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Detection of intestinal parasites by different methods in our type 2 diabetic patients

Tip 2 diyabetik hastalarımızda farklı metotlarla intestinal parazitlerin tespiti

Müge Özsan Yılmaz[1*]

1.Hatay Mustafa Kemal University Faculty of Medicine, Endocrinology and Metabolism Department, Hatay, Turkey

ABSTRACT

Aim: Long term persistently high blood glucose levels result in various complications and conditions in diabetic patients. One of them is gastrointestinal disorders and the other is increased risk of infectious diseases like parasitosis. The aim of the study is to demonstrate of intestinal parasites with various techniques in diabetic patients and **confirm of the frequency of the parasites.**

Methods: A total of 65 patients with type 2 Diabetes Mellitus were included in the study. Laboratory tests were done and gastrointestinal symptoms were recorded. Fecal specimens were evaluated with direct microscopy, Kinyoun acid-fast staining method, trichrome staining method and antigen screening test.

Results: Of the patients included in the study 31 were male and 34 were female. While 53.8% of the patients had no chronic complications of diabetes, 33.8% had multiple complications. Thirty (46.2%) patients had gastrointestinal complaints. Examination of stool samples revealed G. intestinalis in two patients (3.07%) , C. parvum in three patients (4.6%), and G. intestinalis + E. histolytica in six patients (9.2%) by RAT. No association was found between the existence of parasite determined by RAT and any of the patient characteristics of age, sex, duration of diabetes, and dyspeptic complaints (p-values are 0.27; 0.14; 0.90; 0.68, respectively).

Conclusion: This is the first study to explore the prevalence rate of parasitosis detected by RAT in patients with diabetes. In this study, we also compared different parasite detection methods in this patient population and showed that RAT is a more sensitive method.

Keywords: Type 2 Diabetes Mellitus, Parasitic Intestinal Diseases, Gastrointestinal Disorders, antigen, microscopy

ÖZ

Amaç: Uzun süreli kalıcı yüksek kan şekeri seviyeleri diyabetik hastalarda çeşitli olumsuz sonuçlara yol açar. Bunlardan bir tanesi gastrointestinal bozukluklar ve bir diğeri de parazitozlar gibi enfeksiyöz hastalıklardaki risk artışıdır. Bu çalışmanın amacı diyabetik hastalarda bağırsak parazitlerinin çeşitli tekniklerle gösterilmesi ve sıklığının belirlenmesidir.

Yöntemler: Çalışmaya 65 tip 2 diyabet hastası dahil edildi. Rutin laboratuvar testleri yapıldı ve semptomları kaydedildi. Fekal örneklerde direkt mikroskopi, Kinyoun asit fast boyama, trikrom boyama, hızlı antijen tarama(HAT) teknikleriyle intestinal parazit arandı.

Bulgular: Çalışmaya dahil edilen hastaların 31' i erkek, 34'ü kadın cinsiyette idi. Hastaların %53.8'inde diyabetin kronik komplikasyonu mevcut olmayıp, %33.8'inde çoklu komplikasyon mevcuttu. Otuz (%46,2) hastada gastrointestinal şikayetler tespit edildi. Dışkı örneklerinin incelenmesinde HAT ile iki hastada (%3.07) G. intestinalis, üç hastada (%4.6) C. parvum ve altı hastada (%9.2) G. intestinalis + E. histolytica tespit edildi. HAT ile belirlenen parazit varlığı ile yaş, cinsiyet, diyabet süresi ve dispeptik şikayetler gibi hasta özelliklerinden herhangi biri arasında ilişki bulunmadı (p değerleri sırasıyla 0,27; 0,14; 0,90; 0,68'dir).

Sonuç: Bu çalışma diyabetik hastalarda HAT ile parazitoz prevalansını araştıran ilk çalışmadır. Ayrıca bu çalışmada bu hasta popülasyonunda farklı parazit tespit yöntemlerini de karşılaştırdık ve HAT'ın daha sensitif bir yöntem olduğunu gösterdik.

