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Investigation of fecal pancreatic elastase-1 levels in type 2 diabetic patients

Diabetik hastalarda fekal pankreatik elastaz düzeylerinin değerlendirilmesi

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Background/aims: *Due to the close anatomical position between the endocrine system cells and the exocrine system cells in the pancreas, some interactions could be expected in these two different types of cells. This possible exocrine dysfunction may cause difficulties in the management of blood glucose level because of secondary malabsorption which may have resulted from the exocrine dysfunction. Taking this possibility into account, we aimed to investigate the exocrine function of the pancreas in 32 diabetic patients and in 12 healthy control subjects in this study.* ***Methods:*** *Fecal pancreatic elastase-1 (PE1), which has a high sensitivity and specificity, was measured in serum samples by ELISA specifically for this purpose.* ***Results:*** *It was found that the exocrine function declined in 28% of type 2 diabetic patients, while there was no decrease in the control subjects. However, there were no significant correlations between pancreatic elastase levels and the duration of diabetes, glycemic control, or consumption of alcohol.* ***Conclusions:*** *These findings suggest that evaluation of the exocrine function in diabetic patients might be useful for better management of diabetic patients.*

Key words: Pancreatic elastase, diabetes mellitus, exocrine function

Amaç: *Pankreasta endokrin sistem hücreleri ile egzokrin sistem hücreleri arasındaki yakın komşuluk ilişkisi dolayısıyla, bu iki hücre tipi arasında bazı etkileşimlerin olması olasıdır. Bu olası egzokrin fonksiyon bozukluğu malabsorbsiyonla sonuçlanarak kan şekerinin düzenlenmesinde zorluklara neden olabilir. Tüm bunlar dikkate alınarak, çalışmamızda diabet tanısı kesinleşmiş ve insulin kullanan 32 tip 2 diabetli hastadan ve 12 sağlıklı bireyden oluşan kontrol grubunda, egzokrin pankreas fonksiyonları değerlendirmek üzere pankreatik elastaz ölçümünün tip 2 diabetik hastalarda klinik faydasını araştırmayı amaçladık.* ***Yöntem:*** *Egzokrin fonksiyonu değerlendirebilmek için yüksek sensitivite ve spesifiteye sahip fekal pankreatik elastaz-1 testi kullanıldı. Alınan serumlar ELISA metoduyla analiz edildi. Bunlara ilave olarak PE1'in cins, diabet süresi, glukoz düzeyleri, vücut kitle indeksi gibi karakteristikleri ile olan ilişkilerini inceledik.* ***Bulgular:*** *Tip 2 diabetes mellituslu hastaların 9'unda (% 28) azalmış egzokrin fonksiyon saptanırken de kontrol grubunda fekal elastaz düzeylerinde herhangi bir azalma saptanmadı. Fakat, tip 2 diabetes mellituslu hastalardaki elastaz düzeylerinin kontrol grubuyla karşılaştırıldığında anlamlı olarak azalmış olduğu gözlense de, elastaz düzeylerindeki azalmanın diabet süresi, glisemik kontrol ve alkol tüketimi ile ilişkisini gösterebilecek anlamlı bir korelasyona rastlanmadı.* ***Sonuç:*** *Elde edilen bulgular diabetes mellitus gibi sık görülen bir hastalığın uzun vadeli tedavisinde egzokrin yetmezliğin değerlendirilmesinin yararlı olduğunu düşündürmektedir.*

Anahtar kelimeler: Pankreatik elastaz, diabetes mellitus, egzokrin fonksiyon

INTRODUCTION

Diabetes mellitus is quite a common disease in which either insulin secretion is insufficient (type 1 diabetes) or a resistance to insulin action along with/without this insufficient secretion occurs in the target tissues (type 2 diabetes). Hyperglycemia is a well-known result of uncontrolled diabetes. Prolonged hyperglycemia results in damages to tissues, which causes dysfunctions of some organ systems due to micro and macrovascular involvement. Some organ dysfunctions, including enzyme or hormone secretions, can thereby result from this disorder.

