SECTION: FUNCTIONAL EXAMINATION METHODS

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PLASMA CITRULLINE CONCENTRATION AS A MARKER OF SMALL INTESTINE FAILURE

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Introduction

Functional parameters for small bowel damage are not suited to routine examination in daily clinical practice. No blood markers have been identified yet for a functional absorptive bowel length. Plasma citrulline, a non-protein amino acid produced by intestinal mucosa, is one possible candidate. Although produced in the liver, the small intestine is the main source for citrulline in the circulation. In healthy humans, plasma citrulline concentrations range from approximately 20 to 50 µmol/l.
Our study in patients with short bowel syndrome (SBS) was carried out to investigate whether plasma amino acid levels and particularly citrulline concentrations could be a marker of functional absorptive bowel length.

Method

After a minimum follow-up of 10 months adult patients with short-bowel syndrome (n = 20) defined by postduodenal remnant of small bowel length of less than 150 cm were included in the study. Patients were divided into two groups according to their dependence on parenteral nutrition. 10 patients with SBS have continued on long-term parenteral nutrition and 10 patients were without parenteral nutrition that had been interrupted earlier.
Nutritional assessment was performed using anthropometry and laboratory parameters.
Postabsortive plasma concentrations of amino acids were determined by ion exchange chromatography.
Results were compared with a group of normal subjects (n = 9).

Results

Body weight and body mass index (BMI) were significantly lower in patient groups. However, the concentrations of albumin, prealbumin and transferrin were the same in both SBS patients and healthy controls. Total amino acids and nonessential amino acids were same in all groups. Essential amino acid/nonessential amino acid and branched-chain amino acid/total amino acid ratios were significantly lower in the SBS patient group than in the normal controls.
Concentration of citrulline was significantly lower only in the group of SBS patients who had to remain on total parenteral nutrition.

Conclusion

These changes suggest that patients with SBS after a minimum 10 months follow-up have considerably adaptive capacity after intestinal resection.
Only anthropometric differences distinguished significantly when compared with healthy controls.
The lower ratio of essential amino acid/nonessential amino acid reflected some type of nutritional deficit in the SBS group.
Changes in plasma citrulline concentration could very well reflect individual intestinal regeneration or functional absorptive bowel length.

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NONINVASIVE TEST OF EXOCRINE PANCREATIC FUNCTION – ^{13}C-MTG BREATH TEST

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Background

Chronic pancreatitis can well be diagnosed by histopathology. For clinical purposes differential diagnostics and patient follow-up we use mainly imaging procedures and non-invasive pancreatic function tests, if available. In this study we report on the evaluation of two tests of exocrine pancreatic function, fecal elastase and breath test with ^{13}C-mixed triglycerides, and relationship to a new clinically oriented classification of chronic pancreatitis (Buchler and Malfertheiner, Bern 2000).
This classification applies to the following criteria: steatorrhoea (STE), diabetes mellitus (DM) and organic changes on pancreas (ORG). There are five defined grades: grade A (no-STE, no-DM, no-ORG); grade B (no-STE, no-DM, with-ORG); grade C1 (STE or DM, no-ORG); grade C2 (STE and DM, no-ORG); and grade C3 (STE and/or DM, with ORG).

Patients and Methods

Fecal elastase 1 (FELA) was determined by the microplate ELISA method using monoclonal antibody to human pancreatic protein (ScheboTech, Germany) in stool samples stored at –70 °C.
^{13}C -mixed triglycerides (MTG-BT) test was performed with 250mg of Glyceryl-1,3-dioctadecanoate-2-octanoate-1-^{13}C substrate, ^{13}C:^{12}C ratio was analysed by infrared Isomax 4000 breath test analyser. Cummulative recovery of ^{13}C was calculated by two methods, using BSA (Body Surface Area) and BMR (Basal Metabolic Rate).
The study group included 50 patients suspected of chronic pancreatitis covering all groups of new classification and tested by both methods of exocrine pancreatic function determination.

Results

FELA levels (mean values in mg/g of stool) significantly distinguish grade B – 219.4, C1/2 – 224.6 or C3 – 75.6, from non-pancreatic controls – 596.4 or grade A – 462.1, i.e. groups without tissue damage.

Cummulative recovery (MTG-BT) after six hours (PDR – mean values in %) significantly distinguishes only grade C3 – 22.3 from other groups: non-pancreatic controls – 50.8; grade A – 50.7; grade B – 47.3 or grade C1/2 – 41.4. Intraassay variability of measured DOB calculated on 750 quadriplicates of breath samples in range 0–60 DOB is 2.8 % (0,214 DOB value), precission of measurement was checked every 3 months using calibration samples with IRMS reference value. The mean difference NDIRS x IRMS was found to be 6.3%.

Conclusion

Measurement of fecal elastase 1 is a simple, non-invasive, robust test which correlates well with the extent of tissue damage. Breath test using ^{13}C -mixed triglycerides (MTG-BT) is better in evaluating of dynamic and kinetic aspects, real digestive ability and response to stimulation. MTG-BT is, contrary to FELA, also suitable to evaluate pancreatic supplementation therapy.