Anahtar kelimeler: Tip 2 Diabetes Mellitus, parazitik bağırsak hastalıkları, gastrointestinal hastalıklar, antijen, mikroskopİ

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*Corresponding Author: Müge ÖZSAN YILMAZ, Hatay Mustafa Kemal University Faculty of Medicine, Endocrinology and Metabolism Department, Hatay, Turkey, +903262291000, mozsan@mku.edu.tr

ORCİD: 0000-0001-8346-8941

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Introduction

Diabetes mellitus is a chronic disorder of carbohydrate, lipid and protein metabolism which may result from a total or partial deficiency of insulin or resistance against insulin action in peripheral tissues. Long term persistently high blood glucose levels result in various complications. Furthermore, in diabetic patients, the frequency of gastrointestinal (GI) symptoms is increased. Although the exact pathogenesis of diabetes related GI disease is not known clearly, it is thought that the underlying gastroparesis, depression, and anxiety disorders may induce these symptoms [1]. It was concluded that poor glycemic control might increase the frequency of these symptoms in various studies, albeit with conflicting results [2].

Diabetes mellitus increases the risk of infections. Neutrophil chemotaxis, adherence of neutrophil to the vascular endothelium, phagocytosis, intracellular bactericidal activity, opsonization, and cellular immunity are suppressed in case of persistent hyperglycemia [3]. Because of these disturbances diabetes may acceptable as immunodeficiency condition. Immundeficient patients are more susceptible to infections with opportunistic parasites such as Entamoeba histolytica, Cryptosporidium parvum, and Giardia intestinalis. Host-parasite interactions and a decline in or loss of host's resistance to parasites play a role in the transformation of parasites to the pathogen status or increase in their pathogenicity [4].

Amebiasis remains a significant health problem for developing countries [5]. Humans are infected by two species of Entamoeba, which are morphologically indistinguishable. These are infective E.histolytica and nonpathogen E. dispar. The differential diagnosis for E.histolytica and E. dispar can be achieved by the detection of specific antigens.

Cryptosporidium species are one of the commonly detected parasites in humans, domestic animals, and wild vertebrates [6]. While cryptosporidiosis causes mild diarrhea in immunocompetent individuals, it can cause life-threatening severe diarrhea and respiratory system infection in immunocompromised patients [7].

G. intestinalis is one of the leading culprits of endemic and epidemic diarrheas globally. The prevalence of giardiasis varies between 1.9% – 37.7% in studies conducted in Turkey [8,9]. Giardiasis, which might be seen in acute and chronic forms, could be asymptomatic and also cause life-threatening diarrhea.

Several studies were published as to the diagnosis of intestinal parasites in various patient groups in our country [4,10-12]. To the best of our knowledge, there is no study in the literature studying prevalence rates of amebiasis, giardiasis, and cryptosporidiosis by Rapid Antigen Test (RAT) in diabetic subjects. Hence, we aimed to investigate frequencies of amebiasis, giardiasis, and cryptosporidiosis in diabetic patients by means of an antigen screening test and to study whether there is an association between these parasites and diabetic gastrointestinal complaints. We also planned to compare the rate of parasite presence by different laboratory techniques.

Methods

A total of 65 patients with Type 2 Diabetes Mellitus and aged 18 to 65 who were assessed at adult Endocrinology and Metabolism outpatient clinic between January and June 2016 were included in this prospective study. An informed consent form was signed by all patients who were eligible for the study and wished to participate. The patients who have any gastrointestinal malignancy and immunosuppressive condition history or presence of clinically significant chronic disease other than diabetes mellitus were excluded. Age, gender, and concomitant diseases of the patients were recorded.

