Journal of Research in Medical and Dental Science
Volume 5, Issue 5, Page No: 127-133

Available Online at: www.jrmds.in
eISSN No. 2347-2367: pISSN No. 2347-2545

Investigating the Prevalence of Intestinal Parasitic Infections in Kidney Transplant Recipients

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DOI: 10.24896/jrmds.20175520

ABSTRACT

Over the last two decades protozoa are one of the most common causes of infectious diseases in people with impaired immune system. Renal transplant recipients are one of the main groups with impaired immune system. The aim of this study has been to determine the prevalence of intestinal parasitic infections in renal transplant recipients of Mashhad. This descriptive study was conducted from January 23, 2015 to January 18, 2016 on renal transplant recipients of Mashhad. Three stool specimens were collected from each person after filling the questionnaire and informed consent. These samples were examined using four techniques, wet mount, formalin-ethyl Acetate concentration, Ziehl-Neelson staining and trichrome staining. Statistical package for social sciences (SPSS) 11.5 software (SPSS Inc., Chicago, Illinois, USA) was used for all statistical analyses. Standard descriptive statistics were applied to describe the pattern of the data. Chi-square test was used to examine the significance of the association between categorical data. In this study of the 71 patients renal transplant recipients, 41(57.7%) were male and 30(42.3%) were female. The maximum and minimum age of patients respectively was 69 and 14 years and the average age of persons was 39.5 years. In control group too, of the 71 subjects, 41(57.7%) were male and 30(42.3%) were female. The maximum and minimum age of them respectively was 70 and 15 years with the average age of 39.7 years. The results of this research showed that 45.07% of renal transplant recipients and 2.81% of control group were infected with intestinal parasites. The parasites detected among renal transplant recipients included 20(28.2%) Entromonas hominis, 11(15.5%) Giardia lamblia, 3(4.2%) Blastocystis hominis, 2(2.8%) Cyclospora cayetanensis. The intestinal parasitic infections in control group were 1(1.4%) Giardia lamblia and 1(1.4%) Blastocystis hominis. In statistical analysis results of prevalence intestinal parasitic infections, there was a significant difference between the renal transplant recipients and control group (P<0.001). This study indicated that intestinal parasitic infections have relatively high prevalence in renal transplant recipients of Mashhad. So these patients should be tested periodically to prevent morbidity and mortality from parasitic infections.

Key words: Intestinal Parasitic Infections, Renal Transplant, Intestinal Protozoa

HOW TO CITE THIS ARTICLE: Fariba Berenji, Hajar Imanfar, Fateme Nazemian, Veda Vakili, Abdolmajid Fata, Shadi Rahmani Khorasani, Investigating the Prevalence of Intestinal Parasitic Infections in Kidney Transplant Recipients, J Res Med Dent Sci, 2017, 5 (5): , DOI: 10.24896/jrmds.20175520

Corresponding author: Fariba Berenji
Received: 02/08/2017
Accepted: 20/09/2017

INTRODUCTION

Parasitic infections are among problems and obstacles to progress of social and economic development in most countries. It is still one of major health problems in developing countries despite the ongoing efforts and extensive plans of the World Health Organization [1]. About 340 parasitic species have caused different infections with the illness and death of more than 3 billion

people around the world [2]. According to WHO about 40 million death out of 52 million ones in 1996 were in developing countries, 40% of which from infectious diseases (parasitic-bacterial) [3]. Types of host defensive disorders lead to human infection with opportunistic parasites and aggravation of symptoms of common parasitic infections [4].

Patients with weakened immune systems, including AIDS patients, organ transplant recipients, and those being treated with immunosuppressive medication are particularly prone to be affected by opportunistic parasitic diseases [5]. People who have kidney transplants, a group of patients with immune deficiency, are prone to infectious diseases because of immunosuppressive medication [6]. Some intestinal parasites such as *Microsporidia*, *Strongyloides stercoralis, Cryptosporidium,* and *Giardia lamblia* are frequently seen in these individuals. For example, *Cryptosporidium* is one of the causes of infectious diseases in AIDS patients, organ transplant recipients, children, and travelers as well as the elderly [7-9].

Immunosuppressive medication normally alters the usual symptoms of diseases and causes non-timely diagnosis, and is considered one of the major problems after transplant. It is possible to mention chronic and acute diarrhea as an example in this concern [10, 11].

Because of increasing number of AIDS patient, most of studies in recent decade have been dedicated to it. But we decided here to investigate the prevalence of intestinal parasitic infections in kidney transplant recipients who are at risk because of taking immunosuppressive medication among patients in Mashhad, Iran in order to explain the importance of the issue.

