

Review

Fecal Elastase-1 as a Test for Pancreatic Function: a Review

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Pancreatic elastase-1 is a specific human protease synthesized by the acinar cells. Immunoreactive elastase-1 cannot be detected in either porcine or bovine pancreatic enzyme preparations. It is very stable and, in contrast to fecal chymotrypsin, elastase is unaffected by exogenous pancreatic enzyme treatment, and correlates well with exocrine pancreatic function tests. The measurement of this proteolytic enzyme in stool by means of an enzyme-linked immunosorbent assay (ELISA) is a sensitive, specific, and relatively inexpensive non-invasive test. It is an accurate function test for patients with chronic pancreatitis confirmed by endoscopic retrograde cholangiopancreatography and computerized axial tomography. It shows higher sensitivity and specificity for exocrine pancreatic insufficiency than fecal chymotrypsin determination and is comparable to oral pancreatic function tests such as the pancreolauryl test. Clin Chem Lab Med 2002; 40(4):325–332

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Abbreviations: AP, acute pancreatitis; BMI, body mass index; CAT, computerized axial tomography; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; CP, chronic pancreatitis; E1, elastase-1; ERPC, endoscopic retrograde cholangiopancreatography; IBD, inflammatory bowel diseases; NBT-PABA, *N*-benzoyl-L-tyrosyl-*p*-aminobenzoic acid; PI, pancreatic insufficiency; PS, pancreatic sufficiency.

Introduction

Biochemistry of elastases

Mammalian pancreas is a compound gland of endocrine and exocrine tissue derived from the embryonic endoderm. Approximately 90% of the pancreas is exocrine tissue, comprising acinar cells that synthesize and secrete digestive enzymes, and ductal cells that secrete and channel the fluids that transport the acinar enzymes to the duodenum. About 1% of the pancreas is endocrine tissue, comprising four principal cell types synthesizing insulin (β -cells), glucagon (α -cells), so-

matostatin (δ -cells) and pancreatic polypeptide (PP-cells) organized into islets scattered throughout the exocrine pancreas. The endocrine and exocrine compartments are structurally and functionally integrated through an islet-acinar portal blood system that facilitates the regulation of acinar cell functions directly by islet peptide hormones. Historically, any proteinases that hydrolyze elastin, the major structural fibrous protein in connective tissues, and especially elastic fibers, were named elastases. This name is now applied to a group of enzymes that vary in their specificity. The genes coding for the elastases are clustered on chromosome 19, and three main types of enzymes are known: a) pancreatic elastase-1 (E1), or pancreatopeptidase EC 3.4.21.36; b) pancreatic elastase-2 EC 3.4.21.71; c) pancreatic endopeptidase-3 EC 3.4.21.70, also called cholesterol-binding proteinase. These forms of proteases with elastolytic activity differ in respect to their catalytic properties. The proteases, named elastase-1 and elastase-2, have been isolated from activated extracts of human pancreatic tissue: the purification procedure used for both elastases included ammonium sulfate fractionation followed by ion-exchange chromatography on CM Sephadex C-50. E1, which is very similar to another human protease, protease E, which lacks elastolytic activity, was further purified by chromatography on DEAE-Sephadex A-50. Feinstein *et al.* (1) also reported differentiation of these two pancreatic elastases on the basis of esterase activities and kinetic parameters using the synthetic substrates, [Ac(Ala)₃OMe] *N*-acetyl-L-alanyl methyl ester [Ac(Ala)₃OCH₃] and benzoylalanine methyl ester; the latter has been shown to be a specific substrate for the porcine enzyme (2, 3). A comparison of human protease E with porcine elastase revealed a high degree of similarity between the two proteases with respect to the following: inhibition by the active-site directed peptide chloromethyl ketones; stability; reduced susceptibility to naturally occurring proteinase inhibitors; specificity for synthetic substrates and several other physical properties. Degradation of other proteins by pancreatic elastases has been reported to be due to contamination by chymotrypsin or trypsin. Enzymes mimicking pancreatic elastases occur in microorganisms such as *Flavobacterium elastolyticum*, *Clostridium histolyticum* and *Staphylococcus epidermis*. The enzyme is synthesized in porcine pancreas as a pre-proelastase. After processing to proelastase, it is stored in the zymogen granules and later it is activated to elastase by trypsin in the duodenum. The NH₂-terminal amino acid sequences of both human elastase-1 and elastase-2 were shown to be valine-leucine, while those of porcine elastase were valine-valine. Porcine

