INTRODUCTION

The diagnosis of pancreatic exocrine insufficiency at an early stage is challenging, symptoms may be mild with normal imaging findings, pancreas have a large reserve capacity that means more than 90% of acinar tissue must be lost before symptoms like steatorrhea occur. A reliable simple, easy and cheap test for pancreatic insufficiency diagnosis is still lacking; nonetheless, fecal elastase 1 has been used for this purpose(10).

Exocrine pancreatic insufficiency is one of the major complications of chronic pancreatitis(11) that has alcohol as the main etiological cause(10). The diagnosis of chronic pancreatitis nowadays is mainly based on the morphological changes by image findings with more advanced techniques such as magnetic resonance imaging, magnetic resonance cholangiopancreatography and endoscopic ultrasonography with a limited use of the functional tests for exocrine pancreatic insufficiency diagnosis. Nonetheless, functional testing may be important to support the diagnosis, for the indication of enzyme replacement and its effect on fat digestion, in addition exocrine pancreatic secretion impairs progressively along the course of chronic pancreatitis. Thus, fecal elastase test, as a noninvasive test to indicate a reduction in exocrine pancreatic secretion, may be useful for the screening of chronic pancreatitis diagnosis and follow-up(3,11).

Human elastase is synthesized by the acinar cells of the pancreas along with the other proteolytic enzymes, and under normal conditions. It has a concentration in the duodenal juice between 170 and 360 μg/mL (6% of the total pancreatic enzymes); human elastase is transported across the gut while bound to bile salts and if not degraded, is enriched in the stool(10,11,15). An advantage of fecal elastase 1 as a diagnostic marker is its low variability within an individual from day to day, indicating that its measurement in fecal samples is valid diagnostically(8).

The levels of fecal elastase 1 were not affected by substitution therapy or bacterial degradation and showed 94% specificity and 93% sensitivity at a cutoff value of 175 μg/g; its sensitivity increased to 96% in the presence of steatorrhea and decreased to 88% when the pancreatic insufficiency was less severe(21). Fecal elastase 1 had a sensitivity of 16.7%-63% for mild, 12.5%-100% for moderate and 72.2%-100% for severe chronic pancreatitis. Thus, fecal elastase test, as a noninvasive test to indicate a reduction in exocrine pancreatic secretion, may be useful for the screening of chronic pancreatitis diagnosis and follow-up(3,11).

Declared conflict of interest of all authors: none.

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ABSTRACT – Context - Fecal elastase is a noninvasive test for pancreatic insufficiency diagnosis. Objective - Evaluate the usefulness of fecal elastase 1 for the indication of exocrine pancreatic insufficiency among former alcohol addicts and patients with chronic pancreatitis. Methods - Forty-three patients with chronic pancreatitis and thirty-three asymptomatic former alcohol addicts entered the study. The levels of fecal elastase 1 were measured using a commercial kit. Pancreatic imaging findings were used to categorize the groups. Results - The levels of fecal elastase 1 were significantly lower in the patients than in the former alcohol addicts and in the group with tissue calcifications, duct alterations, or atrophy. With a cutoff level of 100 μg/g, the sensitivity of fecal elastase 1 in chronic pancreatitis was 46.51% and its specificity was 87.88% with a positive predictive value of 83.33% and a negative predictive value of 55.77%. When patients were stratified according to the severity of their pancreatitis, the sensitivity was 6.25% for mild pancreatitis and 70.37% for marked pancreatitis. Conclusion - Low level of fecal elastase 1 was associated with marked rather than mild chronic pancreatitis; however, it may be useful to indicate pancreatic exocrine insufficiency in asymptomatic former alcohol addicts.

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severe exocrine pancreatic insufficiency. It had a specificity of 90%-95% with a cutoff value of 200 µg/g, and with this cutoff value, it was less reliable for the detection of some cases of mild and moderate pancreatitis. The sensitivities of fecal elastase 1 for the detection of calcifying pancreatitis (76.5%) and autoimmune pancreatitis (71.4%) were higher than for definite chronic pancreatitis (60%), confirming that it is a reproducible marker for severe pancreatic insufficiency.