MATERIAL AND METHODS

The study was conducted from February 2014 to February 2015 on kidney transplant recipients, as a case group, and healthy subjects without clinical symptoms as a control group. This study was the type of a case-control and case series. A questionnaire and an informed consent were obtained from all participants. Three stool samples were taken from the patients and the control group. The samples were tested using 4 methods direct smear, formalin ethyl acetate, Ziehl-Neelsen staining, and tri-chromium staining. In order to collect the stool samples, we used completely covered plastic containers (specified to collecting stool) containing 10% formalin retainer – by the rate of one part of the stool to three parts of the preservative - and a specimen with no formalin. Then personal information of each participant, such as the name and so on, was placed on the container to collect stool samples for three successive days. Samples were transferred immediately to the central laboratory of Imam Reza Hospital of Mashhad. Four parasitological techniques including direct wet smear with normal saline and Lugol's iodine; concentration technique with formalin ether for detecting eggs and larvae of worms and protozoan cysts; Ziehl-Neelsen staining to recognize Coccidia, and trichrome staining for other protozoa were executed on each sample. Statistical package for social sciences (SPSS) 11.5 software (SPSS Inc., Chicago, Illinois, USA) was used for all statistical analyses. Standard descriptive statistics were applied to describe the pattern of the data. Chi-square test was used to examine the significance of the association between categorical data. All tests were 2-tailed, and probability values _0.05 were considered significant.

RESULTS

71 kidney transplant recipients, 41 men (57.7%) and 30 women (42.3%) participated in the study. The oldest was 69, and the youngest 14, with the average age of 39.5 years old.

71 individuals were tested in control group containing 41 men (57.7%) and 30 women (42.3%). The oldest 70, and the youngest 15, with the average age of 39.7 years old.

45.07% of kidney recipients and 2.81% of control group were infected by intestinal parasites. The detected parasites in the recipients of the kidney transplant included 20 cases (28.2%) of *Entermonase hominis*, 11 cases (15.5%) of *Giardia lamblia*, 3 cases (4.2%) of *Blastocystis hominis*, and 2 cases (2.8%) of *Cyclospora* oocyst. The detected parasites in the control group included 1 case (1.4%) of *Blastocystis hominis*, and 1 case (1.4%) of *Giardia lamblia*. There was a significant difference in the prevalence of intestinal parasitic infections among kidney transplant patients and control group (P <0.001).

Table 1: Comparison of the prevalence of intestinal parasitic infection in the kidney transplant recipient groups and control group

Parasitic infections Groups	*Enteromonas hominis*	*Giardia lamblia*	*Cyclospora*	*Blastocystis hominis*	*Enteromonas hominis +Giardia lamblia*	*Blastocystis hominis +Enteromonas hominis*
Renal transplant recipient group	20(28.2%)	11(15.5%)	2(2.8%)	3(4.2%)	2(2.8%)	2(2.8%)
Control group	0(0%)	1(1.4%)	0(0%)	1(1.4%)	0(0%)	0(0%)

Table 2: Distribution of clinical symptoms in kidney transplant recipients with parasitic infection

Clinical symptoms	**Prevalence**	**Percent**
Diarrhea	12	16.9%
Bloating and abdominal cramps	6	8.45
Abdominal pain	7	9.85
Severe diarrhea	2	2.81
Nausea	2	4

Table 3: Distribution of positive and negative cases of parasitic infections in kidney transplant recipient group according to parasitological technique

Parasitological Technique Parasitic infections	Ziehl-Neelsen staining	Trichrome staining	Concentration technique with formalin ether	Direct wet smear
Positive cases	28 (39.43%)	29 (40.84%)	19 (26.76%)	9 (12.67%)
Negative cases	43 (60.56%)	42 (59.15%)	52 (73.23%)	62 (87.32%)

Table 4: Frequency distribution of intestinal parasitic infection in kidney transplant recipients according to the time after transplant

Time after transplant Parasitic infections	< 1 year	1-3 years	3-6 years	> 6 years
Positive cases	13 (18.30%)	9 (12.67%)	5 (12.67%)	5 (7.04%)
Negative cases	11 (15.49%)	7 (9.85)	9 (12.67%)	12 (16.90%)

At the beginning of the project, the age and sex of two groups of kidney transplant recipients and control group were statistically uniformed. Then the statistical tests were used to ensure the authenticity of the results. Chi-square test showed that there was no significant difference between sex and intestinal parasitic infection in two groups of kidney transplant recipients and control. Also, the results of the Mann-Whitney test showed that there was no significant difference between the two groups in terms of age.

Chi-square test showed a significant correlation between clinical symptoms and parasitic infection in kidney transplant recipients ($P < 0.001$).