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elastase is thought to be derived from proelastase by tryptic activation of the proenzyme. Bovine trypsin, chymotrypsin, thrombin and porcine elastase have been shown to possess a similar activation site, and all four enzymes possess N-terminal sequences beginning with two hydrophobic aliphatic amino acid residues. The fact that human elastase-1 and elastase-2 possess two aliphatic residues at their respective N-terminals suggests that they are activated by a mechanism similar to that of the previously studied serine-proteases. Of the plasma proteinase inhibitors, only α_1 -proteinase inhibitor and α_2 -macroglobulin inhibited human elastase-1 or elastase-2 or porcine elastase to an approximately equal extent.

Human Elastase-1

Human elastase-1 (EC 3.4.21.36) is an anionic protease belonging to the family of serine-proteases, together with digestive enzymes such as chymotrypsin, trypsin and some proteases of the enzyme cascade of blood coagulation and of the complement system; these proteases share more than 40% of homology for the primary and tertiary structure. Pancreatic E1, first described by Balo and Banga in 1950 (4), is a carboxyendopeptidase, which catalyzes hydrolysis of native elastin, with preferential cleavage at Ala-Xaa > Val-Xaa site, but not native collagen and keratin. Cleavage of hemoglobin, casein and fibrin has also been reported. The elastin digestion capability of E1 is a function unique to this enzyme. Because of its unique specificity to degrade elastin, pancreatic elastase has been involved in the pathogenesis of emphysema and atherosclerosis as well as in aging; this protease has also been shown to produce the vascular injury observed in acute pancreatitis (AP). Human E1 is synthesized by the acinar cells of the pancreas along with the other digestive enzymes, and is composed of 240 amino acids, with a molecular weight of about 26 kDa and a special affinity for the carboxyl group (COOH) of alanine, valine and leucine. This acidic endoprotease with properties of sterol-binding protein (5, 6) was described in 1975 by Mallory and Travis (3) as protease E, and further characterized as an elastolytic pancreatic enzyme by Largmann *et al.* (2). Quantitative studies using immunoelectrophoresis have shown that this enzyme, unlike other pancreatic enzymes such as chymotrypsin, is not significantly degraded during intestinal transit where it is mainly bound to bile salts. Its concentration in human feces is about 5- to 6-fold compared with pancreatic-duodenal juice, reflecting exocrine pancreatic function (5–7). The enzyme has been found to be stable in stool samples for up to 1 week at room temperature. Under physiological conditions E1 concentration in pancreatic juice is between 170 and 360 $\mu\text{g/ml}$, which is about 6% of all secreted pancreatic enzymes. The greatest amount of this enzyme has been isolated from necrotic human pancreas and purified by means of ion exchange and gel filtration chromatographic techniques (5). Its concentration was mea-

sured immunochemically by means of quantitative methods such as radial immunodiffusion and rocket immunoelectrophoresis. Using these techniques it was possible to show that elastase-1 and chymotrypsin B as well were not significantly degraded during intestinal passage. Therefore, the amount of concentration of this enzyme in feces with special reference to E1 may represent a good indicator of pancreatic function (5–7).

Elastase-1: Its Role as a Diagnostic Test

Pancreatic diseases may be classified as inflammatory, neoplastic, traumatic or genetic; inflammatory diseases may be subdivided into acute pancreatitis, relapsing acute pancreatitis, relapsing chronic pancreatitis and chronic pancreatitis (CP) (8–10).

AP is an inflammatory disease of pancreas characterized by abdominal pain and increase of pancreatic enzymes in the blood and urine. The criteria used for diagnosis are:

- 1) Increase > 5-fold in pancreatic enzymes' activity in serum (amylase and lipase);
- 2) Evidence of inflammation by imaging methods (computerized axial tomography (CAT), echotomography) and by laparotomy or autopsy. The incidence of AP is 11–35 per 100,000 per year in the countries where the consumption of alcohol is higher.

