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Original Article

Intestinal parasitic infections in patients with Diabetes Mellitus: A case-control study

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Abstract
Introduction: Patients with diabetes mellitus (DM) are at increased risk of certain infections; however, little is known about the prevalence of intestinal parasitic infections in them. The aim of this study was to assess the risk of intestinal parasitic infections in patients with DM in comparison with a healthy control group.
Methods: This case-control study was conducted on 118 patients with DM and 118 healthy people as control group from April to September 2014. Oral glucose tolerance test (OGTT) and hemoglobin A1c level were checked, and checklists including risk factors for parasitic infections were filed for all participants. Three stool samples and one scotch tape were obtained. Samples were examined by direct wet smear, formol-ether concentration, Kinyoun acid-fast staining, and modified trichrome stain. Data were analyzed using chi-square and logistic regression tests.
Results: The rate of parasitic infection was significantly more in the patients (26.3%) than the controls (6.8%) ($P < 0.050$). The most detected infection was Blastocystis hominis (n = 14) followed by Endolimax nana (n = 10) and Giardia lamblia (n = 5). Infection with B. hominis was significantly more in the DM patients (9.3%) than in the controls (2.5%) ($P < 0.050$). DM [odds ratio (OR) = 3.6], female gender (OR = 3.0), and the presence of symptoms (OR = 9.900) were the risk factors for intestinal parasitic infections ($P < 0.050$).
Conclusion: Patients with DM might be at an increased risk of infection with intestinal parasites specifically B. hominis as an opportunistic infection, and routine stool examination should be considered for them.

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Introduction

Intestinal parasites continue to cause significant morbidity and mortality in developing countries, though the increasing population of immunocompromised people, they are now considered as an important health problem around the world.[1] Extensive research showed that apparent immune suppression in human immunodeficiency virus (HIV) infection,[2] primary immunodeficiency,[3] and use of immunosuppressive drugs such as post-transplantation[4] increases the risk of establishment of the infection, chronic carriage states, and morbidity of the intestinal parasites. But any weakness of

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immune system such as chronic internal diseases and metabolic disorders can put the patients at higher risks of infectious diseases.

Diabetes mellitus (DM) is a group of chronic diseases characterized by hyperglycemia that is caused by insufficient insulin secretion, impaired insulin action, or both.[5] Chronic hyperglycemia leads to vascular and neurologic complications, often accompanied by end-organ damages and susceptibility to certain infections in patients with DM such as urinary tract infections, lower extremity infections, tuberculosis reactivation, surgical wound infection, candidiasis, and pneumonia.[6] Probably local and systemic immune defects are responsible for this higher susceptibility.[7]

Recently, it is demonstrated that both innate and acquired immunities are impaired in DM.[8] In mice with DM with urinary tract infection, chemokine expression, neutrophil infiltration, and bacterial clearance are decreased.[9] Functions of neutrophil such as phagocytosis and chemotaxis are impaired in the mice with DM.[8] Because of general immunosuppressive condition, a high prevalence of various infections is expected in DM, but surprisingly, epidemiologic data in this regard are scarce[7] and there are few studies addressing the prevalence of intestinal parasites in patients with DM.[10,11]

The purpose of this study was to assess the rate of parasitic infections in patients with DM and a control group to estimate the risk of intestinal parasitic infection in patients with DM in comparison with healthy people.

Methods

This was a case-control study conducted on individuals referred to Endocrine and Metabolism Research Centre, Isfahan, Iran, from April 2014 to September 2014. The participants were healthy persons without previous chronic internal disease, cancer, or any immunodeficiency diseases (except for DM) who did not currently receive any immunosuppressive drug, based on individual history taking by a health professional. Exclusion criteria were consumption of antibiotics, anti-parasitic drugs, mineral oil, bismuth, or barium during previous 2 weeks. Only individuals who provided at least three stool samples were included in the study. Patients with DM were randomly selected from those with a proven history of DM type II registered in Endocrine and Metabolism Research Centre. The control group were selected and matched with the DM group for age and sex from those referring to the center for routine checkup.