Serum glucose, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), sodium (Na), potassium (K), hemoglobin A1c (HbA1c) and complete blood count (CBC) were measured in all participants. CBC tests were done with Mindray BC 6000 (Mindray Co., Shenzhen, China) a haematology device. Biochemistry parameters (Glucose, creatinin, AST, ALT, Na, K, Albumin, Calcium, Phosphorus, Magnesium, Total cholesterol, HDL cholesterol, LDL cholesterol, Triglyceride) were studied by spectrophotometric method in Siemens Advia 1800 biochemistry autoanalyzer (Siemens, Germany) and HbA1c

levels were studied by HPLC (High Performance Liquid Chromatography).

Patients were questioned in detail in terms of chronic complications of diabetes and the findings were supported by hospital records. Patients for whom appropriate and sufficient information could not be obtained from their anamnesis and records were screened for chronic complications. For this purpose, fundus examination was done for diabetic retinopathy, sensory examination evaluation of orthostatic hypotension for diabetic neuropathy, microalbumin level in 24-hour urine and GFR calculation for diabetic nephropathy, detailed cardiac examination, Doppler USG for carotid and peripheral arteries, and angiographic examinations were performed when necessary.

Patients were questioned regarding gastrointestinal symptoms such as postprandial fullness, early satiety, epigastric pain, epigastric burning, diarrhea, constipation for the last three months. If the patient had at least one of these symptoms, it was deemed that the patients had dyspepsia. Fecal specimens collected from 65 patients were brought to the Parasitology Laboratory in which direct microscopy results were obtained by precipitation with native, Lugol's iodine and formalin ethyl acetate techniques by a consultant parasitologist. Samples were also examined for intestinal parasites using Kinyoun acid-fast staining method, trichrome staining method, and antigen screening test.

1-Kinyoun Acid-Fast Staining Method: Smears were prepared from the collected stool samples and allowed to dry. Then, they were fixed in pure methanol for one minute. The smears were stained with Kinyoun carbol fuchsin for five minutes and then shaken with 50% alcohol. After that, the specimens were washed with tap water and held in a chalet containing 1% sulfuric acid for two minutes and then washed in the tap water again. After leaving for one minute in the methylene blue-containing chalet, they were washed with the tap water, then dried and examined via 100X objective of a microscope [13].

2-Conventional Trichrome Staining Method: Stool samples were spread on the slides. After the edges of slides began to dry, they were held in the Schaudinn fixative at least half an hour. Respectively, they were left in 70% ethyl alcohol for five minutes, in iodine solution of D'Antoni for three minutes, in two chalets containing 70% ethyl alcohol for two and five minutes, and in trichrome staining solution for eight minutes. Then, excess dye on the slides was removed. They were soaked three times in 90% acid-alcohol and shaken in two chalets containing 95% ethyl alcohol. The slides were held in two chalets containing carbol-xylene for two and five minutes, in two chalets containing xylene for two and five minutes, and then they were allowed to dry.

3-RIDA Quick Cryptosporidium / Giardia / Entamoeba Combicasette antigen test (R-Biopharm AG, Germany) was used as an antigen screening test. The rapid antigen test (RAT) is a one-step immunochromatographic lateral flow test. The specific antibodies themselves directed against each parasite bind to green (Entamoeba specific), red (Giardia specific), or blue (Cryptosporidia specific) latex particles. Other antibodies specific to these three pathogens bind firmly to the membrane. The stool sample is suspended in the extraction buffer and then precipitates. Clear supernatant part of the sample is placed on the test area.

Ethics committee approval of the study was obtained from Hatay Mustafa Kemal University Tayfur Ata Sökmen Faculty of Medicine Clinical Research Ethics Committee with the decision number 131, dated 17.11.2015.

Statistical analysis

Data were recorded to SPSS 21 System with double check and analyzed using SPSS 21 with 95% confidence. After evaluating normality with Shapiro Wilk test, Student-t test was used for normally distributed data and Mann Whitney U test was used for data not normally distributed. In categorical data, chi-square tests were used. The significance limit for all tests was set at 0.05. ROC analysis was performed to evaluate whether there would be parasitosis according to the WBC value. The performance of the assay was calculated by the area under the curve (AUC) sensitivity and specificity values. In addition, PPV (positive predictive value), NPV(negative predictive value), Sen (Sensitivity) and Spe (Specifity) values were calculated for RAT when direct microscopy was

accepted as gold standard.