The chronic form of pancreatitis is a progressive inflammatory process of pancreas characterized by pain and morphological, irreversible alterations of the function of exocrine and endocrine components of the gland. The criteria used for diagnosis are:

- 1) Pancreatic function tests which indicate reduced exocrine function of pancreas;
- 2) Morphological alterations evidenced by CAT, wirsunography or histology (biopsy).

The incidence of CP is relatively low: in the Western countries it is 3.5–4.0 per 100,000 per year, and the main cause (85–90%) of CP is excessive alcohol consumption. During an acute inflammatory process of the pancreas many enzymes are released into the blood, so that their quantification in serum allows confirmation or exclusion of AP (9–11). On the other hand, the diagnosis of CP is hampered by the absence of easily available histological confirmation and is therefore based on morphological and functional variables.

For diagnosis of pancreatic diseases, pancreatic function tests are needed in order to assess the amount of functional damage. In contrast to the chronic form of the disease, pancreatic function returns to normal after attacks of acute or relapsing acute pancreatitis (11). Therefore, pancreatic function tests are necessary to differentiate between acute and chronic presentation of the disease (10, 11). In the course of the last decades a variety of exocrine pancreatic function tests have been developed. Such tests are described in an exhaustive review (12), which classifies them into different categories. The tests mentioned there have been

used for many years. The different test categories mentioned in the review (12) are:

1. Direct tests, in which the flow rate, bicarbonate and enzyme secretion are measured in duodenal or pure pancreatic juice after exogenous hormonal stimulation of the pancreas (secretin-pancreozymin and secretin-coerulein tests).
2. Indirect tests, which make use of nutrients for endogenous stimulation of pancreatic enzyme secretion; they are subdivided into tests requiring duodenal intubation (Lundh test, small intestinal infusions), and tubeless tests (*N*-benzoyl-L-tyrosyl-*p*-aminobenzoic acid (NBT-PABA) test, pancreolauryl test).
3. Fecal tests, which include microscopic examination of stools and estimation of fecal trypsin, chymotrypsin, fat and nitrogen content.
4. Serum enzyme or isoenzyme estimation, with or without previous hormonal stimulation (12).

Exocrine pancreatic functions include the hydrokinetic function of duct cells and the ecbolic function of acinar cells. Both of these are best estimated with the secretin-pancreozymin test and secretin-coerulein test, which have been considered the gold standard in the evaluation of pancreatic function. Direct pancreatic function tests such as the secretin-pancreozymin or secretin-coerulein tests have the highest sensitivity and specificity for the detection of exocrine pancreatic insufficiency and remain the "gold standard" for testing the pancreatic function. Direct function tests have various, practical disadvantages: they are time-consuming, invasive, expensive, uncomfortable, not standardized, and require fluoroscopic tube placement. Therefore, they are unsuitable for routine application and are confined to a few academic centers. In the last few years, several simple indirect pancreatic function tests suitable for clinical practice have been suggested, such as the fluorescent dilaurate test (pancreolauryl test), NBT-PABA or bentiromide test, fecal chymotrypsin determination, or different breath tests. In the pancreolauryl test, the fluorescein dilaurate, a synthetic ester poorly soluble in water, is hydrolyzed by specific arylesterases from pancreatic juice to lauric acid and free water-soluble fluorescein (12). This is readily absorbed in the small intestine, partly conjugated in the liver, and excreted in the urine. The test procedure is standardized: on the first day the test capsules are given orally during a standard breakfast. The urine is collected for 10 hours. To evaluate individual absorption, conjugation and excretion, the test is repeated on the third day using a control capsule which contains free fluorescein only. The recovery rate on both days is expressed as a ratio and taken as an index of pancreatic function (12). However, these tools proved to have limited sensitivity in mild and moderate exocrine pancreatic insufficiency and are interfered with by some drugs, diarrhoea, pH variations and gastrointestinal surgery, which lower their specificity.

Simple, less invasive and indirect methods such as

fecal chymotrypsin activity, pancreolauryl test, and fecal fat content determinations, are less frequently used in daily practice. For example, the PABA/bentiromide test which is based on the specific hydrolysis of a synthetic tripeptide (NBT-PABA) by chymotrypsin in the duodenum is essentially an indirect test of chymotrypsin secretion and is no longer available in most European countries.

