Faecal pancreatic elastase - 1 a non invasive measure of exocrine pancreatic function

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Summary

Background:- **Faecal pancreatic elastase-1 is a laboratory based test used for the diagnosis or exclusion of exocrine pancreatic insufficiencies. Pancreatic elastase-1, is released into blood circulation during inflammation of the pancreas, but unlike most pancreatic enzymes it is stable during intestinal passage and not degraded.**

Objectives:- **The major objective of this work was to establish the assay of faecal pancreatic elastase-1 in spot stool samples as an exocrine pancreatic function test at Korle-Bu (a referral) hospital in Ghana, for the diagnosis of pancreatic diseases.**

Method:- **Twenty-five apparently healthy persons; mean age of 43.4 years and thirty-two patients with various pancreatic diseases, mean age 51.4 years were referred for the test based on clinical presentation, imaging studies and biopsy findings. The male to female ratio was 6.4:3.6 and 8.1:1.9 respectively. An ELISA technique which recognizes human pancreatic elastase-1 from spot stool samples was employed for the test and read photometrically at 405nm.**

Results:- **Elastase-1 activity in spot stool samples from apparently healthy group ranged from 165 to 870mg/g with a mean of 379 (SE 41)mg/g, and a range of 20 to 285mg/g with a mean of 112.9 (SE 11.6)mg/g obtained for the pancreatic disease group. Disease severity was classified as mild to moderate with elastase-1 concentration between 100 and 200mg/g stool and the severe pancreatic insufficiency group with elastase-1 concentration of less than 100mg/g stool. The pancreatic elastase-1 was found to be stable in faeces for several weeks when stored frozen, hence the convenience for batch determinations.**

Conclusion:- **The test is non invasive and can assist with the diagnosis of inflammatory conditions of the pancreas where imaging results are equivocal.**

Keywords: ***Chronic pancreatitis, Faecal pancreatic elastase 1(E1), Exocrine pancreatic insufficiency, Secretin-cholecystokinin, Steatorrhoea.***

Résumé

L'Arrière-plan:- Pancreatic elastate fécal-1 est un laboratoire un test basé utilisé pour le diagnostic ou l'exclusion d'insuffisances de pancreatic de exocrine. Pancreatic elastase-1, is a relâché dans la circulation de sang pendant l'inflammation du pancréas, mais contrairement à la plupart d'enzymes de pancreatic c'est stable pendant le passage intestinal et pas dégradé.

Les objectifs:- L'objectif majeur de ce travail était d'établir l'essai de pancreatic elastate fécal-1 dans les échantillons de tabouret d'endroit comme un test de fonction de pancreatic de exocrine au Korle-Bu (une référence) l'hôpital dans Ghana, pour le diagnostic de maladies de pancreatic.

La méthode: Vingt-cinq personnes apparemment saines; l'âge moyen de 43.4 malades d'années et trente-deux avec les diverses maladies de pancreatic, signifier vieillir 51.4 années ont été référées pour le test basé sur la présentation clinique, les études de imaging et les conclusions de biopsie. Le mâle à la proportion femelle était 6.4:3.6 et 8.1:1.9 respectivement. Une technique de Elisa qui reconnaît pancreatic elastate humain-1 des échantillons de tabouret d'endroit a été employé pour le test et a lu photométriquement à 405nm.

Les résultats:- Elastase-1 activité dans le tabouret d'endroit essaie du groupe apparemment sain étendu de 165 a 870mg/g avec un moyens de 379 (SOI 41)mg/g, et une gamme de 20 a 285mg/g avec un moyens de 112.9 (SOI11.6) le mg/g a obtenu pour le groupe de maladie de pancreatic. La sévérité de maladie a été classifiée comme doux pour modérer avec elastate-1 concentration entre 100 et 200 tabouret de mg/g et le groupe d'insuffisance de pancreatic sévère avec elastate-1 concentration de moins que 100mg/g tabouret. Le pancreatic elastate-1 a été trouvé pour etre l'écurie dans les matieres fécales pour plusieurs semaines quand emmagasiné gelé, par conséquent la convenance pour les déterminations de fournée.

