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Frequency of Intestinal Parasites in Patients with Diabetes Mellitus Compared with Healthy Controls in Fasa, Fars Province, Iran, 2018

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Abstract

Background: Parasitic diseases are medically important, especially among immunocompromised patients. Recently, diabetes mellitus (DM) has become one of the most important clinical diseases, and the combination of this disease with parasitic infections can result in serious consequences.
Objectives: The aim of this study was to evaluate the infection of intestinal parasites in patients with DM and compare them with healthy individuals in Fasa city and surrounding health centers.
Methods: In this case-control study a total of 501 patients and healthy individuals (254 patients with DM and 247 healthy controls) referred to health centers in Fasa, Fars province. Fecal specimens of all of these individuals were collected in a special container. These specimens were investigated using direct smear, concentration method, acid-fast and trichrome staining, agar plate cultivation, and Baermann technique.
Results: In this study, 254 diabetic patients (50.7%) and 247 (49.3%) healthy controls were included. The age range of these people was between 25 to 89 years old, with an average age of 57.7 ± 10.7. Our findings revealed that the frequency of intestinal parasites in the control group (18.6%) is higher than the patient group (12.6%).
Conclusions: Although the frequency of intestinal parasites in the control group was higher than the patient group, this does not reduce the importance of preventative programs for these patients. Considering the significant effect of unpurified water on intestinal parasites, it is better to consider this issue more in health programs.

Keywords: Diabetes Mellitus, Intestinal Parasites, Epidemiology, Iran

1. Background

Parasitic infections are among the most widespread of all chronic human infections worldwide. Intestinal parasitic infections remain a significant health problem in both developed and developing countries. These infections can be varied in a broad-spectrum manner of benign to complex situations, and cause mortality, especially in immunocompromised patients such as diabetes disease group (1, 2).

Diabetes mellitus (DM) is a group of metabolic disorders in which a patient has high blood glucose, either because the body does not produce enough insulin, or the cells do not respond to the insulin that is produced (3). Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. These immune or autoimmune disorders can facilitate the risk of parasitic infection in these patients (4).

Some studies conducted in this field and the published data showed a paradoxical picture after the comparison of frequencies in the two groups of patients and controls. Some of them reported higher infections in diabetic patients compared with the control group and some reported contradictory results (5-8). On the other hand, most of these studies are not so inclusive to cover all parasites e.g. opportunistic organisms that have much importance.

2. Objectives

This study was conducted to determine the prevalence of intestinal parasitic infections in patients with

DM in comparison to the healthy individuals in Fasa, Fars province, Iran.

3. Methods

3.1. Study Area

The study area was in Fasa city and rural areas that have medical health centers and medical record units for resident people. It is located at 28.94 latitude and 53.65 longitudes and situated at 1366 meters above sea level (9). We announced all medical health centers to refer the patients with DM in favor of participating in this study.

3.2. Population Study

In this case-control study, according to prevalence Formula and consulting with statistics specialist, a total of 501 cases (161 men and 340 women) recruited to this study that were divided into 254 cases with DM and 247 healthy controls (non-diabetic volunteers). Given the confidence interval of 95%, which is conventional, Z value was 1.96, and d was calculated as 0.05. In this study area, the number of women suffering from diabetes mellitus was higher than men (10) and our diabetes positive cases belonged to the female gender. The age of all participants ranged from 25-89 years. patients suffering from diabetes were approved by physicians and plasma glucose higher than 126 mg/dL level considered to be a patient with DM according to Iran Health Ministry glucose range (11). For the control group, we relied on the physician's diagnosis in the medical centers to approve that the controls do not present upper amounts of glucose limit in routine laboratory tests. Specimens were taken from main medical health centers that monitor and examine population for diabetes and other medical issues in the spring and summer 2018. These rural centers include Fadashkouyeh, Fatemyeh, Myanshahr, and Zahedshahr centers.