Recently, in attempt to identify a simple but yet sensitive and specific test for exocrine pancreatic function, determination of the fecal concentration of pancreatic E1 with an ELISA method has been introduced, and attention has been focused on the possibilities offered by the measurement of E1 in feces (13).

Recently published studies comparing fecal E1 with the fluorescein dilaurate test and fecal chymotrypsin activity in patients with CP showed a comparable sensitivity of fecal E1 with that of the fluorescein dilaurate test, whereas determination of fecal chymotrypsin activity was less sensitive. A study published by Loser *et al.* (13) based on a group of patients with various well defined degrees of exocrine pancreatic insufficiency as measured by the secretin-coerulein test, together with other observations by various investigators, showed that: a) fecal E1 determination with a cut-off <200 µg/g of stool is highly sensitive (93%) and specific (93%) for the detection of exocrine pancreatic insufficiency; b) in accordance with the results of the secretin-coerulein test and fecal fat excretion (13), fecal E1 determination exhibited a very good sensitivity for moderate and severe insufficiency (100%), while its sensitivity for mild exocrine insufficiency appeared to be limited (63%); c) overall sensitivity of fecal E1 (93%) is much higher than that of fecal chymotrypsin (64%); d) E1 shows an excretion pattern similar to that of the other pancreatic enzymes, and its concentration is significantly correlated with duodenal enzyme and volume output; e) the analysis of measurement of fecal E1 proved to be a practical clinical method with low individual day-to-day variability (14, 15), and the enzyme showing excellent stability under various storage conditions. Decreased concentration of fecal E1, up to <100 µg/g is found in mild and moderate as well as in severe exocrine pancreatic insufficiency and therefore is not characteristic only for severe cases. On the other hand, sensitivity in mild cases is limited, with a cut-off <200 µg/g, as three out of eight patients reported in the study by Loser *et al.* had E1 concentrations above this value (13). Nevertheless, a sensitivity of 63% in mild cases is much higher compared with other indirect pancreatic function tests available. In a well documented meta-analysis, overall sensitivity for mild and moderate CP assessed by the secretin-coerulein test was: for fluorescein dilaurate test 39%, for NBT-PABA test 46% and for fecal chymotrypsin determination 49%, whereas sensitivities for severe cases were 79%, 71% and 85%, respectively. Different results were presented by Amann *et al.* who found normal concentration (>200 µg/g) of fecal E1 in four out of seven patients with mild to moderate exocrine pancreatic insufficiency (16). This low sensitivity could be due to the dif-

ferent subclassification criteria used in this study. In fact, Amann subclassified his patients according to a combined parameter score with morphological (calculifications, endoscopic retrograde cholangiopancreatography (ERPC), surgery) and functional (secretin test) criteria, whereas in the study by Loser *et al.* subclassification was performed only according to functional criteria, although morphological criteria had to be included to confirm the diagnosis (14–18). Evaluation of a novel function test should be based, in principle, on the comparison with a gold standard. Significant correlations were found between fecal as well as duodenal E1 concentrations, and duodenal lipase, α -amylase, trypsin, volume and bicarbonate; this shows that secretion patterns of E1 are similar to those of other pancreatic enzymes (17–20). Furthermore, fecal E1 was highly correlated with duodenal E1 concentrations, confirming clearly the suggestions of other investigators that measurement of fecal E1 is representative of pancreatic E1 secretion (21, 22). Some practical aspects should be mentioned here. Determination of fecal E1 concentration does not require analysis of different stool samples, as a single analysis of a 100 mg stool sample proved to be sufficient and should be repeated only in uncertain cases with fecal E1 concentrations of approximately 200 $\mu\text{g/g}$ of stool. Furthermore, fecal E1 was found to be very stable over a storage period of 1 week, even at room temperature, making easy the handling and mailing of small stool samples. E1 is measured in stool by an ELISA method, utilizing two monoclonal antibodies against human pancreatic E1, which bind to two different epitopes of the enzyme. This means that the determination is not affected by simultaneous oral enzyme replacement therapy with pancreatin of animal origin, in contrast to fecal chymotrypsin determination. Moreover, the test is not affected by previous gastrointestinal surgery, gastric dysmotility or mucosal diseases of the small intestine (23–26). As mentioned, fecal E1 is measured immunochemically by ELISA technique in the microplate sandwich format. Most of the available data described here have been obtained using commercially available reagent kits (7). Results are reported as micrograms/per gram ($\mu\text{g/g}$) of stool. The intraassay and interassay imprecision values (as CV%) are in the 3.3–6.3% and 4.1–12.2% interval, respectively (7, 13, 29, 33, 41, 44, 47). Limits of analytical sensitivity are not well defined, but a detection limit of 15 $\mu\text{g/g}$ (corresponding to 1/7 of the lower limit of the reference range is generally reported. The time required is strongly dependent on available instrumentation. The duration of the preanalytical preparation of a series of about 20 samples is 30 minutes; printed results are available in 2 $\frac{1}{2}$ hours. The net cost of the reagent kit, in microplate format, is less than 10 Euro per patient, including the calibrators and quality control.