La conclusion:- Le test est non invasif et peut aide avec le diagnostic de conditions incendiaires du pancréas ou les résultats de imaging sont équivoque.

Introduction

The measurement of faecal immunoreactive elastase is an innovative test with high diagnostic sensitivity and specificity when compared with the secretin-pancreozymin test in the diagnosis of exocrine pancreatic insufficiences.[1] Clinically, it is well known that obstruction of the pancreatic duct, whether by cancer, ampullary or panceatic calculi or inflammation, is a cause of pancreatic insufficiency and malabsorption. However, the diagnosis of chronic exocrine pancreatic insufficiency is hampered by the absence of easily available histological confirmation and is therefore based on the morphology and functional variables.[2,3]

Exocrine pancreatic function includes the hydrokinetic function of duct cells and the ecbolic function of acinus cells, and are best estimated with the secretin-cholecystokinin test which is the gold standard of pancreatic function. Though the secretin- cholecystokinin test is a direct function test frequently used in Northern America, it is invasive, time consuming, expensive, uncomfortable to the patient and requires fluoroscopic tube placement.[4, 5,6] Due to these disadvantages the secretin-cholecystokinin test is regarded unsuitable for routine application in Europe and therefore confined to few academic centres only.[7] Lundh's test, another direct measure of pancreatic function which requires exogenous substrates to stimulate the pancreas is also invasive as it involves the intubation of the patient. These direct, together with indirect pancreatic function tests which depend on the

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exogenous simulation of the pancreas with synthesis peptides as well as measurement of the tryptic activity in stool have constituted the main laboratory based tests for pancreatic function. Generally these tests lack the specificity and sensitivity to diagnose patients with chronic pancreatitis[3,8] since most of them are affected by gastrointestinal operations, faecal pH and drugs which lower their specificity. Faecal fat analysis used as a measure of steatorrhoea, is also both insensitive and non-specific in the diagnosis of mild to moderate degrees of chronic pancreatitis and is unpopular among laboratory staff.

Breath tests using stable isotopes of carbon or hydrogen for the measurement of exocrine pancreatic insufficiency are not sufficiently validated for clinical application.[9]

Discrepancies concerning the relative diagnostic value of these laboratory tests have resulted in confusing opinions expressed by clinicians and which are attributed to variability and absence of standardization of chemical substrates used in different centres.[10] Lack of sensitive and specific old laboratory based tests, failed to diagnose and characterise the degree of severity of patients with pancreatic disease. Neither the secretin-cholecystokinin nor other laboratory based pancreatic function tests are performed at the Korle Bu Teaching Hospital to aid diagnosis and management of patients who have recurrent abdominal pain and whose imaging studies are equivocal to support clinical findings. It has become necessary to employ tests that are organ specific and whose methods are sensitive. These criteria are achieved by using faecal elastase-1 which uses a simple spot stool from a patient for the assay.

Faecal elastase-1, a highly sensitive proteolytic, pancreas specific enzyme is present in human pancreatic juice at a concentration of between 170 and 360µg/ml.[11] During intestinal passage it is bound to bile acids and has been shown to be a transport protein for cholesterol. In contrast to most of the other pancreatic enzymes, elastase-1 is stable during intestinal passage and it is not degraded. Its concentration in stool is found to be five to six times those determined in pancreatic juice and thus reflect pancreatic function.[12]

The secretion patterns of elastase are similar to those of other pancreatic enzymes such as lipase, amylase, and trypsin as highly significant correlation were found between faecal elastase and duodenal elastase concentration, duodenal lipase, amylase and trypsin,[5] stressing the fact that faecal elastase-1 concentration reflects the exocrine pancreatic capacity. Human faecal elastase-1 is immunologically specific; the test is not affected by enzyme replacement therapy, and quite stable between the passage of stool and time of analysis as there is no loss of immunoreactivity in samples stored frozen for several months.[1,7] Faecal elastase-1 determination as evaluated on the basis of the secretin-cholecystokinin test as the "gold standard" of pancreatic function test is highly superior compared to faecal chymotrypsin activity.[5] Its superiority in the diagnosis of exocrine pancreatic insufficiency has been pointed out by several studies.[14, 15, 16]

Materials and methods

The present study was aimed at establishing the assay of faecal elastase-1 as a laboratory diagnosis tool for assessment of patients with chronic exocrine pancreatic diseases at the Korle-Bu Hospital.