Low levels of fecal elastase 1 were reported in patients with celiac disease; thus, exocrine pancreatic insufficiency may be associated with celiac disease and may be the cause of chronic diarrhea in gluten-free diet adherent patients; conversely, diarrhea may provoke a dilution effect, falsely lowering fecal elastase levels. Cases of inflammation of the duodenal mucosa caused by autoimmune reactions, infections, short gut syndrome, and Crohn's disease with normal secretin-cholecystokinin tests were correlated with lower fecal elastase levels in children by the reduced stimulatory capacity in the intestine of pancreatic exocrine secretion. Fecal elastase 1 fails to distinguish primary exocrine pancreatic insufficiency from other causes of diarrhea; thus, the integrity of the intestinal mucosa should be evaluated when primary causes of pancreatic insufficiency were excluded. In spite of that, low level of fecal elastase 1 may indicate pancreatic insufficiency.

Although fecal elastase 1 was thoroughly studied by other authors and subject of review; the purpose of this prospective study was to evaluate the usefulness of fecal elastase 1 for the indication of pancreatic insufficiency to introduce this test in our routine clinical practice (Hospital of the Public Service). For this purpose a group of patients with chronic pancreatitis and a group of former alcohol addicts were chosen for this analysis.

METHODS

This study was approved by the local Ethics Committee, and the patients were invited to participate and those who participated gave their written informed consent. Forty-three patients (mean age of 54.61 ± 9.68 years) with chronic pancreatitis diagnosed by abdominal imaging procedures (ultrasound, computed tomography, nuclear magnetic resonance, and/or endoscopic ultrasound) and clinical evaluation (steatorrhea-related symptoms such as bloating, abdominal cramps, bulky, sticky and fatty stools and diabetes) entered the study. Twenty-nine (67.4%) patients were men, twenty-five (58.1%) were Caucasians, seventeen (39.6%) were African-Brazilians and one (2.3%) was Japanese-Brazilian. Most (67.4%) of the cases of chronic pancreatitis were caused by alcohol consumption, followed by idiopathic causes (20.9%), cystic fibrosis (4.6%), pancreas divisum (2.3%), autoimmune causes (2.3%), and hypertriglyceridemia (2.3%). The chronic pancreatitis patients were divided into two groups according to their image findings: the first group contained patients with tissue calcifications, duct alterations, or atrophy (marked alterations), and the second group contained those with mild alterations (normal gland size and shape or slight enlargement of less than two times the normal, main pancreatic duct <2 mm or main pancreatic duct between 2 and 4 mm, and or heterogeneous parenchyma). Pancreatic enzyme replacement therapy was initiated based on the clinical suspicion and pancreatic imaging findings of pancreatic insufficiency diagnosis according to International guidelines.

Thirty-three patients (mean age of 54.61 ± 9.68 years) that were previously alcoholics (ethanol consumption of more than 100 g/day for men and more than 80 g/day for women for at least six years, abstinent for at least six months prior the study) and had a normal pancreatic evaluation by abdominal imaging procedures entered the study. Most of them (11; 33.3%) had normal liver by imaging procedures, eight (24.2%) had a component of steatosis, eight (24.2%) had nonspecific signs of chronic hepatopathy, three (9.1%) had liver calcifications, one (3%) had gall bladder stones, one (3%) had hepatic cyst, and one (3%) had undergone cholecystectomy. However, biochemical tests showed that all of these patients had normal hepatic function, amylase, triglycerides, lipase and glucose in spite of being ex-alcoholics. Among them, thirty (90.9%) were men, twenty-four (72.7%) were Caucasians, and nine (27.3%) were African-Brazilians.

Quantitative determination of the concentration of pancreatic elastase 1

Stool samples with firm consistency (not loose or watery) were collected at home, delivered to the lab the same day and immediately stored at -20°C. All samples were analyzed for their fecal elastase 1 concentrations (Schebo® Biotech, Giessen, Germany) at the same time by means of an enzyme linked immunosorbent assay, according to the manufacturer's instructions. The basic principle of this assay is that a monoclonal antibody that only recognizes human pancreatic elastase 1 is coated on a plate, and when human pancreatic elastase 1 binds to the monoclonal antibody there is an observable reaction. The results were reported in µg/g of stool; values >200 µg/g were considered normal, values between 100 and 200 µg/g were considered to indicate mild-to-moderate exocrine pancreatic insufficiency and values less than 100 µg/g were considered to indicate severe exocrine pancreatic insufficiency.