Results

Sixty-five patients with type 2 Diabetes Mellitus were included in the study. Of all participants, 47.7% (n=31) were males, and 52.3% (n=34) were females. The mean age of the patients was 51.5 ± 12.3 years. 53.8% of the patients had hypertension and 52.3% had hyperlipidemia. The median duration of diabetes mellitus diagnosis was 7 (1-20) years. While 53.8% of the patients had no chronic complications of diabetes, 33.8% had multiple complications. Thirty (46.2%) patients had gastrointestinal complaints, of which nineteen (n=19) had dyspepsia and the rest had constipation or diarrhea. The frequency of gastrointestinal complaints was 44.1% (n=15) in women and 45.1% (n=14) in men (p=0.87).

Examination of stool samples revealed C. parvum in one patient (1.5%) by Kinyoun method, G. intestinalis + E. histolytica in six patients (9.2%) by trichrom method, G. intestinalis in seven patients (10.7%) by direct microscopy, G. intestinalis in two patients (3.07%) , C. parvum in three patients (4.6%), and G. intestinalis + E. histolytica in six patients (9.2%) by RAT. No association was found between the existence of parasite determined by RAT and any of the patient characteristics of age, sex, duration of diabetes, and dyspeptic complaints (p-values are 0.27, 0.14, 0.90, 0.68, respectively). No association was found between HbA1c and other biochemical parameters and the existence of parasite (Table 1). There was not an association between the presence of parasite and hemoglobin, eosinophil, and lymphocyte counts (p>0.05). The association between the number of white blood cells (WBC) and the existence of parasites was not statistically significant. However, the frequency of parasite positivity increased as the number of WBCs decreased (Table 1). The area under the curve (AUC) was calculated as 0.711 and p=0.023 in the ROC analysis based on the presence or absence of parasites for the WBCs. The cut-off value for the WBCs was calculated as 6860 /μL with 66% sensitivity and 77% specificity (Figure 1).

No significant association between the parasite positivity and diabetic complications such as diabetic neuropathy, retinopathy, coronary artery disease, cerebrovascular disease and comorbidities was found, either (p>0.05) (Table 2).

When the RAT was compared with other methods in terms of parasite evaluation, the samples deemed as negative by direct microscopy was negative in 91.4% of the samples studied with RAT method, as well. All samples that were considered as positive by direct microscopy were also found to be positive with RAT (Table 3).