In a recent study in more than 500 obese patients (body mass index; BMI 35) at high risk of developing biliary microlithiasis, a decreased level of stool E1 (<200 $\mu\text{g/g}$) in almost 20% of the patients was observed; almost 10% had levels <100 $\mu\text{g/g}$, *i.e.* levels

frequently associated with grade II or III CP (data presented at Chronic Pancreatitis 2000 – Novel Concepts in Biology and Therapy; Berne, Switzerland). Similar results have been obtained in a study on patients with gallstones where 31% had reduced fecal E1 levels. Recently, the measurement of fecal elastase was also used as an indirect marker of damage of duodenum-jejunum in a group of 56 children aged 4 months–14 years affected by acute enteritis (27). In this study the results indicated a severe or moderate pancreatic insufficiency in 13 patients out of 56 (23%), and lower E1 levels were present in 17 out of 56 (30%) individuals. Statistical analysis showed a significant difference between the enteritis group and the control group ($p < 0.01$).

Probably the impairment of elastase secretion was related to the reduced bowel cholecystokinin-pancreozymin secretion.

Elastase-1 and Cystic Fibrosis

Cystic fibrosis (CF) is a common, life-shortening, autosomal recessive disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) located on the long arm of chromosome 7 (7q). Exocrine pancreatic insufficiency (PI) develops in 85% of CF patients and is a major clinical manifestation of the disease; it is caused by the obstruction of the small ducts by viscous secretions leading to necrosis of acinar and ductal cells and to eventual fibrosis of the pancreatic lobules. More than 90 different CFTR mutations have been described, and specific mutations have been shown to correlate closely with disease severity, including PI (28–32). Recognition of PI is important for proper diagnosis and management (28, 29). The loss of exocrine pancreatic function leads to maldigestion and malabsorption. Classical tests for the exocrine pancreatic function in children with CF are difficult to perform and lack reliability; therefore the availability of an immunoassay to measure human E1 in stool is helpful. In a pilot study published by Wallis *et al.* (30) on stool obtained from 30 children with CF (aged 1 day to 16 years), three neonates with CF had serial testing performed during the first 3 weeks of life. Patients were selected to include those with a broad range of CFTR mutations and enzyme requirements. CF is a heterogeneous disorder and the rare milder mutations associated with pancreatic sufficiency (PS) are not necessarily detected by E1 testing. Stool from 10 non-cystic fibrosis children were included as controls. In this study, all the normal children had elastase levels within the normal range, and the majority of CF patients (87%) had no detectable elastase. In another study Gullo *et al.* (31) have shown that the enzyme is present in the meconium, and that its concentration increases rapidly after birth reaching values above 500 $\mu\text{g/g}$ within the first month; after this rapid initial increase there is only a slight further age-dependent increase. In the same study most of the children with CF (20/22) had extremely low concentrations of E1 (less than 20 $\mu\text{g/g}$), re-