Statistical analysis

The values of fecal elastase 1 were compared between former alcohol addicts and the patients with chronic pancreatitis. Within the patient group, the values were also compared between patients with steatorrhea and treated with pancreatic enzymes, patients with diabetes, patients that had undergone surgical procedures, and patients with differing imaging findings by the Likelihood Ratio by SPSS 15.0 (Chicago, U.S.A). A P-value <0.05 was considered significant. The power of the statistical analysis was performed by Post hoc: Compute achieved power, considering the sample size group 1 of chronic pancreatitis patients (43 patients) and the sample size group 2 (33 former alcohol addicts), proportion
p1=0.465 and proportion p2=0.121 (as shown in Table 1: 46.5% of patients and 12.1% of former alcohol addicts had fecal elastase levels <100 µg/g, α error probability = 0.05, resulting in Power (1-β error probability) = 0.9388323 and actual α=0.0202700. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) with 95% confidence intervals (95% CI) were obtained by R version 2.12.1 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org), comparing the patients with chronic pancreatitis with former alcohol addicts (group with high risk of chronic pancreatitis and exocrine pancreatic insufficiency).

TABLE 1. Comparison of fecal elastase 1 levels in chronic pancreatitis patients with former alcohol addicts

<table>
<thead>
<tr>
<th>Fecal elastase 1</th>
<th>Chronic pancreatitis patients</th>
<th>Former alcohol addicts</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;200 µg/g</td>
<td>22 (51.2%)</td>
<td>28 (84.8%)</td>
<td>50 (65.8%)</td>
</tr>
<tr>
<td>100-200 µg/g</td>
<td>1 (2.3%)</td>
<td>1 (3%)</td>
<td>2 (2.6%)</td>
</tr>
<tr>
<td>&lt;100 µg/g (P&lt;0.05)</td>
<td>20 (46.5%)</td>
<td>4 (12.1%)</td>
<td>24 (31.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>43 (56.6%)</td>
<td>33 (43.4%)</td>
<td>76 (100%)</td>
</tr>
</tbody>
</table>

RESULTS

The levels of fecal elastase 1 were significantly (P<0.05) lower in the patients with chronic pancreatitis than in the former alcohol addicts (Table 1). Patients were usually split into two distinct groups: one group with levels >200 µg/g (normal) and one group with levels <100 µg/g (severe exocrine pancreatic insufficiency). Only one patient and one former alcohol addict had fecal elastase 1 levels between 100 and 200 µg/g (mild-to-moderate exocrine pancreatic insufficiency). Thus, considering the former alcohol addicts versus the patients with chronic pancreatitis with a cutoff level of 100 µg/g, the sensitivity of fecal elastase 1 was 46.51% [95% CI = 32.54-61.07] and its specificity was 87.88% [95% CI = 71.91-95.66], with a positive predictive value of 83.33% [95% CI = 63.37-93.77] and a negative predictive value of 55.77% [95% CI = 42.34-68.38]. When patients were stratified according to the severity of their pancreatitis, the sensitivity was 6.25% [95% CI = 0.30-36.65] for mild pancreatitis and 70.37% [95% CI = 51.84-2] for marked pancreatitis.

Four former alcohol addicts had fecal elastase 1 levels lower than 100 µg/g; two of these subjects had unspecified signs of chronic hepatitis indicated by imaging, one had undergone cholecystectomy, and the other had a simple hepatic cyst.

The levels of fecal elastase 1 among chronic pancreatitis patients distributed according to the presence of steatorrhea, occurrence of diabetes, and pancreatic surgery were not significant (Table 2).

The chronic pancreatitis patients were divided into two groups according to their image findings: the first group contained patients with tissue calcifications, duct alterations, or atrophy (marked alterations), and the second group contained those with mild alterations. The levels of fecal elastase 1 were significantly (P<0.05) lower in the group with marked alterations than in those with mild alterations. The fecal elastase 1 levels were >200 µg/g in fifteen (93.7%) of the sixteen patients that had mild changes and in seven (25.9%) of those with marked alterations. In contrast, the fecal elastase 1 levels were <100 µg/g in 19 (70.4%) and > 200 µg/g in 7 (25.9%) of the patients with marked alterations (Table 3).