Although the endocrine system seems to be mostly affected in diabetes, exocrine functions should

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also be expected to be involved due to the exocrine system's close proximity to the endocrine system. For example, the fibrosis in chronic calcified pancreatitis that develops secondary to chronic alcoholism blocks the function of islet cells by damaging vascular drainage (1). In the same way, the other disorders of the pancreas (e.g. acute or chronic pancreatitis, cystic fibrosis, hemochromatosis, pancreas carcinomas) mainly disturb the exocrine system, but the endocrine system could also be affected, which causes diabetes, although the rate is only 0.5-1.15% (2). However, the rate of endocrine system deterioration is much higher in diabetic patients (3). Varying and often-seen complications such as autonomic neuropathies (AN) and gastroparesis secondary to AN, which all develop in time in diabetes, raise difficulties in the control of metabolism. In addition, exocrine insufficiency of the pancreas disturbs the absorption of foods, which in turn causes further deregulation of the blood glucose. As is well known, the exocrine capacity of the pancreas is responsible for the production and secretion of digestive enzymes. A properly working exocrine function is therefore vital for digestion and absorption of food in the gastrointestinal tract. It is thought that fat malabsorption and malabsorption-related vitamin deficiencies in patients with exocrine insufficiency of the pancreas may occur if these patients are not diagnosed initially and treated immediately. An efficient digestive process is especially important in diabetics due to the fact that the regulation of blood glucose and the drugs used in these patients are firmly associated with caloric intake, which is directly proportional to food digestion. It was found that the size of the pancreas in diabetics was smaller compared to healthy individuals (4). In type 1 diabetics, it was observed that the Langerhans islets and their surrounding tissue appeared normal when they died at an early age. However, both the islets and the surrounding tissue become atrophic if they died at a later age (5). In type 2 diabetics, such changes are still, to some extent, observable but not as severe as in type 1 diabetics. Ultrasonographic examinations already proved the smaller pancreas in diabetic subjects compared to those in the control subjects (6). Therefore, the evaluation of the exocrine function in diabetic patients seems to be important. There are a number of direct and indirect tests used to prove this. However, it appears that the direct ones in particular are mostly invasive and too complicated to be performed on all patients, as well as not being well standardized. For example, the secretin-pancreozymin test is invasive, hard to apply, and expensive (7), while one of the non-invasive tests, the fecal chymotrypsin test, has a broad range of sensitivity (72 to 90%) and specificity (49 to 90%) (8-11).

Szeigoleit and co-workers discovered the endoprotease, which was fecal pancreatic elastase-1 (PE1). This novel parameter has been used to detect the exocrine insufficiency of the pancreas. PE1, a member of the acidic elastase family (12), is present in both feces and pancreatic fluid. PE1 has a number of advantages: it is not affected by intestinal transit and it is accumulated at a 5-6 times higher rate in feces than pancreatic fluid (13, 14); it is stable for a long time (15); it is not disturbed by medications, gastric surgery, dysmotility, or small intestine diseases (16); and it is detected by sandwich type ELISA quantitatively. For these reasons, PE1 seems to be a non-invasive and simple test that can be used in the diagnosis of pancreatic exocrine insufficiency (17). As an indirect test, PE1 measurement seems to be helpful in the evaluation of exocrine sufficiency of the pancreas in some disorders, such as cystic fibrosis (18) or chronic pancreatitis (19). Moreover, PE1 was reported to be superior to both fecal lipase and fecal chymotrypsin tests in the assessment of exocrine pancreatic function (20, 21).

The aim of this study was to investigate the exocrine function of the pancreas in diabetic patients by measuring PE1 levels in feces. In addition to this, it was also aimed to analyze possible correlations between PE1 levels and some characteristics of the groups such as gender, the duration of diabetes and the levels of glucose. It was found that

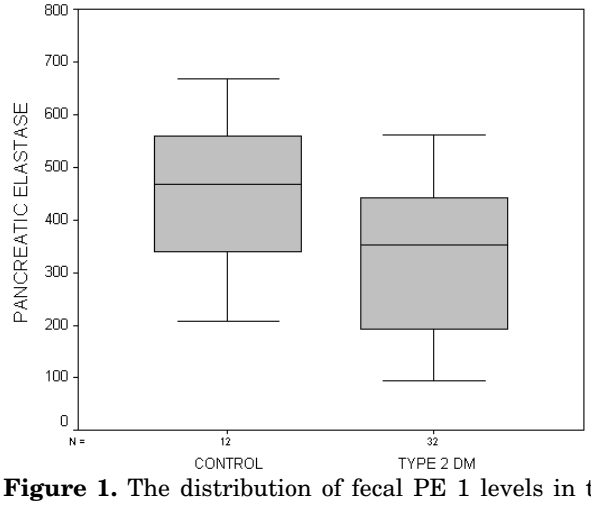

Figure 1. The distribution of fecal PE 1 levels in the groups of control and diabetic subjects

diabetic patients had statistically significant lower PE1 levels compared to those in normal subjects.

