was much lower than our study [13]. The differences in results can be attributed to differences in regions, using dissimilar methods, variations in patient status (age, sex, residency, immunological state). In another study in Saudi Arabia, overall prevalence of protozoan infection in cancer patients was 88.9% which is in contrast with results of current study [14]. Also, dissimilar results compared to current study observed in Bagai et al., study, they found that 80% of cancer patients infected with protozoan parasites [15]. Totally, the higher prevalence of parasitic infection among cancer patients in current study (39%) showed the vulnerability of cancer patients to parasitic infection due to their impaired immune system, although no significant difference observed between patient group and control one (p value=0.07).

Among 187 cancer patients, 33.7% (63 patients) infected with protozoan parasites, while only 6.4% (12 patients) infected with helminth parasites. Thirty two of control group (22.2%) infected with protozoan parasites and six of them (4.2%) had helminth infection. This finding showed that protozoan infection (33.7%) was more prevalent than helminth ones (6.4%) and the difference between case group and control group was significant ($p=0.001$). Currently, based on reports, the incidence of helminth infection decreased in Iran [16]. In recent years, broad advances in sanitation and hygiene status led to decrease of helminth infection in Iran but infection with *Hymenolepis nana* and *Enterobius vermicularis* still occur in many parts of the country [16]. In current survey, infection with *Ascaris lumbricoides* (0.5%), *Hymenolepis nana* (2.7%) and *Enterobius vermicularis* (4.8%) observed in patient group, which is in agreement of current status of helminth infection in Iran [16]. Furthermore, several investigations indicated that protozoan infections were more prevalent than helminth infection which confirms the results of current study

[12,14,17]. In Iran, in 2017, Rasti et al., did not observe helminth infection among patients with cancer or in control group [13].

Blastocystis hominis was the most frequent parasite in current survey in both cancer patients and control group. Among cancer patients, rate of infection with *Blastocystis hominis* was 18.7% while rate of infection in control group was 13.2%, no significant difference observed between patient group and control group (p value=0.2). This is in accordance with Esteghamati et al., results conducted in similar population which showed that *Blastocystis hominis* with the prevalence of 22.3% was the most prevalent parasitic infection among cancers patients [12]. In another similar study performed by Mohamed et al., 2017, it revealed that 33.3% of cancer patients infected with *Blastocystis hominis*, which is identical to the results of current study [18]. Also, *Blastocystis hominis* considered as the most prevalent parasite in cancer patients in literature [19]. Overall, the Prevalence of *Blastocystis hominis* is high in developing countries, with the prevalence rate of about 50-60% in developing countries [20]. Recently, in Iran, *Blastocystis hominis* considered as the most prevalent parasite like the results of current study in both cancer patients and healthy individuals [21]. Also, in a study performed in Isfahan, in patients with diabetes mellitus and control group, *Blastocystis hominis* infection was the most detected infection [22]. Although, pathogenic role of *Blastocystis hominis* is controversial, several studies confirmed the pathogenicity role of *Blastocystis hominis* and related it to some disorders while in other studies, it is assumed as a commensal organism [23]. The doubtfulness of pathogenesis of *Blastocystis* attributed to subtype variations in virulence and other factors [23]. Previous investigation suggested that the presence of greater than five parasites per high-power field ($\times 400$) for wet mounts or under oil immersion is associated with acute gastrointestinal signs in patients [24]. Since the pathogenesis of *Blastocystis* is controversial and symptoms are self-limited, specially in immune-compotent patients, treatment option prescribed for *Blastocystis* infections only when other etiologies excluded [25].

Other parasitic infections in the current study in patients were as follows: *Entamoeba coli* (10.2%), *Endolimax nana* (6.4%), *Giardia lamblia* (4.8%), *Cryptosporidium spp.* (4.3%) and *Chilomastix mesnili* (4.3%). No infection with *Isospora* and *Cyclospora* detected in current study. Only 2 cases with *Microsporidia* infection detected in cancer patients and none of the control group infected with *Microsporidia*. In a similar study performed by Esteghamati et al., in 2018 in Tehran, the prevalence rate of intestinal parasites among 85 cancer patients were as follows: *Blastocystis hominis* (22.3%), *Giardia lamblia* (2.3%) and *Dientamoeba fragilis* (1.2%) [12], totally the results of both study showed that *Blastocystis hominis* was the most prevalent parasite in cancer patients, no *microsporidia* and *isospora* detected in patients. In previous study in Kashan and Qom, the most prevalent parasites were *Giardia lamblia* and *Blastocystis hominis* [13]. *Giardia lamblia* and *Blastocystis hominis* are among the most prevalent protozoan parasites in Iran and worldwide [26]. In Mashhad, north east of Iran, Zabolinejad et al., found that 35.9% children with hematological malignancies infected with parasitic infection, their results showed that the most prevalent infections were *Giardia lamblia* (18%), *Ent. coli* (6.7%) and *B. hominis* (5.6%) [27]. In a dissimilar study, the most prevalent parasite in cancer patients was *Cryptosporidium* followed by *Isospora* and *microspora* [14], which is in contrast to the results of current study.

The prevalence rate of *Cryptosporidium spp.* infection in present study was 4.3% in cancer patients and 0.7% in control group, which

was relatively low. In a similar study in Iran, in 2010, 4% of cancer patients infected with *Cryptosporidium* spp. [11]. Similarly, studies conducted by Sreedharan et al., and Radrapatna et al., on patients with neoplasms showed lower infection rates, 1.3% and 0.3%, respectively [11]. Higher prevalence rates (17-61.1%) have been reported in India and Turkey, among immunocompromised patients [11,28]. The above mentioned differences may be due to several reasons including: variations in diagnostic methods for cryptosporidiosis, different hygienic life styles and living environmental.

Only a case with *Entamoeba histolytica/dispar* infection observed in current study, since *Entamoeba histolytica/dispar* is not endemic in Isfahan, this observation confirmed the previous study [29]. None of the healthy individuals infected with *Entamoeba histolytica/dispar*. Furthermore, in agreement with the results of current study, other studies in Iran showed a very low prevalence of *Entamoeba histolytica/dispar* in Iranian individuals [30].

Conclusion

In current study, a refreshed epidemiological survey of parasitic infection in Isfahan carried out. The results of prevalence rate of parasitic infections in cancer patients and control group showed that 39% of cancer patients and 28% of control group infected with parasitic infections. Although a significant decline in the prevalence of helminths occurred, high prevalence of protozoan parasites is still a considerable public health problem. The trend of parasitic infection in Isfahan was compatible with Iran and global changes and shifted to higher prevalence of *Blastocystis hominis* in population.

Advantages of the current survey were as follows: three stool samples obtained from each patient and control one, unique and specific method used to detect microsporidium which seldom carried out by previous studies. There was one limitation in this study; we did not use molecular techniques to detect *Cryptosporidium*. For subsequent investigations, molecular techniques could be carried out to more accurate detection of parasites like *Cryptosporidium* and microsporidium. Careful differentiation of *Entamoeba histolytica* and *Entamoeba dispar* should be considered.

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Authors' Contribution

Nader Pestechian designed the study protocol; Hosein Ali Yousefi conducted the experimental tests; Fariborz Mokarian provided cancer patients and conducted clinical studies; Mohamad Javad Tarrahi analyzed and interpreted the data; Sanaz Tavakoli wrote the article, all the contributors revised the article critically for important intellectual content and finally approved the version to be published.

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