Written informed consent was taken from all participants and the project was approved by Isfahan University of Medical Sciences Ethical Committee (Project number: 293052).

A 75 g oral glucose tolerance test (OGTT) was performed for all participants and hemoglobin A1c level was checked. Diagnosis or exclusion of DM was based on the criteria from the American Diabetes Association:[5] fasting plasma glucose $\geq$ 126 mg/dl, or 2 hours plasma glucose $\geq$ 200 mg/dl during OGTT, or A1c $\geq$ 6.5%.

A checklist including demographic data and risk factors for parasitic infections was filled for each participant. Three stool samples and one scotch tape were obtained from individuals after the full explanation of the process to them. The samples were transported immediately to the Department of Parasitology, School of Medicine, Isfahan University of Medical Sciences, where lab assessments were done.

Stool samples were examined by direct wet smear and formol-ether concentration method for the routine screening of ova and parasites. Each sample was examined separately. Smears were prepared from sediments of formol-ether concentration for specific staining: Kinyoun acid-fast staining was used to detect Cryptosporidium, and modified trichrome stain (Ryan-Blue) was employed for detection of Microsporidia as described elsewhere.[12,13] Slides were examined by light microscopy at × 400 magnification. Rate of parasitic infection was calculated as the ratio of the number of participants with at least one positive parasitological test to the number of total participants in each group.

Data were analyzed by SPSS software

(version 16, SPSS Inc., Chicago, IL, USA). Rate of parasitic infection and the risk factors were compared between the two groups by chi-square or Fisher's exact test when appropriate. Logistic regression analysis determined the degree of relationship between the rate of parasitic infection and the identified risk factors. Odds ratios (OR) and 95% confidence intervals (CI = 95%) for OR were calculated. P < 0.050 was considered as statistically significant.

Results

Three stool samples were obtained from 236 persons (118 controls and 118 patients with DM) with an age range of 4-73 (male/female: 82/154).

The rate of parasitic infection was significantly more in patients with DM (26.3%) than in controls (6.8%) (P < 0.001). In this study, the most detected infection was Blastocystis hominis (14 cases), followed by Endolimax nana (10 cases) and Giardia lamblia (5 cases) (Table 1). Only the rate of B. hominis infection was significantly different between the two groups (2.5 and 9.3% for control and DM patients, respectively, P < 0.050). Infection with two or more parasites was seen in three patients with DM and two controls, from those four were co-infected with B. hominis and E. nana.

Fisher's exact test showed significantly more parasitic infection in females (20.1%) than males (9.8%) (P < 0.050). Also the rate of infection was more in patients with symptoms such as diarrhea, abdominal Pain, and abdominal discomfort (70.6 vs. 12.3%) (P < 0.001), in those who kept animal at home (53.8 vs. 14.3%) (P < 0.050), and in undereducated persons (less than Diploma: 42.9%, Diploma and more: 14.9%) (P < 0.050). Although parasitic infection was more in persons under 10 years old (20.0%) and over 50 (28.0%) in comparison with ages 10-50 (14.9%), the difference was not statistically significant. The number of households was not related to the rate of parasitic infection.

The distribution of risk factors in the two groups was not different except for the presence of symptoms and keeping animals at home (Table 2). In logistic regression analysis, independent risk factors for parasitic infection were identified as DM (OR = 3.6, 95% CI: 1.5-8.8), female gender (OR = 3.0, 95% CI: 1.1-8.3), and the presence of symptoms (OR = 9.9, 95% CI: 2.5-39.1) (Table 3).