3.3. Ethical Aspects

The aim of the study was described to patients or their parents and they signed the written informed consent. The patients with DM were followed by the medical physician, and all infected individuals were treated at the end of the research. All protocols of the study were approved by the Ethics Committee of Hormozgan University of Medical Sciences, in HUMS.REC.1397.71 number.

3.4. Specimen Collection and Processing

3.4.1. Direct Examination

Stool samples were collected from all participants in wide-mouthed containers. All freshly voided samples were examined directly in the beginnings of study. The wet mount was applied using normal saline and iodine solution. A more precise method of direct examination is the concentration method. In addition, Formalin ethyl acetate concentration method helps to find ova and protozoan cysts.

3.4.2. Staining Methods

To detect intestinal parasite trophozoites, permanent stains such as trichrome were performed. Small protozoa, neglected by wet mount tests (either unconcentrated or concentrated samples) are often seen in the stained smears. A portion of stool samples was stained by the acid-fast method to recognize coccidian parasites that may be causative agents of intestinal disturbances.

3.4.3. Baermann Method

To diagnose the Strongyloides stercoralis, some studies suggest the Baermann method because of high sensitivity in the detection of the parasite (12). The Baermann technique is based on the active migration or movement of larvae. A peanut size of stool sample wrapped in gauze and inserted into a glass funnel soaked with warm water. Figure 1 shows the facility required for Baermann method.

Figure 1. Baermann method requirements to collect the *S. stercoralis* larvae

3.4.4. Agar Plate Cultivation for Opportunistic S. stercoralis

The nutrient agar plate method can improve the detection of *S. stercoralis*. The medium was prepared according to the manufacturer's instructions. Movement, copulation, and hatching of the larvae and adult worms may be seen across the medium. A portion of stool would be placed in sealed plates with adhesive tapes to prevent the escape of the larvae to the outside.

A questionnaire was filled by all patients that included questions about the duration of the disease, the drugs used by each patient, the place of residence, age, and gender.

3.5. Statistical Analysis

Data were analyzed using statistical package for social sciences (SPSS) version 20. Summary statistics (frequencies, proportions) in the form of tables, texts, and figures were used to present the findings. The chi-square and related P value were used to check the presence of an association between dependent and independent variables. The $P < 0.05$ was considered statistically significant.

4. Results

A total of 501 participants (Female = 161 ~ 32%, male = 340 ~ 68%) were analyzed during the study. Here, 254 (50.7%) were suffering from diabetes and 247 (49.3%) considered the control group according to the physician's diagnosis. Their age ranged from 25 - 89 years with a mean age of 57.7 ± 10.7 years.

Stool examination showed that in diabetic patients 32/254 were infected at least by one of the intestinal parasites. In the control group, 46/247 were positive for intestinal parasites. Multi-infection occurred in 18 participants of the population, which concomitant infection with 2 organisms, 3 organisms, and 4 organisms were seen in 14, 3, and 1participants, respectively. Table 1 shows the status of the intestinal parasite frequency in the patients with DM and control group.

All patients were classified into 3 age groups. Our data showed that the group of 66 - 79 years old had a higher frequency of the intestinal parasites, which 19 cases (out of 9 participants) were diagnosed.

Comparison of the two groups according to gender revealed that female participants are more infected (45 cases) than males (33 cases) by the intestinal parasites. Also, this frequency difference between males and females exist in the patient group and statistically is significant ($P < 0.05$).

Frequency according to the residence place revealed that 5 cases were resident of the urban area and 73 cases reported from rural areas. Statistical analysis showed a significant difference between the two groups ($P < 0.05$).

Water sources for consumption can have an effect on the rate of infection. Contaminated or unpurified water can cause public health concerns (13). Our data indicated that urban-sourced water consumers showed fewer rates of infection compared to rural-sourced water consumers, 7 (5.6%) cases in urban areas in comparison to 71 (19%) cases in rural areas that were significant ($P < 0.05$). Table 2 represents the intestinal parasitic infections, according to the occupation of the participants.