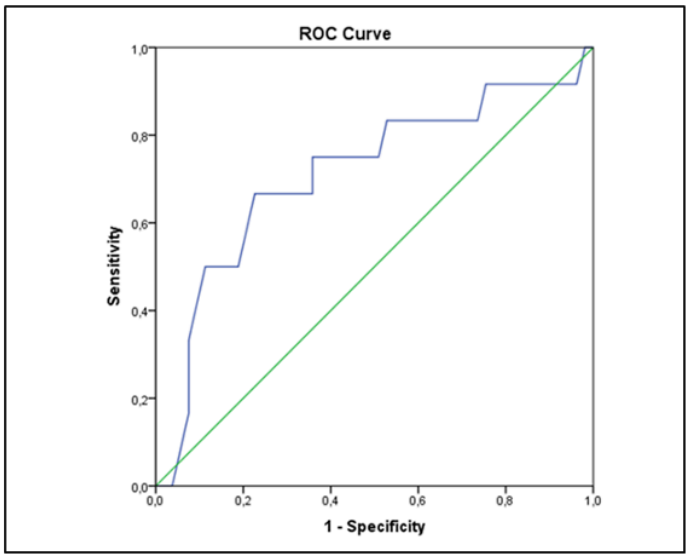

Figure 1. Association between existence of parasites and white blood cell count

Discussion

As the number of people with diabetes increases rapidly the associated complications of diabetes will increase inevitably. In the long term, diabetes leads to chronic complications including various gastrointestinal symptoms in which neuropathy is an important causative factor. The prevalence of these symptoms varies according to ethnic groups and the type of diabetes [1]. While it has been reported that gastric emptying is delayed in 25-55% of type 1 diabetic patients, and in 30% of type 2 diabetic patients, the prevalence of gastroparesis in the community has been reported to be approximately 5% in Type 1 diabetes and 1% in Type 2 diabetes [14]. In our study, 46.2% of diabetic patients had gastrointestinal symptoms, most common of which were dyspepsia and constipation/diarrhea. Gastrointestinal complaints related to diabetes are known to be more prevalent among women. The reason for this difference could

Table1. Association between existence of parasites and biochemical and hemogram results

	Parasite (+)*			Parasite (-)*			p
	Mean±SD	Median	Min-Max	Mean±SD	Median	Min-Max	
A1C (%)	8.97±2.24	8.80	6.4-16.4	8.85±1.34	8.80	6.8-11.5	0.899
Glucose (mg/dL)	198.81±79.84	193.00	87-391	176.83±84.49	145.00	100-400	0.290
Creatinin (mg/dL)	0.82±0.14	0.79	0.56-1.39	0.97±0.26	0.90	0.56-1.39	0.029
LDL-chol (mg/dL)	118.58±63.33	104.00	7-363	91.92±42.38	89.50	7-163	0.099
HDL-chol (mg/dL)	41.88±16.61	41.00	13-129	37.83±8.93	40.50	19-51	0.388
Total chol (mg/dL)	209.24±110.08	188.00	78-838	243.42±190.63	192.00	144-838	0.806
Triglyceride (mg/dL)	192.11±144.05	157.00	49-846	218.08±179.2	163.00	68-711	0.919
AST (U/L)	22.21±12.48	18.00	9-67	21.67±5.66	22.00	11-31	0.330
ALT(U/L)	23.79±13.1	17.00	7-60	24.58±7.9	26.00	13-38	0.466
Na (mmol/L)	137.53±2.4	138.00	132-141	136.33±1.72	137.00	134-139	0.058
K (mmol/L)	4.54±0.44	4.40	3.6-5.5	4.45±0.38	4.45	3.6-4.9	0.568
Albumin (g/dL)	3.64±0.25	3.60	3.2-4.3	3.7±0.29	3.75	3.2-4.3	0.388
Ca (mg/dL)	8.97±0.37	9.10	8.4-10.4	9.11±0.51	9.10	8.5-10.4	0.490
P (mg/dL)	3.74±0.62	3.70	2.4-5.2	3.85±0.71	3.66	3-5.2	0.965
Mg (mg/dL)	1.84±0.26	1.80	1.4-2.72	1.84±0.13	1.80	1.56-2.05	0.413
WBC /µL	7191.33±2041.92	6595.00	5200-12070	8669.02±2134.01	8150.00	4400-12970	0.023
Eosinophil /µL	186.67±130.55	160.00	0-450	236.79±170.05	190.00	0-660	0.462
Lymphocyte/µL	2324.17±883.4	2405.00	950-3590	2790.94±1094.55	2830.00	70-6490	0.148
Platelet /µL	255.83±52.82	249.50	176-344	292.62±96.6	265.00	150-554	0.374

*with Rapid Antigen Test, SD: Standard deviation, Min: Minimum, Max: Maximum, LDL-chol: Low density lipoprotein cholesterol, HDL-chol: High density lipoprotein cholesterol, Total chol:Total cholesterol, AST: Alanine aminotranspherase, AST: Aspartate aminotranspherase, Na:Sodium, K:potassium, P:Phosfor, Mg:Magnesium, WBC: White Blood Cell, p:Statistical significance for Student-t and Mann-Whitney U tests, p≤0,05

Table 2. Association between existence of parasites and comorbidity, complications