DISCUSSION

There are several studies on intestinal parasitic infections in patients with suppressed immune system, but regarding the increase of kidney transplant as well as the susceptibility of these patients to opportunistic infections, more studies are needed in this area. The present study was carried out on February 2014 to February 2015 on kidney transplant recipients as a case group and healthy subjects with no clinical symptoms as a control group in Mashhad, northeast of Iran. The criteria for entry into the study was to be recipient of kidney transplant using immunosuppressive medications who had received at least 6 months earlier. The exclusion criteria were the simultaneous use of other anti-parasitic drugs such as tetracycline.

In the present study, most recipients of kidney transplant were in the age group of 20-40 years and the youngest ones were less than 20 years old. Again, most of the patients with intestinal parasitic infections were in the age group of 20-40 years, indicating that recipients of kidney transplants at this age group are most at risk for parasitic diseases.

In this study, from 71 recipients of kidney transplant, 41 (57.7%) were men and 30 (42.3%) women. The results of statistical tests showed no significant difference between participant's sex and the intestinal parasitic infection.

According to the results of this study, 45.07% of kidney transplant recipients had at least one intestinal parasite infection, which is a high prevalence in comparison to other studies in Iran or any other countries. Azami (2006) reported the prevalence of intestinal parasitic infections among kidney transplant recipients in Isfahan was 33.3% [2]. Intestinal parasitic infections in Tehran kidney transplant recipients according to Rostami *et al.* (2005) were 4.5% [5]. Valar *et al*., (2007) showed in Brazil, in 2001-2005, 2.4% of all recipients of kidney transplant had intestinal parasitic infections [12]. Intestinal parasitic infections in Sudan were reported 24% on kidney transplant recipients by Eltayeb *et al.*, (2012) [13]. The high prevalence of intestinal parasites in our study indicates the potential importance of these organisms in developing clinical manifestations in patients with immune system deficiency; because, such patients are not only more vulnerable than healthy people to common infections, they are also prone to the establishment and pathogenesis of opportunistic parasites, due to their insufficient immune system. The disease manifestation is therefore much sever in these individuals.

In the present study, the most common pathogenic parasitic infections were *Giardia lamblia* (15.5%) and then *Blastocystis hominis* (4.2%), respectively, which were in accordance with those found by Berenji F. in 2005, about the HIV positive patients in Mashhad (*Giardia lamblia* 22.6%, *Blastocystis hominis* 22.6%) and with those found by Zabolinejad (2013), about the children with lymphohematopoietic malignancies (*Giardia lamblia* 18%, *Blastocystis hominis* 6.6%) [14, 15]. It is therefore concluded that the most prevalent pathogenic intestinal parasites in patients with immune deficiency in Mashhad are first *Giardia lamblia* and then *Blastocystis hominis.* While the highest parasitic infection in recipients of kidney transplant in Isfahan reported by Azami (2006) is *Entamoeba coli* (10.6%) [2].The most common parasitic infections according to Rostami in kidney transplant recipients of Tehran are first *Blastocystis hominis* (1.7%) and then *Giardia lamblia* (1.4%) [5]. It is reported that the highest parasitic contamination in hemodialysis patients in Turkey in 2012 was first *Blastocystis hominis* (23.9%) and then Giardia *lamblia* (8.5%) [16]. Giardiasis reduces the mucosal absorption in the small intestine and leads to disaccharidases deficiency, and consequently reduces the absorption of electrolytes, food, and water, resulting in malabsorption and indigestion. The global prevalence of *Giardia lamblia* is 20-60% whose occurrence in developed countries is 2-7% [17]. According to Gil *et al*., (2013) in Brazilian hemodialysis patients, the incidence of *Giardia lamblia* was seen less than other intestinal infections (0.9%) [18]. The prevalence of Blastocystosis in humans in developing countries (30-50%) is higher than in developed countries (1-10%) (19). *Blastocystis hominis* is one of the most important opportunistic intestinal parasites in individuals with suppressed immune system that causes diarrhea in them [20]. Ok *et al*., (1997) reported the prevalence of *Blastocystis* (39.1%) among the recipients of kidney transplants in Turkey, and the incidence of Cryptosporidium 18.8% [21].

It is seen that the overall outbreak of infection due to these factors varies in different studies, which can be out of differences in the studied population, the time of the study, the observance of health, education, climate, and geographical location of the study. Nevertheless, similar to other studies, in our one, *Giardia lamblia* and *Blastocystis hominis* were more prevalent than others which indicate the important role of these parasites in infecting, and in digestive complications.