Subjects were classified under two groups. Group 1 which consisted of 25 apparently healthy persons aged 25 to 63 years with no history of alcoholism, diabetes nor evidence of gastroduodenal diseases, were enrolled after giving informed consent.

Group 2 consisted of 32 patients with various pancreatic diseases (26 males and 6 females) between the ages of 23 and 78 years and an average age of 51.4 years. The patients were referred for the test having been seen and diagnosed of various inflammatory conditions of the pancreas by the surgeon and others with unexplained causes of recurrent upper abdominal pain. Diagnosis made included cancer of the head of pancreas, obstructive jaundice, calcification of the pancreas and idiopathic causes. The diagnosis of chronic pancreatitis was based on the clinical presentation of patients and findings of the following diagnostic aids; plain radiography, ultrasonography, computerized tomography as well as biopsy findings and routine laboratory tests for stool, urine and blood. Laparotomy was done for both exploratory and treatment of cases requiring surgical intervention.

Labelled spot stool samples received from patients were stored frozen at - 20°C until ready for the assay of the pancreatic elastase-1 enzyme. An ELISA (enzyme linked immunosorbent assay) plate in the form of wells coated with monoclonal antibody which only recognises human pancreatic elastase-1 (E1) binds with E1 from samples and standards is immobilized in the wells. This is sandwitched by a second monoclonal antibody, which is biotinylated and binds to E1 during incubation at room temperature. A conjugate of peroxidase and streptavidin binds to the biotin moiety which then reacts with the substrate solution (2,2-Azino-bis-(3-ethylbenzothiazolin-6 sulphonic acid) diammonium salt) to give a dark green colour determined photometrically at 405nm.[13] Stool masses between 30mg and 80mg were accurately weighed and homogenized in extraction buffer.

Results

The mean elastase-1 activity in spot stool samples from apparently healthy subjects in the study was 379 (SE.41)µg/g; whereas a mean of 112.9(SE.11.6)µg/g was observed in patients with various pancreatic diseases. Enzyme concentrations were found to be stable when stool samples were stored frozen at -20°C for periods ranging from 1 to 4 weeks irrespective of the state of health of the subject as shown in figure 1.

Faecal elastase-1 concentration of 100µg/g and less was observed in 18 of the 32 (56%) patients with pancreatic disease while 11 out of 32(34%) for the same group of patients had values > 100 and <200µg elastase -1/g stool; as shown in figure 2.

The characteristics of patients with pancreatic disease and unexplained causes of recurrent upper abdominal pain showing their elastase-1 values are presented in table 1.

In accordance with these results a sub-classification of patients with pancreatic disease was made as follows:
(A), mild to moderate pancreatic insufficiency (MMPD) with faecal elastase-1 values between 100 and 200µg stool, and

Table 1 Characteristics of the patients with suggested pancreatic insufficiencies showing elastase 1 values between 20 and 285µg/g stool