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SUCROSE PERMEABILITY – NON-INVASIVE MARKER OF GASTRODUODENAL DAMAGE

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Background

Absorption of sucrose from the gastrointestinal tract is minimal under normal condition, but in patients with gastric and duodenal impairment increases. Thus, it can be a good non-invasive marker for diagnosis of damage to the stomach and duodenum, including inflammation and ulcer.

Patients and Methods

The aim of the study was to compare absorption of sucrose using endoscopic findings in the oesophagus, stomach and duodenum. 28 patients (17 men, 11 women, average age 63.6 years) with dyspeptic syndrome were examined, 10 healthy volunteers (5 men, 5 women, average age 22 years) served as controls. Gastric permeability was measured using absorption of sucrose from the gastrointestinal tract in a urine sample after 5 hours. The test solution contained 25 g sucrose, 10 g lactulose, 2 g mannitol, and 2 g D-xylose in 100 ml water. Presence of the sugars was measured in the urine using capillary gas chromatography. Patients were divided into 2 groups following endoscopic examination: group N (normal) and P (pathological) – patients with oesophageal, gastric and duodenal inflammation and/or ulcer.

Results

Absorption of sucrose was 0.178 ± 0.09 in patients with normal endoscopy (N, n = 9), 0.527 ± 0.414 in patients with oesophageal, gastric and duodenal inflammation and/or ulcer (P, n = 19) (p 0.01) and finally 0.1 ± 0.06 in control subjects (n = 10) (p 0.001). The value of normality was 0.22. In the normal group (N) six patients were below and three patients over the critical value, in the P group five patients were below and 14 over the critical value 0.22. Absorption of sucrose is a good marker for gastroduodenal damage with the sensitivity 0.74 and specificity 0.67.

Conclusion

Gastroduodenal permeability (measured as sucrose absorption) is a good non-invasive marker of oesophageal, gastric and duodenal damage with sensitivity 0.74 and specificity 0.67 as compared to endoscopy and could be used in examination of patients with dyspeptic syndrome.

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FAECAL ELASTASE ACTIVITY IN RELATION TO GRADING OF CHRONIC PANCREATITIS

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Introduction

For the diagnosis of chronic pancreatitis (CP), we currently use the following main imaging methods: ERCP, MRCP. CT, Endosonography and Abdominal Ultrasonography. These imaging methods give no information on the function of pancreas. However, they show the main morphological changes in the pancreatic ducts and its parenchyma. The classification of CP currently comes after examination of the morphological changes in the pancreatical duct system (Cambridge, Marseille, 1984) or examination of patho-morphological changes (Marseille, Rome, 1988). Faecal elastase I (FELA, mean values in µg/g of stool) is a simple, non-invasive method which correlates well with the damage of pancreatic tissue, stemming from CP. This test is routinely used in the diagnosis of CP.

Patients and Methods

In our studies we have used a newly proposed classification system created in Bern, 2000 (Buchler and Malfertheiner). This new system encompasses morphological and functional changes including: Steatorrhea (STE), Diabetes mellitus (DM), and organic complications of chronic pancreatitis (ORG). This classification system is broken down into the following levels: Grade A (no STA, no DM, no ORG), Grade B (no STE, no DM, with ORG), Grade C1 (STE or DM, no ORG), Grade C2 (STE and DM, no ORG) and Grade C3 (STE and/or DM, with ORG). The FELA occurring in the stool was taken from 67 patients who had been diagnosed with CP stemming from alcoholism. The presence of FELA was classified according to the levels assigned by the classification system.

The control group used in this study included 51 patients diagnosed with a different kind of disease. FELA was determined by a microplate ELISA method using monoclonal antibody to human pancreatic protein (Schebo Tech, Germany).

Results

The occurrence of FELA in the control group (579 µg/g) was similar to a group of patients with CP Grade A (490 µg/g). In patients classified Grade B (325 µg/g) and C1 (351 µg/g) the occurrence of FELA was significantly lower. Levels of FELA were even lower in patients classified Grades C2 and C3 (69 µg/g and 99 µg/g, respectively).

Conclusion

The results demonstrate a very good correlation of FELA with the grading of the newly proposed classification system of CP. Patients with the highest levels (C2 and C3) had a significantly lower occurrence of FELA in comparison with the non- pancreatic control group and patients with CP who have no clinical complications or damage of endocrine and exocrine functions of the pancreas.

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^{13}C-XYLOSE BREATH TEST IN DIAGNOSIS OF SMALL BOWEL BACTERIAL OVERGROWTH – PRELIMINARY RESULTS

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Background

Contemporary diagnostic methods of small bowel bacterial overgrowth are based primarily on small bowel content cultivation and bacterial presentation in amounts of more than 10^3/ml. Diagnostic methods were improved by bacterial reaction with a defined administered substrate. After substrate metabolism by bacterial flora it was possible to detect a marker in exhaled air (H_2 or ^{14}C breath tests) or in urine (indican test). A new, completely non-invasive method for determination of small bowel bacterial overgrowth is ^{13}C –xylose breath test.