TABLE 2. Fecal elastase 1 levels among chronic pancreatitis patients according to the presence of steatorrhea, diabetes, and undergone pancreatic surgery

<table>
<thead>
<tr>
<th>Chronic pancreatitis patients</th>
<th>&gt;200 µg/g</th>
<th>100-200 µg/g</th>
<th>&lt;100 µg/g</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steatorrhea (P&lt;0.05)</td>
<td>14 (48.3%)</td>
<td>1 (3.4%)</td>
<td>14 (48.3%)</td>
<td>29 (67.4%)</td>
</tr>
<tr>
<td>No steatorrhea</td>
<td>8 (57.1%)</td>
<td>6 (42.9%)</td>
<td>14 (32.6%)</td>
<td></td>
</tr>
<tr>
<td>Diabetes (P&lt;0.05)</td>
<td>8 (36.4%)</td>
<td>1 (4.5%)</td>
<td>13 (59.1%)</td>
<td>22 (51.2%)</td>
</tr>
<tr>
<td>No diabetes</td>
<td>14 (66.7%)</td>
<td>7 (33.3%)</td>
<td>21 (48.8%)</td>
<td></td>
</tr>
<tr>
<td>Pancreatic surgery (P&lt;0.05)</td>
<td>4 (36.4%)</td>
<td>7 (63.6%)</td>
<td>11 (25.6%)</td>
<td></td>
</tr>
<tr>
<td>No pancreatic surgery</td>
<td>18 (56.3%)</td>
<td>1 (3.1%)</td>
<td>13 (40.6%)</td>
<td>32 (74.4%)</td>
</tr>
</tbody>
</table>

TABLE 3. Fecal elastase 1 levels in chronic pancreatitis patients according to the image findings scored as mild or marked alterations

<table>
<thead>
<tr>
<th>Image findings</th>
<th>&gt;200 µg/g</th>
<th>100-200 µg/g</th>
<th>&lt;100 µg/g</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild alterations</td>
<td>15 (93.7%)</td>
<td>-</td>
<td>1 (6.3%)</td>
<td>16 (37.2%)</td>
</tr>
<tr>
<td>Marked</td>
<td>7 (25.9%)</td>
<td>1 (3.7%)</td>
<td>19 (70.4%)</td>
<td>27 (62.8%) (P&lt;0.001)</td>
</tr>
<tr>
<td>Total</td>
<td>22 (51.2%)</td>
<td>1 (2.3%)</td>
<td>20 (46.5%)</td>
<td>43 (100%)</td>
</tr>
</tbody>
</table>

DISCUSSION

In the present study we compared fecal elastase 1 levels in patients with chronic pancreatitis with former alcohol addicts, showing that severe exocrine pancreatic insufficiency was more frequent (46.5%) in the patients with chronic pancreatitis than in the group of former alcohol addicts (12.1%). Most importantly, when the patients with chronic pancreatitis were stratified into mild and marked alterations by imaging findings (tissue calcifications, duct alterations, or atrophy), the levels of fecal elastase 1 indicated that those with marked alterations more frequently (70.4%) had severe exocrine pancreatic insufficiency than the patients with mild
Comparison of fecal elastase 1 for exocrine pancreatic insufficiency evaluation between ex-alcoholics and chronic pancreatitis patients

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The overall sensitivity (46.51%) and specificity (87.88%) of fecal elastase 1 was lower than those previously described, 77.3% and 95.8%, respectively(8). The specificity of fecal elastase 1 for the detection of pancreatitis may have been falsely decreased because the comparison was with former alcohol addicts, different from other report that compared to healthy nondrinking subjects, showing 100% specificity(24).

In patients with concomitant diabetes mellitus, the sensitivity (59%) did not reach the level found by other authors, 91.7%(16); however, the data for patients with concomitant diabetes mellitus agreed with previous studies that showed no significant difference among groups with or without diabetes mellitus and steatorrhea(24).

The sensitivity of fecal elastase 1 for mild pancreatitis was too low (6.3%) in comparison to that reported by other authors(8) (22.2%); nonetheless, of among studies that compared fecal elastase 1 with a direct test of pancreatic function, only one study suggested reasonable sensitivity and specificity for mild disease(10). Early diagnosis of exocrine pancreatic insufficiency is challenging as symptoms may be mistaken for those of functional gastrointestinal disorders and may not be detected in patients even the gold standard (secretin-cholecystokinin or secretin-cerulein test by duodenal intubation) has low sensitivity(8). In this setting, the noninvasive detection of fecal elastase 1, although it provides higher sensitivity and specificity than chymotrypsin(8), has some limitations(10), such as difficulties in diagnosing mild-moderate chronic pancreatitis and in differentiating pancreatic from non-pancreatic malabsorption(22). Thus, fecal elastase 1 may be more useful for follow-up examinations of patients than for chronic pancreatitis diagnosis, as chronic pancreatitis is a progressive condition and fecal elastase 1 levels decreases accordingly(24).