Discussion

This study demonstrated that the risk of infection with intestinal parasites was 3.6 times greater for patients with DM than healthy people. This is in agreement with a previous study in two cities near Tehran (Karaj and Savojbolagh) Iran, in which the rate of intestinal parasitic infections in patients with DM was more than healthy controls (5.6 vs. 10.0%).[10] In another study in Egypt, patients with DM were examined among other immunocompromised groups, and high risk of parasitic infection was found among them.[11]

Table 1. Different parasitic infections detected in patients with DM (diabetes mellitus) and control group

Type of parasite	patients with DM	Control	Total
Giardia lamblia	4	1	5
Cryptosporidium spp.	2	0	2
Microsporidia	2	0	2
Blastocystis hominis	11	3	14
Entamoeba coli	3	1	4
Endolimax nana	6	4	10
Chilomastix mesnili	1	0	1
Iodamoeba butschlii	2	1	3
Trichomonas hominis	1	0	1
Enterobius vermicularis	2	0	2
Hymenolepis nana	1	0	1

DM: Diabetes mellitus

Table 2. Distribution of risk factors for parasitic infection in patients with DM (diabetes mellitus) and control group

Risk factors for parasitic infection	Patients with DM [n (%)]	Control [n (%)]	Total (n)	P*
Gender				
Male	42 (35.6)	40 (33.9)	82	0.440
Female	76 (64.4)	78 (66.1)	154	
Age (year)				
≤ 10	7 (5.9)	3 (2.5)	10	
10-50	95 (80.5)	106 (89.8)	201	0.125
≥ 50	16 (13.6)	9 (7.6)	25	
Level of education				
< Diploma	9 (7.6)	5 (4.2)	14	0.205
≥ Diploma	109 (92.4)	113 (95.8)	222	
Symptoms				
Yes	15 (12.7)	2 (1.7)	17	0.001
No	103 (87.3)	116 (98.3)	219	
Keeping animals				
Yes	11 (9.3)	2 (1.7)	13	0.010
No	107 (90.7)	116 (98.3)	123	
Households				
≤ 4	54 (45.8)	58 (49.2)	112	0.348
> 4	64 (54.2)	60 (50.8)	124	

*By chi-square or Fisher's exact test, DM: Diabetes mellitus

Table 3. Risk factors for intestinal parasitic infection

Risk factors for parasitic infection	Infected/total	OR	95% CI for OR	P*
Diabetes (%)				
Yes	31/118 (26.3)	3.67	1.51-8.87	0.004
No	8/118 (6.8)	-	-	
Gender [n (%)]				
Female	31 (20.1)	3.06	1.12-8.35	0.029
Male	8 (9.8)	-	-	
Age (year) (%)				
≤ 10	2/10 (20.0)	0.98	0.09-10.05	
10-50	30/201 (14.9)	1.23	0.34-4.41	0.936
≥ 50	7/25 (28.0)	-	-	
Education (%)				
< Diploma	6/14 (42.9)	2.87	0.66-12.38	0.157
≥ Diploma	33/222 (14.9)	-	-	
Symptoms (%)				
Yes	12/17 (70.6)	9.95	2.53-39.14	0.001
No	27/219 (12.3)	-	-	
Keeping animals (%)				
Yes	7/13 (53.8)	2.54	0.53-12.12	0.240
No	32/223 (14.3)	-	-	
Households (%)				
> 4	21/124 (16.9)	1.13	0.50-2.56	0.762
≤ 4	18/112 (16.1)	-	-	

*By logistic regression test, OR: Odds ratio; CI: Confidence interval

The results indicate that the clearance of parasites and also commensals from intestine might have been impaired in DM; however, the exact mechanisms are not clear. Resolving of intestinal parasitic infections is dependent on both innate and adaptive immune responses, but cell-mediated immunity, specifically T-cells, plays the main role in pathogen clearance from intestine.[1]