flecting a severe pancreatic insufficiency as indicated by the presence of macroscopic steatorrhea prior to enzyme supplementation in all patients, and confirming a very high sensitivity of the test. When fecal E1 measurement is used to assess pancreatic function in neonates, the age-dependent behavior of the enzyme must be considered. In an appropriate study, measurement of fecal E1 in a group of 16 human preterm infants, of whom seven were classified as small-for-gestational-age and nine appropriate-for-gestational-age, was performed in order to study the development of the pancreatic secretory capacity in the first weeks of life, compared to 11 full-term infants as controls. The conclusions of this study were that preterm infants in the small-for-gestational-age group initially have insufficient pancreatic exocrine secretion, which normalizes within 2–4 weeks. The behavior of fecal chymotrypsin activity, also measured by means of a colorimetric assay, was similar. Regarding specificity, it is important to report that all 23 children with non-pancreatic digestive disorders who participated in the study as controls, had normal fecal E1 values, indicating excellent specificity of the enzyme determination for pancreatic insufficiency. In contrast, two of the above mentioned 23 children had decreased fecal chymotrypsin activity, suggesting that the determination of this enzyme may be influenced by extrapancreatic factors which do not affect the elastase assay. In a study by Deffert *et al.* (32), fecal E1 concentration was measured in order to screen a large CF population and establish a correlation between CFTR mutations and pancreatic phenotype (genotype-phenotype at the CFTR locus). These authors studied 75 CF patients: 56 with PI and 19 without; pancreatic status was evaluated on the basis of clinical criteria and/or presence of steatorrhea, the mean E1 concentrations were in PI and PS: 50 and 649 $\mu\text{g/g}$, respectively ($p < 0.001$). For patients with normal E1 concentrations, CFTR allele distribution was in agreement with that of previous studies using pancreatic stimulation tests. (33–38).

Elastase-1 and other Diseases

Elastase-1 and HIV infection

In the last few years the measurement of fecal E1 has been performed for the evaluation of pancreatic dysfunction and its association with fat malabsorption in patients infected with immunodeficiency virus (HIV). Intestinal malabsorption of nutrients has been reported (39–41) in symptomatic HIV infection both in adults and in children. In a cohort of 47 children with HIV infection without apparent pancreatic disease, pancreatic function was evaluated by measuring E1 concentration and chymotrypsin activity in stool and compared to 45 age- and sex-matched healthy controls. Intestinal function was evaluated by measuring fat and protein loss using the steatocrit method and measuring fecal α_1 -antitrypsin concentration. Thirty patients had abnormal pancreatic function tests, of them seven

had an isolated deficiency of E1, three had a chymotrypsin deficiency and four had fecal deficiencies in both enzymes. Low fecal pancreatic enzymes were not associated with symptoms; 12 children had steatorrhea and four had increased α_1 -antitrypsin. A significant correlation was found between steatorrhea and reduced fecal pancreatic enzymes, and a negative correlation was observed between E1 concentration and steatocrit. Similar results were obtained in a group of 35 HIV-infected adult patients (41).

Elastase-1 in celiac disease and inflammatory bowel disease

Exocrine pancreatic function was studied in relation to nutrient malabsorption occurring in celiac disease which is attributed mainly to impaired morphology and absorptive function of damaged intestinal mucosa (42–44). In this study enzyme values obtained from celiac disease patients with normal mucosa were significantly higher than those obtained from patients with villous atrophy ($p < 0.001$) and comparable to those obtained from the control group. The results of the study showed that exocrine pancreatic function was abnormal in celiac disease when mucosal atrophy was present. Furthermore, exocrine pancreatic function parameters were associated with the changes of intestinal mucosal morphology in three consecutive phases of the disease. Last but not least, attention has been focused, by some investigators, on the relationship between exocrine pancreatic function and inflammatory bowel disease (IBD: Crohn's disease and ulcerative colitis) in order to assess the clinical and morphological features of so-called idiopathic pancreatitis associated with IBD and to define its physiopathological characteristics (45, 46). Pancreatitis is considered a rare extraintestinal manifestation of IBD; the pancreatic function in these patients was assessed either with secretin-coerulein test, Lundh meal test, or by the presence of steatorrhea, together with imaging methodology (ERCP, ultrasonography). Present studies are in course with the assessment of the exocrine pancreatic function in IBD patients by measuring the fecal E1 concentrations.