Other indirect tests to assess pancreatic function with malabsorption are fecal fat determination and 13C-mixed triglyceride breath test(2,11). The fecal fat determination by the classical Van de Kamer test(25), which is the gold standard for fat malabsorption diagnosis, have some limitations. Adherence of the patients to the requirement of limiting their fat consumption to 100 g a day for six days, which should be initiated three days prior to the three-day total stool collection, is not always high. Additionally, the long period of total stool collection and handling is unpleasant for the patient and for the lab personnel, and the total stool-collection homogenization and manual extraction can be cumbersome(4,11,15).

The 13C-mixed triglyceride breath test was shown to be an accurate method to evaluate the effect of enzyme therapy on fat digestion, using the coefficient of fat absorption as gold standard, therapy was modified to obtain a normal test(9). However, for the patient 6-hour exam may be a long lasting test in comparison to fecal elastase test with a single stool collection(10). For the lab personnel, a 3-hour ELISA of fecal elastase test may be easier(10) than handling complaining patients during 6-hour exam. In Brazil, fecal elastase decreased in patients who had undergone pancreaticoduodenectomy; nonetheless, 13C-mixed triglyceride breath test was similar to healthy controls(14). The promising results of 13C-mixed triglyceride breath test to evaluate enzyme therapy replacement(25) should be further evaluated in our patients.

Pancreatic dysfunction was previously shown as rare among patients who stop drinking after the first episode of alcohol-associated pancreatitis. Fecal elastase levels returned to normal after two years of abstinence except for one patient (6%) that maintained low elastase-1 activity(17). In contrast, in our study four (12.1%) ex-alcoholics had fecal elastase 1 levels lower than 100 μg, indicating that they might have exocrine pancreatic insufficiency although asymptomatic and with normal pancreas by imaging procedures. Two of these subjects had unspecific signs of chronic hepatopathy indicated by imaging with normal biochemical hepatic function tests.

Alcohol intake usually is associated with either pancreatic or liver disease; however, both conditions may coexist in alcoholic subjects. Some reports(1,18) have studied the prevalence of exocrine pancreatic insufficiency in patients with liver disease. Using fecal elastase test, 7% of patients with cirrhosis had exocrine pancreatic insufficiency, compared with 14.8% of asymptomatic alcoholic patients(8). High prevalence of exocrine pancreatic insufficiency (55.2%) and chronic pancreatitis (44%) was observed in subjects with chronic alcoholic liver disease by 13C-mixed-triglycerides breath test. Nonetheless, exocrine pancreatic insufficiency was more common in the early stages of the liver disease (70%) with lower prevalence in patients with cirrhosis (46.2%)(18). The authors(18) suggested possible explanations for this finding such as, the state of pancreatic hypersecretion(19,23) with less protein and calcium, measured by secretin test in patients with alcoholic liver disease. Pancreatic juice with less protein and calcium would protect the pancreas from protein plugs and calcification formation(19). The mechanism for this hypersecreatory state would be impaired secretin removal rate in the abnormal liver(29).

In conclusion, low level of fecal elastase 1 was associated with marked rather than mild chronic pancreatitis; however, it may be useful to indicate exocrine pancreatic insufficiency in asymptomatic former alcohol addicts.

ACKNOWLEDGMENTS

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RESUMO - Contexto - O teste de elastase fecal é um teste não invasivo para diagnosticar insuficiência pancreática. Objetivo - Avaliar a utilidade da elastase fecal 1 como indicador de insuficiência pancreática entre ex-alcoólatras e pacientes com pancreatite crônica. Métodos - Quarenta e três pacientes com pancreatite crônica e 33 ex-alcoólatras assintomáticos entram no estudo. Os níveis de elastase fecal 1 foram medidos usando kit comercial. Os achados de imagem pancreática foram usados para categorizar os grupos. Resultados - Os níveis de elastase fecal 1 foram significativamente menores nos pacientes que nos ex-alcoólatras e no grupo com calcificações teciduais, alterações de ductos, ou atrofia. A sensibilidade da elastase fecal 1 na pancreatite crônica foi de 46,51% e a especificidade foi de 87,88%, com valor preditivo positivo de 83,33% e valor preditivo negativo de 55,77%. Quando os pacientes foram estratificados segundo a severidade da pancreatite, a sensibilidade foi de 6,25% para pancreatite crônica leve e 70,37% para pancreatite crônica severa. Conclusão - Baixo nível de elastase fecal 1 foi associado com pancreatite crônica severa mais do que com a leve; entretanto, pode ser útil para indicar insuficiência pancreática exócrina entre os ex-alcoólatras.


REFERENCES