Subject	Sex	Age(yrs)	Pancreatic diseases	E1(µg/g)
CB	M	39	Chr pancreatitis, D. M.	20
E. A.	M	31	Chr. Pancreatitis	40
K-J C	M	60	Duodenitis recurrent abd.pain	45
SL	M	34	Chr. pancreatitis with steatorrhoea	46
AA	M	39	Chr pancreatitis	46
DD	M	48	Obstructive jaundice	55
JA	M	59	Calcified pancreas. Chr. pancreatitis	63
FKS	M	70	Chr. Pancreatitis	65
NKA	M	39	Chronic pancreatitis	70
WK	M	70	Non specific abd. pain? Chr. pan.	70
OA	M	32	Chronic pancreatitis with steatorrhea	70
GD	M	32	D.M.2° to chr. pancreatitis	70
TF	F	59	Obstructive jaundice/ Ca gall bladder	71
RA	M	40	Chr. Pancreatitis	74
MB	M	78	Ca. Bile duct	76
MBO	F	64	Ca head of pancreas/ Obstr. jaundice	80
MI	M	23	Obstructive jaundice	90
SAM	M	63	Ca head of pancreas/ Obstr. Jaundice	100
TH	F		Chr. Pancreatitis	120
HE	M	58	Diabetes mellitus	125
AS	M	41	Non specific abd. pain? Chr. pan	140
CA	F		Non specific abd., pain? Chr. pan	140
IB	M	43	Chr pancreatitis	153
ATA	M	53	Non specific abd. pain? Chr. pan	160
CA	F	69	Diabetes mellitus	160
EA	F	64	Pancreatic tumour	168
AG	M	55	Diabetes mellitus	170
DN	M		Obstructive jaundice	180
TOA	M	64	Obstructive jaundice	195
MD	M	28	?Chr. pancreatitis	215
BA	M	55	DM2° to Chr. pancreatitis	285

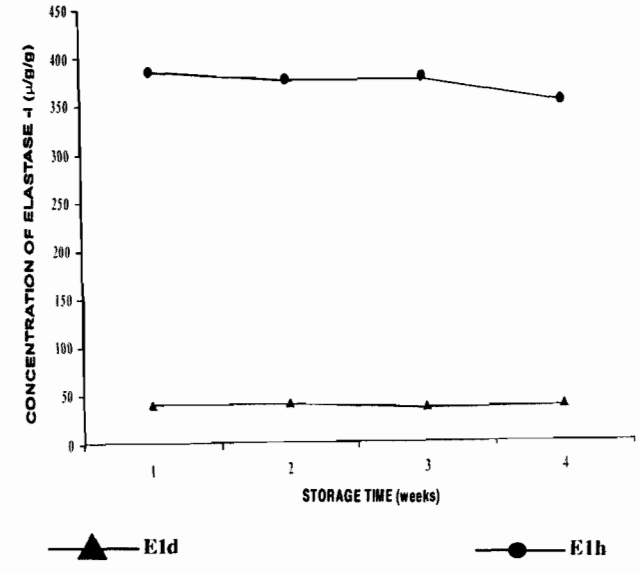

Fig. 1 ***Change in faecal elastase-1 with storage at -20°C in different stools of (a) one patient with exocrine pancreatic insufficiency and (b) one apparently healthy person. E1d refers to pancreatic insufficiency. E1h refers to apparently healthy.***

(B), severe pancreatic insufficiency (SPD) with faecal elastase -1 values up to 100µg/g stool.

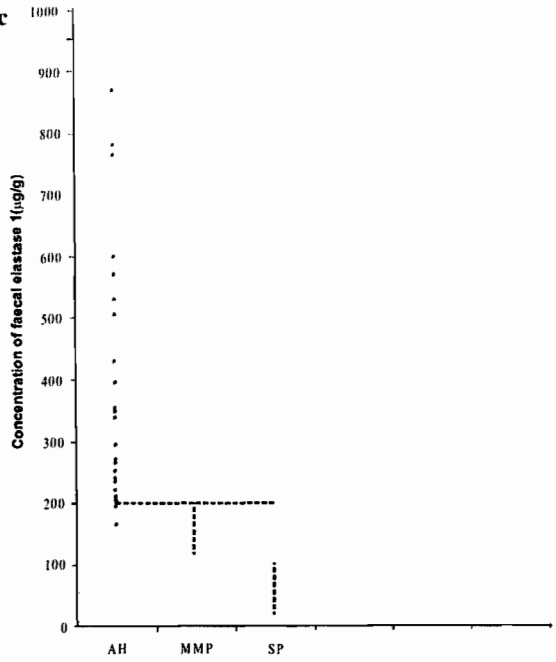

Fig. 2 ***Individual values of faecal elastase-1 concentration of the apparently healthy (AH) group and patients diagnosed for pancreatic insufficiencies. Mild to moderate pancreatic insufficiency (MMP) and severe pancreatic insufficiency (SP). The horizontal broken line represents the lower limit of normal.***