Although some defects in the function of neutrophil and macrophage are documented in several studies,[6] there are controversies about the defective T-cell function in type II DM. Spatz et al. found that the expression of cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) (involved in down regulation of immune response) on CD4+ T cells is increased in type I DM.[14] Likewise, in response to stimulation, CD4+ T-cells from DM type I patients secreted elevated levels of the regulatory cytokine transforming growth factor-beta 1 and their monocytes produced more inhibitory cytokine interleukin-10 (IL-10) in comparison with cells from healthy people or DM type II patients.[14] Thus, it seems that T-cell function is intact in DM type II.[7] Local intestinal immune response to parasites is an important factor which can explain high parasitic infection in our patients; however, basic studies in this regard are scarce and actually there is no experiment on the mechanisms of intestinal infections in DM.[7] Also impaired mucosal integrity, due to defective microcirculation in DM,[15] can predispose parasitic infections although it is not evaluated in any study.

The most infection detected in this study was B. hominis followed by E. nana and G. lamblia. We found that the rate of infection with pathogenic and opportunistic agents as well as commensals was not different between the two groups except for B. hominis which was significantly more in with DM. We found only four cases of infection with Cryptosporidium spp. and Microsporidia (each 1.6%) in patients with DM. The rate of Cryptosporidium infection was lower in our setting in comparison with a similar study (2.4%) in which only Cryptosporidium infection was meaningfully more in DM.[10] As the cryptosporidiosis is a zoonotic infection, the reason might be little contact of our patients with reservoir animals.

While there have been many doubts about pathogenic role of Blastocystis in humans, now it is accepted as a potential pathogen which can specifically trouble immunocompromised host.[16] Symptoms associated with Blastocystis are more likely to develop in HIV-infected patients and transplant recipients than in healthy hosts.[17,18] Studies of intestinal parasitic infections show different parasites as the dominant infectious agents; however, Blastocystis nearly always has been among the most prevalent parasites in immunocompromised patients. For example, in Ethiopia, the prevalence of Cryptosporidium and Blastocystis spp. was significantly associated with lower CD4+ T-cell count in patients with HIV/AIDS (acquired immune deficiency syndrome).[19] In Laos, Blastocystis was the most frequent protozoa (26.3%) compared with Cryptosporidium spp. (6.6%).[20]

In another study in Iran, B. hominis (4.4%) was the most prevalent parasite after G. lamblia (7.3%) in HIV-positive individuals.[21] B. hominis (16. 7%) and Cryptosporidium parvum (8. 3%) were the most infections in HIV (+) patients in south of Iran.[22] Also in another study in Iran, B. hominis was the most prevalent intestinal parasite in hemodialysis patients.[23] It is suggested that the pathogenesis of Blastocystis depends upon subtype; subtypes 1-4 are more common and have a cosmopolitan distribution.[16] Mucosal invasion and intestinal inflammation have been shown in animal models of subtypes 3 and 4.[24,25] The theory of impaired intestinal mucosal integrity in DM might explain the increased rate of Blastocystis infection in these patients.

In addition to DM, female gender and the presence of symptoms were the risk factors identified for infection with intestinal parasites. The reason for the increased risk of infection in females is not clear for us. Several studies including a national survey of the prevalence of intestinal parasitic infections in Iran showed no sex related significant difference,[26-28] however, in some regions, these infections predominated in either males or females.[29,30] Sex dependent distribution of intestinal parasitic infections depends on cultural, social, and environmental factors which are different in each area, thus those inconsistencies are expected.

Limitations

We used conventional microscopic methods for diagnosis of intestinal protozoa and helminthes rather than the molecular methods. The combination of both methods would lead to more strict results; however, conventional methods are steel and the most cost-effective methods for detection of intestinal parasites.[12] Small sample size was another limitation of this study, but the power was high enough to show the difference in intestinal parasitic infection rate between patients with DM and healthy people.

Conclusion

DM patients are at higher risk of infection with intestinal parasites than normal population. In this regard, B. hominis specifically is an important opportunistic infection which can cause gastrointestinal symptoms; therefore patients with DM should be screened for this parasite routinely.

Conflict of Interests

Authors have no conflict of interest.

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