MATERIALS AND METHODS

Patient Selection

In the study, 32 outpatients who were admitted to the Medical School Hospital of Uludağ University were included. They were all type 2 diabetics. All the patients were using insulin for the regulation of their blood glucose level. The control group consisted of 12 healthy, age- and sex-matched volunteer subjects with no known medical problems. Subjects with a history of alcohol abuse, gastrointestinal surgery, cancer or inflammatory bowel diseases were not included. None of the patients involved in this study were using acarbose or orlistat. The local Ethical Committee, Uludağ University Medical Faculty Ethics Committee (approval number 24.03.2003, 2003-7 decision number 8) approved the study. Consent was obtained from all the subjects and they were not charged for the pancreatic elastase tests.

Method

Feces samples were stored at -20°C until analysis. The duration of storage was about two months. Fecal elastase levels were measured using a commercial kit (Elastase 1 stool kit, ScheBo Biotech, Wettenberg-Giessen, Germany) which was ordered by the Central Laboratory of the Medical School Hospital of Uludağ University. The kit contains two different monoclonal antibodies that specifically recognize different epitopes on human elastase. The results were presented in mg/g stool. According to the company's instruction booklet, it was accepted that levels of <100 μg/g, 100-200 μg/g, and >200 μg/g were considered as severe pancreatic insufficiency, mild pancreatic insufficiency, and normal pancreatic function, respectively. In addition, we analyzed the possible correlations between PE1 levels and some characteristics of the groups such as gender, the duration of diabetes and the levels of glucose.

Statistics

Mann-Whitney U test was used to compare the means of elastase levels between the groups, while correlations were analyzed using Pearson's correlation test. A package program, SPSS 11.0, was used to perform the analysis, and a value of $p<0.05$ was considered as significant.

RESULTS

The characteristics of the groups were as follow: the diabetic group consisted of 17 (53.1%) women and 15 (46.9%) men (n=32). Two (6.3%) of all diabetic patients were alcohol consumers and six (18.8%) were cigarette smokers. In the control group, the ratio of women to men was similar (50%). In this group, the number of alcohol consumers and cigarette smokers was one (8.3%) and one (8.3%), respectively. In both groups, the average age was approximately the same (Table 1).

Table 1. Features of the patient and control groups

	Control (n=12) n (%)	**Type 2 DM (n=32) n (%)**
Age (mean)	55	58
Sex		
Women	6 (50)	17 (53.1)
Men	6 (50)	15 (46.9)
BMI (kg/m^2)		
≥25	5 (41.7)	19 (59.4)
<25	7 (58.3)	13 (40.6)
Duration of diabetes		
< 5 years	N/A	4 (18.8)
≥ 5 years	N/A	28 (81.2)
HbA_{1c}		
< 7 %	12 (100)	3 (10)
≥ 7 %	0 (0)	27 (90)
Insulin use	N/A	32 (100)

N/A: nonapplicable. DM: diabetes mellitus. Values in parentheses show the percentage of subjects in the relevant group

The level of elastase in the diabetic group was significantly lower than in the control group (p=0.015). In eight (25%) of the type 2 diabetic patients, their mean PE1 level was between 100-200 mg/g, suggesting mild pancreatic insufficiency, while it was lower than 100 μg/g in only one (3.1 %) patient, suggesting a severe pancreatic insufficiency. In the remaining 23 diabetic patients (71.9%) and in the control subjects, the mean level of PE1 was higher than 200 μg/g, which is considered as a normal exocrine function of the pancreas (Table 2).

Table 2. The number (percentage) of patients classified into three different groups according to their fecal PE 1 levels

Groups	**<100 μg/g**	**100-200 μg/g**	**>200 μg/g**
Control (n=12)	0	0	12 (100%)
Type 2 DM (n=32)	1 (3.1%)	8 (25%)	23 (71.9%)

DM: diabetes mellitus. Values in parentheses show the percentage of subjects in the relevant group

Table 3. Comparison of fecal pancreatic elastase-1 levels in control subjects and type 2 diabetics

	Control μg/g	Type 2 DM μg/g	p
Mean±SD	455.2±154.1	331.3±138.6	0.015
Median	467.5	352.7	

P value was assessed by Mann-Whitney U test. DM: diabetes mellitus

It was found that the PE1 level of the diabetic group was significantly lower than that of the control group ($p<0.01$). The mean levels of PE1 were 331.3 μg/g (138.6 ± SD) (median: 352.7 μg/g) in the diabetic group and 455.2 μg/g (154.1 ± SD) (median: 391.1μg/g) in the control group (Table 3).