The range for the faecal elastase-1 concentration in the patients with mild to moderate pancreatic insufficiency was 120-285µg with a Mean of 175.8 (SE 12.6)µg/g whereas patients classified as having severe pancreatic insufficiency (SPD) with faecal elastase-1 values up to 100µg/g stool had a Mean value of 63.9 (SE 4.5) µg/g with a range of 20 - 100µg/g.

Discussion

The ultimate aim of this study was to establish the assay of faecal elastase-1 as a laboratory based diagnostic test for chronic exocrine pancreatic insufficiency at the Korle-Bu Teaching Hospital. The assay of faecal elastase-1 as a diagnostic tool was to help in the differential diagnosis of patients presenting with upper abdominal pain and other abdominal disturbances for which results of imaging tests as well as the clinician's findings were equivocal.

The diagnostic sensitivity and specificity of faecal elastase-1 for the detection of exocrine pancreatic insufficiency had previously been determined[1,5] with the secretin - cholecystokinin test as the gold standard pancreatic function. Scheefers-Borchel et al[13] proposed a cut off value of 200mg elastase-1/g stool for normal cases; however Glassbrener et al[15] showed that faecal elastase-1 assay achieved optimal discrimination at a cut off value of 175µg/g stool, yielding a specificity of 94% and a sensitivity of 93% and inspite of the high cut off value of 200µg/g, the specificity of the faecal elastase determination was reported to be excellent when compared to healthy controls[15] The stability of the enzyme has been discussed elsewhere[1,11] and results of this study confirm that faecal elastase-1 stored

frozen at -20°C was highly stable as there was no appreciable loss of enzymic activity over periods ranging from one to four weeks irrespective of the health of the individual. This suited batch analysis (figure 1).

Faecal elastase-1 results are not affected by pancreatic enzyme replacement therapy[1] which is in contrast with faecal chymotrypsin whose measurement has widely been accepted as a useful indirect test for pancreatic function.

The ability of a simple non invasive but sensitive and specific test of pancreatic function to diagnose or exclude exocrine pancreatic insufficiency or pancreatic involvement in abdominal pain has led to the widespread use of faecal elastase-1 as a follow up study of patients with mild, moderate and severe pancreatic insufficiencies.

The results of the study showed that all but 2 of the 25 apparently healthy control subjects enrolled for the study had faecal elastase value of 200µg/g or more giving a 92% specificity. With a cut off point of 200µg/g stool, the sensitivity of faecal elastase-1 was 89.3% in all patients with cystic fibrosis studied by Walkowiak et al[4] and a sensitivity of 93% was reported by Loser et al[9] for all their subjects with exocrine pancreatic insufficiency classified according to the secretin-cholecystokinin test.

Our results showed a sensitivity of 90.6% on all the subjects with pancreatic diseases. This was in agreement with published work mentioned above, indicating that faecal elastase-1 determination is a sensitive test for the detection of decreased exocrine pancreatic function. The subjects within the pancreatic disease group diagnosed as chronic pancreatitis with steatorrhoea all had elastase-1 concentrations <100µg/g stool suggesting that faecal elastase-1 may not be able to differentiate pancreatic from non pancreatic causes of steatorrhoea. Steatorrhoea as part of the malabsorption syndrome, occurs at the absorptive state in chronic pancreatic disease and also after massive resection of the gut or severe disease of the small intestine.[17]

The concentration of faecal elastase-1 in subjects previously diagnosed with cancer of the head of pancreas suggest severe exocrine pancreatic insufficiencies (table 1) and may be due to obstructive processes as well as destruction of pancreatic tissue resulting from the tumour; this may play an important role in diminished secretion of the pancreas. In any case, the secretory capacity is concentrated largely in the head of the gland and up to 85% of the pancreas can be removed surgically in some patients without severe impairment of enzyme secretion.[18] In chronic pancreatitis, obstruction of the pancreatic duct by calculi, or an inflammatory mass can be a contributing factor in diminished pancreatic secretion, which could lead to lower enzyme levels in faeces.