Data depicted in Table 2 shows that the rate of infection is significantly higher in housewives group ($P = 0.038$).

Various methods were applied to have a correct and precise diagnosis in the enrolled population. Table 3 shows the rates of parasitic infections, according to the diagnostic method.

5. Discussion

The findings of the current study revealed that the frequency of intestinal parasites in the control group is higher than patients with DM that was 18.6% and 12.6%, respectively. After a long survey in databases, we found controversial results about significant differences between the patient and control groups. Tangi (8) in Cameroon carried out a hospital-based study to show the difference between patients and controls. The study results are similar to our study. Overall, the prevalence of intestinal parasites in the control group (23.5%) was higher than the patient group (10%) (8). Altogether, our data were similar to studies of Nazligul et al. in Turkey, Ali et al. in Egypt, and Akinbo et al. in Nigeria (3, 14, 15).

Fattahi Bafghi et al. studied 250 patients with DM and 250 healthy controls in Yazd, Iran, showed higher rates of infection in patients with DM with a higher Odds ratio (16). The same results obtained by the study of Akhlaghi et al. in Karaj, Iran, by direct smear and staining methods, 15.6% in the patient and 10% in the control groups (17). Elnadi et al. in Egypt, after tabulating and analyzing the two groups, showed that the frequency of intestinal parasites in the patient group (25%) was higher than the controls (7%) (1). In the same year, Sabah and Temsah indicated that there is no significant difference between the two groups, but *E. histolytica* was the prominent parasite in the control group (43%) (18). Alemu et al. assessed the magnitude of intestinal parasitic infections and associated risk factors among the patients suffering from DM without the control group. They reported prominent infection with the parasite of *Cryptosporidium* spp. (18.5%) (19).

The main reason for differences between frequencies reported in our study and results of other studies that obtained higher parasitic infections in the patient group compared with control group may be the periodic and monthly survey and examination of documented patients with DM by medical staffs and physicians of health centers in Fasa city.

We exploited the methods mentioned in these studies and used complementary procedures e.g. agar culture and Baermann method to detect opportunistic parasites such

Table 1. Frequency of the Intestinal Parasites in the Patients with Diabetes Mellitus and Control Group[a]

Parasites	Patients with DM	Control	Total	P Values
Blastocystis hominis	23 (9.1)	25 (10.1)	48 (9.1)	0.40
Entamoeba coli	5 (0.2)	12 (4.9)	17 (3.4)	0.06
Strongyloides stercoralis	6 (2.4)	4 (1.6)	10 (2)	0.39
Giardia lamblia	3 (1.2)	8 (3.2)	11 (2.2)	0.10
Chilomastix mesnilli	0	1	1	
Endolimax nana	10 (4)	10(4)	20 (4)	0.56
Entamoeba hartmani	0	1	1	
Iodamoeba bustchlii	0	2	2	
Total	47 (18)	63 (24)	110 (21)	

[a]Values are expressed as No. (%).

Table 2. Frequency of Intestinal Parasites in the Participants Referred to the Rural and Urban Clinics of Fasa[a]

Infection	Occupation						P Value
	Housewives	Employee	Worker	Farmer	Other Jobs	Total	
Infected	45 (57.7)	3 (3.8)	7 (9)	7 (9)	16 (20.5)	78 (100)	0.038
Non infected	316 (74.7)	12 (2.8)	27 (6.4)	19 (4.5)	49 (11.6)	423 (100)	
Total	361 (72.1)	15 (30)	34 (6.8)	16 (5.2)	65 (13)	501 (100)	

[a]Values are expressed as No. (%).