The prevalence of *cyclospora* in this study was 2.8% (2 cases), the patients suffered from severe and chronic diarrhea, but it was not reported in direct smear method, and we were able to detect it using tri-chrome and Ziehl-Neelsen staining. *Cyclospora* is nowadays a novel pathogen in humans and one of the most common causes of diarrhea worldwide [22]. *Cyclospora* seems a fecal-oral infection and the parasite transmits to the host through water and food [23]. Eating unclean vegetables and fruit can cause transmission of parasites. Drinking contaminated water has been the main route of transmission up to now [24]. The first case of cyclosporiasis in Iran was in 1996, an HIV-infected hemophilic teenager, and the other one, a young boy living in Tehran (with severe watery diarrhea) who was reported by direct smear, trichrome staining [25]. In a study in Tehran in 2002, among children less than 10 years with diarrhea who referred to the pediatric medical center showed 31 cases (8.3%)

infected with *Giardia* and *Cryptosporidium*, but no case of *Cyclospora* was detected [26]. Vahabzadeh et al (2008) tested 617 patients with gastroenteritis in Gilan and diagnosed 4 cases of *Cyclospora* (0.6%) and 6 cases of *Cryptosporidium* (1/1%) through direct smear and then staining methods such as Auramin Phenol Fluorescence, acid fast staining and giemsa staining [27]. Just one case of *Cyclospora* was observed in Mashhad following the use of contaminated vegetables, but not officially reported.

The prevalence of *Cryptosporidium* in our study population was zero. *Cryptosporidium* is one of the most important intestinal pathogenic parasites that causes diarrhea both in human and animal [28]. *Cryptosporidium* has been reported in a wide range of patients with impaired immune system. Typically, as long as the patient's immunity impairment is not remedied, the disease will continue to return with attacks for several years and even to the end of the patient's life. In a study on children with diarrhea in Mashhad Medical School hospitals, 3% of samples were positive for *cryptosporidium* oocytes (by modified Ziehl-Neelsen staining method) [29]. Berenji *et al.,* (2007) testing one hundred stool samples collected from children and adolescents with lymphohematopoietic malignancies (Ziehl-Neelsen and ELISA staining for fecal antigen) in Mashhad found 22 positive cases, just half of them showing clinical symptoms [30]. In another study on 206 individuals in Tehran who were HIV positive, 1.5% of their stool samples were positive for *Cryptosporidium* [9]. Raja *et al*., reported 343 cases of cryptosporidium oocytes (53%) from a total of 644 kidney transplant recipients suffering from severe diarrhea in Pakistan [31]. Guk *et al*., reported *Cryptosporidium* the most prevalent intestinal parasitic infection (10.5%) in Korean patients with immune deficiency during 1995-2003 [32]. Contacts with some domestic animals such as caws, calves, and sheep, important carriers of the disease, are one of the most important causes of contamination with *Cryptosporidium*. The absence of *Cryptosporidium* parasite in our study might be due to a number of reasons such as less contacting with animals, raising the level of education and knowledge, increasing the community's health, respecting personal hygiene, clean washing of vegetables, and using more refined water.

In the present study, 28.2% of kidney transplant recipients were infected with *Entermonase hominis* which is a high rate compared to (3.2%) obtained in the study by Berenji on HIV positive patients in Mashhad [14]. The contamination of water, food and/or hands with infected cysts causes transmission of this parasite.

The most commonly observed clinical symptoms in kidney transplant recipients infected with intestinal parasites are diarrhea, most of which related to *Giardia lamblia*. This parasite caused diarrhea (16.9%) and abdominal pain (85.9%) in some of these patients. *Blastocystis hominis* in 4.22% and *Cyclospora* in 2.8% of patients caused diarrhea which were in chronic condition in some cases.

The most positive cases for intestinal parasites in the studied population of transplanted recipients were among those with lower health and economic level, indicating the need for further consideration and giving better awareness to these patients.

According to Table 2, the best diagnostic method used in this study was first tri-chrome staining and then Ziehl-Neelsen staining. Though providing direct smear is good for observing the active form of amoeba and flagellate movements, but in mild intestinal parasitic infection is not helpful enough in diagnosis.

According to Table 3, the most risk for intestinal parasitic infections is in the first year after kidney transplant. Because during this period, the patient's body is more susceptible to viral, bacterial, fungal, and parasitic infections due to continuous use of repressive medication.

CONCLUSION

It is generally concluded that the prevalence of intestinal parasitic infections, in a variety of levels is evident in different developing countries. The findings of the present study in Mashhad showed that the rate of intestinal protozoan parasites in kidney transplant recipients, due to the use of immunosuppressive medication is more than the control group.

Because of high probability of intestinal parasitic infections, especially opportunistic parasites, in kidney transplant recipients, it is necessary to pay more attention to controlling and preventing these

infections. It is therefore recommended to conduct similar studies in other provinces to achieve the true rate of such infections. It is also suggested to physicians and nephrologists to periodically test kidney transplant recipients for parasitic infections in order to control mortality and morbidity after kidney transplant in these patients.

Acknowledgement
The authors gratefully thank the research deputy of the Mashhad University of Medical Sciences for the funding of this research project. This article is part of the thesis by Hajar Imanfar, under the permission No.809.

Conflict of interest
None

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