There were no statistically significant correlations between PE1 levels and the duration of diabetes ($p=0.855$, $r=0.032$), consumption of alcohol ($p=0.820$, $r=0.040$) or HbA_{1c} levels ($p=0.337$, $r=-0.173$) of diabetic patients. There were also no statistically significant differences between PE1 levels when we grouped diabetic patients according to sex ($p=0.865$), duration of diabetes ($p=0.210$) and HbA_{1c} levels ($p=0.351$). There were no significant differences between the diabetics whose body mass index (BMI) was higher or lower than 25 ($p > 0.05$) (Table 4).

Table 4. Comparison of fecal pancreatic elastase-1 levels in type 2 diabetics according to sex, duration of diabetes and BMI

Characteristics	Fecal Elastase μg/g Mean±SD	p
Sex		
Women	325.1±159.7	0.865
Men	338.3±115.4	
Duration of diabetes		
< 5 years	399.0±144.5	0.201
≥ 5 years	321.6±137.7	
BMI		
<25	336.0±154.8	0.863
≥25	328.1±130.7	

p value was assessed by Mann Whitney U test. BMI: body mass index

DISCUSSION

In this study, we investigated the level of PE1 in feces in order to understand whether or not the exocrine function of the pancreas is disturbed in diabetic patients. It was found that the level of PE1 was significantly lower in nine (28%) diabetic patients compared to the healthy (control) subjects ($p<0.01$). It is thought that the fecal PE1 test has better specificity and sensitivity in the evaluation of pancreas exocrine function than the direct tests, which are considered as gold standard. For example, the performance of fecal PE1 was tested for this purpose in one study using the secretin-cerulein test as a reference method (gold standard) in the patients with exocrine insufficiency of the pancreas (22). They found that when the cut-off level for PE1 was 200 μg/g faces, the sensitivity was 63% in patients with light pancreatic insufficiency, and increased up to 100% in those with moderate and severe insufficiency. In the same study, the specificity of the fecal PE1 test was 93%. In another study, PE1 was also reported to have higher sensitivity than chymotrypsin in the stool and to be moderately correlated with duodenal enzyme outputs (23). Taken together, it seems that the fecal PE1 might be considered as a reliable parameter for the evaluation of the exocrine function of the pancreas. When compared with healthy control subjects it was found that there was pancreatic insufficiency in nine (28%) of the diabetic patients whose PE1 levels were lower than 200 μg/g ($p<0.01$). In addition to this, one of 32 patients (3.1%) had severe insufficiency. This ratio may represent the group of patients who may be at severe risk of developing some other disorders that are secondary to exocrine insufficiency in the pancreas, such as vitamin deficiencies. Thus, it may be vital to detect, and monitor, these patients before the development of such disorders. Furthermore, Hardt and co-workers even suggested in their study and observations on autopsies that, because chronic pancreatitis might be a common problem as a result of the diabetes, which is secondary to the exocrine disease, it could be much more frequent than believed up until today (24). They determined pancreatic insufficiency in 56.7% of type 1 diabetics and in 35% in type 2 diabetics. Contrary to their study, we did not determine any pancreatic insufficiency in the control group, while they found that 18.1% of the control subjects had an insufficiency. This may imply assay-relevant problems which in turn produces underestimation of the results. As a similarity between these two studies, there were no significant associations between PE1 levels and the duration of diabetes or consumption of alcohol. Rathmann and coworkers (25) reported that the level of PE1 was lower in diabetic patients than in control subjects, and they also stated that poor glycemic control (HbA_{1c}) was associated with this outcome. However, there was no such association in our study. In another study (26), there seemed to be a correlation between duration of diabetes and PE1 levels, but our study did not confirm this result. In addi-

tion, it was also reported that there were weak associations between PE1 and diabetes duration, age at onset of diabetes and BMI (27). It is clearly seen that the results regarding the PE1 status in diabetics is debatable.

In view of the above, even though the specificity of PE1 was reported by Luth and co-workers to be low (23), PE1 measurement seems to be a very helpful parameter for the evaluation of the exocrine function of the pancreas. If desired, PE1 may also be combined with other invasive tests. For example, secretin-stimulated endoscopic function testing with the measurement of bicarbonate and fecal elastase-1 has been suggested to provide a simultaneous assessment of pancreatic insufficiency (28).

Although it has been suggested that individual changes from day to day should be taken into consideration (29), in one recent study it was found to be enough to collect one single stool at random (30).

In conclusion, because the proper regulation of blood glucose in diabetics is directly related to the exocrine function of the pancreas, it should be noted that the exocrine function is to be maintained through the replacement of enzymes as soon as diabetic patients are diagnosed with any insufficiency of the pancreas. However, more extensive studies are needed to better understand why some diabetics have varied levels of insufficiency while others do not.

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