In conclusion, several imaging procedures with reported diagnostic sensitivities of up to 80% and specificities up to 90% are usually the first diagnostic step when pancreatic disease is suspected. The introduction of faecal pancreatic elastase-1 as a laboratory based diagnostic tool would enable pancreatic function tests with similar diagnostic efficiency to be used directly or indirectly to assess pancreatic exocrine function.

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References

1. Stein J Jung and Sziegoleit A et al: Immunoreactive elastase 1; Clinical evaluation of a new non-invasive test of pancreatic function. Clin. Chem. 1996; 42: 222 - 226.

2 Niederau C and Grendell JH: Diagnosis of chronic pancreatitis. Gastroenterology 1985; 88: 1973 - 1995.

3. Steer ML, Waxman I and Freedman S: Chronic pancreatitis N. Engl. J. Med. 1995; 332: 1482 - 1490.

4. Walkowiak J, Citchy W and Herzig KH: Comparison of faecal elastase 1 determination with the secretin-cholecystokinin test in patients with cystic fibrosis. Scand. J Gastroenterology 1999; 34: 202 - 207.

5. Loser C, Mollgard A and Folsch U: Faecal elastase 1; a novel, highly sensitive and specific tubeless pancreatic function test. Gut 1996; 39: 580 - 586.

6. Katshinski M, Schiraa J and Bross A et al: Duodenal secretion and faecal excretion of pancreatic elastase 1 in healthy humans and patients with chronic pancreatitis. Pancreas 1997; 15: 191 - 200.

7. Loser C: Clinical relevance of faecal elastase-1. Determination in the diagnosis of chronic pancreatitis. Gastroenterology Int. 1997; 10: 66 - 70.

8. Lankisch PG: Exocrine pancreatic function test. Gut 1982; 23: 777 - 798.

9. Loser C, Brauer C, Aygen S, Hennemann O and Folsch UR: Comparative clinical evaluation of the 13C-mixed triglyceride breath test as an Indirect pancreatic function test. Scand. J Gastroenterol 1998; 3: 327 - 334.

10. Clavien PA, Burgan S and Moosa AR: Serum enzymes and other laboratory tests in acute panceatitis. Br. J. Surg. 1989; 76: 1234 - 1243.

11. Sziegoleit A: A novel proteinase from human pancreas. Biochem J. 1984; 219: 735 - 742.

12. Sziegoleit A, Knapler H and Peters B: Elisa for human pancreatic elastase 1. Clin. Biochem 1989; 22: 79 - 83.

13. Scheefers-Borchel U, Scheefers H, Arnold R, Fischer P and Sziegolet A: Pancreatic elastase1: Test for the chronic and acute pancreatitis lab. Med. 1992; 16: 427 - 432.

14. Dominguez-Munoz JE, Hieronymus C, Sauerbruch T and Malferheiner P: Faecal elastase test: Evaluation of a new non invasive pancreatic function test. Am J. of Gastroenterology 1995; 90: 1834 - 1837.

15. Glasbrenner B, Schom A and Klatt S, et al: Clinical evaluation of the faecal elastase test in the diagnosis and staging of chronic pancreatitis. Eur. J. Gastroentology and Hepatology 1996; 8: 1117 - 1120.

16. Gullo L, Ventrucci M, Tomassetti P, Migliori and Pezzilli R: Faecal elastase-1 determination in chronic pancreatitis. Digestive diseases and science 1999; 44: 210 - 213.

17. Baron DN, Whitcher JT and Lee KE: A new short textbook of chemical pathology, 5th Edition pp 170 Edward Arnold London. 1989.

18. Di Magno EI: Pancreatic adenocarcinoma. In Yamada T. Eds. Textbook of Gastroenterology pp 1893 - 1911, JB Lippincott, Philadelphia 1991.