Table 3. Frequency of Intestinal Parasites in the Participants Referred to the Rural and Urban Clinics of Fasa, Fars, Iran According to the Diagnostic Methods[a]

Positive/Negative	Methods					
	Direct Smear	Formalin-Ether	Trichrome Stain	Ziehl-Nelsen Stain (Coccidian)	Baermann For (*S. stercoralis*)	Agar Plate (*S. stercoralis*)
Positive	66 (13.2)	39 (7.8)	54 (10.8)	0	7 (1.4)	0
Negative	435 (86.8)	462 (92.2)	441 (89.2)	501 (100)	494 (98.6)	501 (100)
Total	501	501	501	501	501	501

[a]Values are expressed as No. (%).

as *Strongyloides stercoralis* that were detected in 6 cases of the patient group, while 4 cases were diagnosed in the control group.

According to the age and gender, our study revealed that there were no statistically significant differences between age groups (P = 0.319), but the females had more infection rate compared to the males (P = 0.04). In the category of age variable, our study was not consistent with findings of Fattahi Bafghi et al. (16), but in agreement with Akhlaghi et al. (17) and Mohtashamipour et al. (5). Our study indicated that the infection increases in the age of 66 - 79 years old, which may be related to the reduction of the immune system activity. In the gender category, the study of Mohtashamipour et al. did not show significant differences between groups but reported that the risk of parasitic infection is higher in females rather than males. Some studies found controversial results in this issue and showed that male participants had more infection rates compared to females (19). We concluded that female households are at more risk of parasitic infections and had more infected numbers in comparison to males. The main cause of higher reported frequency of infections in female households may be that female housewives have more contacts with infected fruits, vegetables, and children in these regions. The other reason may be differences in population studies, sample size, and different methods. Our study used more methods and larger samples to diagnose intestinal parasites.

Water sources for consumptions can be a transmission route of infectious diseases. In developing countries, access to both clean water and sanitation are not the rule and waterborne infections are common (20). Our investi-

gation illustrated that the people who consumed purified and treated water supplies were less prone to be infected by intestinal parasites. About 18.9% of the patients had consumed rural (unpurified) water resources and only 5.6% of positive cases had used urban (purified) water supplies.

Comparison of the method in order to diagnose *S. stercoralis* showed that Baermann is a better method to detect the parasite. Direct smear detected 66 cases (out of 76 positive cases) while 27 cases missed in the Formalin-Ether concentration method. The event may be related to trophozoites that were destroyed across concentration. Ziehl-Neelsen method could not find any coccidian-infected case in both groups, whereas some studies reported 1% - 6% among patients with DM (8, 16, 17). Although the plate-agar technique is the gold standard method for the diagnosis of *S. stercoralis*, our study was not able to find any positive samples. As mentioned previously, the best way to detect opportunistic Strongyloidiasis in our study was the Baermann technique that is consistent with Mohammadi-Meskin et al. (12).

5.1. Limitations to This Study

The limitations to this study were comprised of high prices of equipment and diagnostic solutions, the large sample size, and the need for coordination and programming among health centers to invite patients and obtaining the samples.

5.2. Conclusions

It has now been proven that diabetes mellitus is a type of immunodeficiency and we focused on opportunistic parasites in this study. The main point in our study is the association between contaminated water and the frequency of parasitic infections that emphasize the need for clean water supply and water treatment infrastructures.

Supplementary Material

Supplementary material(s) is available here [To read supplementary materials, please refer to the journal website and open PDF/HTML].

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Footnotes

Authors' Contribution: Zahra Poorkhosravani: researcher, Jebreil Shamseddin and Mahmoud Agholi: advisors, Khojasteh Sharifi-Sarasiabi: consultant, Mehrgan Heydari-Hengami: laboratory technician.

Conflict of Interests: The authors certify that they have no conflict of interests.

Ethical Approval: Diabetes patients were followed by the medical physician, and all infected individuals were treated by the end of the research. All protocols of study approved by Hormozgan University of Medical Sciences Ethics Committee, in HUMS.REC.1397.71 number.

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Patient Consent: The aim of study was described to patients or their parents and they signed the informed consent.

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