Elastase-1 and diabetes mellitus

Some studies from the 1970s deal with the endocrine-exocrine axis, which describes the interactions between islet cells and exocrine pancreas. A relationship was discovered between these; when the islet cells produced less insulin, the exocrine cells were stimulated to a lesser extent. This is the reason why diabetic patients, even those without CP, may have pancreatic secretory insufficiency; patients with CP gradually develop diabetes mellitus as a result of alterations in the exocrine tissue; the endocrine compartment begins to be affected at some stages of disease. Several large prevalence studies in Germany, the UK and Canada showed reduced elastase levels in diabetic populations. A high prevalence of pathological changes in the exocrine function in type 1 and type 2 diabetes mellitus

was observed by the measurement of fecal E1 concentration. This confirmed similar data obtained in previous studies, which used direct secretory tests and demonstrated an impairment of exocrine function in 43–80% of diabetic patients. In the study by Hardt and co-workers (47, 48), 114 diabetic patients (31 classified as type 1 and 83 as type 2) were studied and compared with 105 healthy controls. E1 concentration was normal ($>200 \mu\text{g/g}$) in 82% of controls, and in 43% of type 1 and 65% of type 2 diabetic patients. It was reduced ($<200 \mu\text{g/g}$) in 13% of controls, and in 27% of type 1 and 18% of type 2 diabetic patients. The concentration was markedly reduced ($<100 \mu\text{g/g}$) in 4.8% of controls, and in 30% of type 1 and 17% of type 2 diabetic patients. E1 concentrations were found reduced in 57% of patients with type 1 diabetes, in 35% of patients with type 2 diabetes and in 18% of controls. The differences between controls and type 1 diabetic patients ($p<0.01$), and between controls and type 2 diabetic patients were statistically significant ($p<0.05$). E1 activity seemed not to be affected by diabetes duration or by alcohol consumption. The mode of diabetes therapy also had no influence on exocrine pancreatic function tests. In diabetic patients pancreas was smaller than in healthy controls, mainly due to involution of exocrine parenchyma. Changes in exocrine tissue seemed to be more pronounced in type 1 diabetes, and they correlated inversely with C-peptide concentration in type 2 diabetes. It was shown that about 50% of diabetic patients had pancreatic fibrosis, and pathological observations in exocrine tissue were twice as frequent as in controls (49–55).

Elastase-1 and osteoporosis

Vitamin D deficiency is common in the elderly. The level of 25-hydroxyvitamin D $25(\text{OH})\text{D}_3$ declines with aging, due to lesser exposure to sunshine and decreased production in the aging skin (56). Nutrition does not compensate for this. However, intestinal disorders and exocrine pancreatic insufficiency could be responsible for worsening of the vitamin D supply. In a recent study, the measurement of concentration of pancreatic E1 in stool, as a marker of the vitamin D status, was evaluated in a group of 167 elderly postmenopausal women with X-ray-documented fractures. In 34% (57 subjects), stool E1 values were $<200 \mu\text{g/g}$ (below the normal range), and the median levels of serum $25(\text{OH})\text{D}_3$ were significantly lower in subjects with decreased stool E1 content ($p<0.01$) compared to those with a pancreatic exocrine marker within the normal range. There was a statistically significant correlation between pancreatic E1 in stool and the $25(\text{OH})\text{D}_3$ levels in serum. Since the pancreatic enzymes are involved in digestion and absorption of important nutrients, reduced exocrine function could be an important risk factor for the fractures of hip and vertebral bodies.

In the evaluation of nutritional status the simple estimation of BMI is a poor indicator of this state, as even though BMI was in the normal range, a significant loss

of body mass and fat mass, especially in subjects with steatorrhea, was observed.

Conclusion

In conclusion, the simple performance, the noninvasiveness, and the diagnostic efficiency of the fecal immunoreactive elastase-1 assay make it a satisfactory pancreatic function test for screening of pancreatic insufficiency. Furthermore, this test appears to be useful not only in adults, but also in children, to screen and follow-up the residual exocrine pancreatic function after the diagnosis of cystic fibrosis. Similarly to all pancreatic function tests, the assay for determination of elastase-1 in stool is not able to differentiate between pancreatic insufficiency due to chronic pancreatitis and that due to pancreatic cancer.

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