Comorbidity / Complications		n/ percent	Parasite (-)*	Parasite (+)*	p
Hypertension	(-)	n	24	6	0.767
		%	80.0	20.0	
	(+)	n	29	6	
		%	82.9	17.1	
Dislipidemia	(-)	n	24	7	0.414
		%	77.4	22.6	
	(+)	n	29	5	
		%	85.3	14.7	
Complications	(-)	n	29	6	0.263
		%	82.9	17.1	
	Neuropathy	n	3	0	
		%	100.0	0.0	
	Retinopathy	n	0	1	
		%	0.0	100.0	
	Coronary arter disease	n	2	0	
		%	100.0	0.0	
	Cerebrovascular disease	n	1	1	
		%	50.0	50.0	
	Multiple complications	n	18	4	
			81.8	18.2	

Table 3. Association between rapid antigen test and the other tests

Comorbidity / Complications		n/ percent	Parasite (-)*	Parasite (+)*	p
Kinyoun	Negative	n	53	11	0.185
		%	82.8%	17.2%	
	C. parvum	n	0	1	
		%	0,0%	100,0%	
Trichrom	Negative	n	53	6	0.001
		%	89.8%	10.2%	
	G. Intestinalis + E. histolytica	n	0	6	
		%	0.0%	100.0%	
Direct microscopy	Negative	n	53	5	0,001
		%	91.4%	8.6%	
	G. Intestinalis	n	0	7	
			0.0%	100.0%	

*with Rapid Antigen Test, PPV=0.58, NPV=1.00, Sen=1.00 and Spe=0.91 for RAT, PPV: Positive predictive value, NPV: Negative predictive value, Sen: Sensitivity, Spe: Spesifity, RAT:Rapid Antigen Test, C. parvum: Cryptosporodium parvum, G. Intestinalis: Giardia intestinalis, E. histolytica: Entamoeba histolytica n: Number of patients, p:Statistical significance for Chi-square test, p≤0,05

not be explained clearly yet, but it is associated with a high prevalence of abdominal bloating/ fullness in women [15]. However, our results revealed that the prevalence of these complaints was similar between females and males (44.1%; 45.1%, respectively).

Although the entire pathogenetic process of gastrointestinal complications of diabetes mellitus is not well understood; gastroparesis, depression, and anxiety disorders may impact these symptoms. The effect of poorly controlled diabetes on these symptoms is not clear. However, it is known that the level of glycemic control affects gastric emptying [1]. Persistent poor glycemic control may lead to damage to the vagus nerve, and autonomic neuropathy in diabetic patients. This process usually takes about ten years [16]. Poor glycemic control can shorten this duration. However, it is controversial whether it increases symptoms [1,2]. Although the median duration of diabetes was seven years in our patients, higher HbA1c mean value could explain the more frequent gastrointestinal symptoms in our study population.

The pathogenesis of functional gastrointestinal disorders is poorly understood in healthy population as well as in diabetic patients, however, factors such as prolonged gastric emptying, tenderness in stomach tension, and infiltration of the duodenum with inflammatory cells might impact pathogenesis [17,18].

Chronic dyspeptic complaints can be observed following bowel infections. Many pathogens including G. intestinalis were held responsible for the development of these complaints [19]. While E. histolytica causes acute abdominal pain and diarrhea, it may also give rise to abscess formation throughout the body, especially in the liver. C. parvum may present as a diarrheal illness in immunocompromised individuals [4,5]. In our study, we did not show any association between the presence of any of the parasites studied and the symptoms such as dyspepsia, constipation, and diarrhea. This may be in part due to the small number of participants in this study.

Parasitic diseases still pose a significant health problem for underdeveloped and developing countries. These diseases are among the important causes of the morbidity and the mortality in these regions. For example; every year, 50 million people in the world are infected with amebiasis, only 10% of them are symptomatic and 100 000 people die [5]. In Turkey, intestinal parasitosis is common in regions where infrastructure problems could not be solved, and compliance with personal hygiene is poor.