Patients and Methods

Small bowel is not able to absorb xylose, the used substrate, under physiological conditions. In case of bacterial overgrowth in small bowel degradation of administered substrate – xylose occurs. ^{13}C labeled xylose (100 mg) in 200 ml of water is administered to a patient. The amount of exhaled $^{13}CO_2$ is detected. Analysis of exhaled $^{13}CO_2$ proceeds by ISOMAX 4000 breath test analyser during 6 hours after administration. Samples of exhaled air are analysed every hour. We evaluate maximum value of ^{13}C reached after 120 or 180 minutes and the percentage of bulk eliminated after six hours.
Preliminary results include 36 patients with suspicion of small bowel bacterial overgrowth and 7 healthy volunteers as a control group.

Results

Average maximum value of exhaled ^{13}C after 120/180minutes in healthy volunteers (2 women and 5 men) was 4.518. Average percentage of the total administered substrate exhaled after six hours was 11.7 %. In five patients (4 women and 1 man – 3 patients after by-pass bowel surgery, 1 patient with Crohn's disease on small bowel and one patient with small bowel hypoganglionosis) unambiguously pathologic values were reached. Average maximal value of exhaled ^{13}C after 120/180 minutes was 14.49 ($p < 0.001$) and average percentage of the total administered substrate exhaled after six hours was 28.46 % ($p < 0.001$). The small bowel bacterial overgrowth in this group was confirmed by a small bowel content cultivation. Thirty-one patients (19 women and 12 men) were examined by ^{13}C-xylose breath test because of long-term dyspeptic complaints. In this group average maximum value of exhaled ^{13}C after 120/180 minutes was 5.90 and average percentage of the total administered substrate exhaled after 6 hours was 13.67 %.

Conclusion

^{13}C-xylose breath test is an entirely new method for diagnosis of small bowel bacterial overgrowth. Literature data concerned with reference normal values is very poor, groups of patients are very small, and results differ depending on methodology of examination. However, this new method is very promising and our first experience indicates this. Use of this method in routine practice is necessary to confirm our results in large group of patients and healthy volunteers.

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COELIAC DISEASE SCREENING WITH RESPECT TO DIFFERENT SCREENING ALGORITHMS

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Background

Coeliac disease (CD) is one of the most underdiagnosed diseases with an overall incidence 1:100 in the general population. Finding the most efficient screening algorithm is very important from both the medical and economic point of view to lower the number of patients undergoing small bowel biopsy (well performed, with complex histopathological and immunological evaluation) which is still needed to make the diagnosis of CD.

Patients and Methods

We present the results of 3239 consecutively tested samples by panel of CD markers: antigliadin antibodies in classes IgA (AGA) and IgG (AGG), antiendomysium antibodies (EmA) and antibodies against tissue transglutaminase (atTG). AGA, AGG and atTG were tested by ELISA methods, EmA by immunofluorescence on smooth muscle samples. 237 patients underwent small bowel biopsy.

Results

Of 237 biopsed patients 97 were found to bear active CD, in 17 CD status of remission was found and 123 were found to be negative for CD. The number of positive serological markers (AGA, AGG, EmA, atTG) in these subgroups was the following [in %]: all 4 markers

positive in 71.1, 5.9 and 4.1, at least 3 positive in 87.6, 23.5, and 8.1, at least 2 positive in 92.8, 41.2, 26.0, at least 1 positive in 97.9, 70.6, 60.2, none positive in 2.1, 29.4 and 39.8 respectively.
Specificity, sensitivity and accuracy of individual markers was following [in %]: AGA 75.6, 87.6, 80.9, AGG 51.2, 90.7, 68.6, AGA and AGG combined 43.1, 94.8, 65.9, EmA 95.9, 80.4, 89.1, atTG 78.9, 90.7 and 84.1 respectively.
Retrospectively we analysed different screening algorithms: 1. Our routine approach of parallel determination of all 4 markers revealed 95/97 (97.9 %) bioptically proven active CD probands. 2. One step screening (just EmA) finding 78/97 (80.4 %) and 86 patients undergoing enterobiopsy. 3. Sequential determination (AGA and AGG in the first step, in case of positivity of at least one of them; EmA as the second step) revealed 77/97 (79.4 %), with 174 patients entering the second step and 85 undergoing biopsy. Sequential determination (like the previous but atTG as the second step) finding 88/97 (90.7 %) with 174 patients entering the second step and 113 undergoing biopsy.

Conclusion

None of the serologic methods itself is specific and sensitive enough to find all CD cases. Of the screening algorithms tested we recommend using parallel testing of antigliadin, antiendomysium and antitissue transglutaminase antibodies

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