Diabetes increases the risk of various infections. Hyperglycemia and hyperglycemia-induced reduction in immune response, vascular insufficiency, peripheral and autonomic neuropathy, colonization of skin and mucosa with some microorganisms are among the causes of this predisposition. Hyperglycemia affects chemotaxis of neutrophils, adherence to vascular endothelium, phagocytosis, intracellular bactericidal activity, opsonization, and cellular immunity favorably [3]. Immunosuppressed patients are more likely to be infected with opportunistic parasites such as E. histolytica, C. parvum, and G. intestinalis. Host-parasite associations and decrease or complete loss of host resistance to parasites play a role in the transformation of parasites into a pathogen or increased pathogenicity [4]. Our results demonstrated that as the number of WBCs, which is an indicator of the host's reaction to parasites, was lower than approximately 7000/μL, the prevalence of parasitosis increased.

There are various studies in the literature reporting the prevalence of different parasites in diabetic patients. In a study of 100 diabetic patients from Egypt, G. intestinalis was detected in 22%, E. histolytica in 7% and C. parvum in 5% of the patients [20]. In another study involving more patients, the prevalence of C. parvum was reported as 8.4% [21]. In another study, the prevalence of G. intestinalis was 13%, while E. histolytica was seen in 1% [22]. In a study conducted in our country, the prevalence of G. intestinalis in diabetic patients was found to be 15% [23]. In our study, the prevalence of all parasites combined was 16.9% studied by RAT, which had the highest sensitivity among the techniques we utilized. The prevalence rates of parasites in our study were 4.6% for C. parvum and 3% for G. intestinalis. The prevalence of G. intestinalis was less than other studies reported in the literature, for which a relatively small sample size of our study could

account. In our research, while E. histolytica was not seen alone, the prevalence of it with G. intestinalis was 9.2%. The prevalence of multiple parasitic infestations was higher in our study, as in other studies [21,22].

Uyar and Taylan Ozkan reported that the diagnosis of G. intestinalis and other protozoa was usually made by direct microscopic examination and it was a cheap technique [24]. However, they stated that the microscopic examination, especially by augmentation methods, was demanding and required experienced staff, and intensive work. On the contrary, antigen detection methods (Direct fluorescent antibody-DFA, enzyme immunoassay-EIA, rapid antigen tests-RAT) are useful in the diagnosis of protozoa because they are rapid and do not require experienced staff [24].

Aziz et al. emphasized that immunological methods such as DFA were more sensitive, useful, faster, and cost-effective than traditional microscopic techniques in the diagnosis of G. intestinalis [25]. Also evident in our patient group, RATs can provide a practical, early, and safe diagnosis for immunocompromised patients in centers that do not have adequate laboratory equipment and experienced staff.

Limitations of the Study:

Despite the interesting findings, the main and the first limitation of our study was the sample size. The sample size available was small. If we had a larger number of patients, we could have divided them into subgroups according to the characteristics of the patients especially for diabetic neuropathy existence. We could obtain more significant results in these subgroups in terms of parasitosis. Second we did not have a control group to account for the prevalence of parasitosis in the general population in our region. In addition, we did not use any objective measure to diagnose specific types of diabetic gastrointestinal complications such as gastric emptying study.

Conclusion

Since cellular immunity is defective in diabetic patients it has been assumed that frequency of parasitic infestations might increase among diabetic patients, which is confirmed in some but not all studies. Our results revealed a similar frequency of intestinal parasitic infections reported in the literature; however, it seems that the most sensitive method to detect these infections is antigen screening test. With such easy to use tests, diabetic patients can easily be screened with this regard. Hence, we can distinguish gastrointestinal symptoms related to diabetic intestinal autonomous neuropathy from those related to intestinal parasites. Parasitosis such as E. histolytica, C. parvum, G. intestinalis may have an effect on gastrointestinal problems in diabetic patients, but large sample studies are required to demonstrate these associations.

Although we have some limitations in this study on the other hand it has some strength as well. This is the first study to explore the prevalence rate of parasitosis detected by RAT in diabetic patients. Furthermore, we also compared different parasite detection laboratory methods in this patient population.

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