



Clinical Evaluation of The Breath Test With Mixed Triglycerides in Chronic Pancreatitis

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ABSTRACT

The main objective of this study was to determine sensitivity and specificity of C13 labeled breath test with mixed triglycerides (¹³C-MTG BT) in patients with chronic pancreatitis (CP), and to compare results to imaging CT/MRCP data and Fecal-elastase-1. Study encompassed 40CP patients (26 males); mean aged 53.29 ± 14.09. Patients were divided according to CT/MRCP Cambridge classification. ¹³C-MTG BT3 was analyzed by a mass-spectrometry and FE-1 by ELISA (ScheBo® Biotech). Results: 30% of the patients were positive for ¹³C-MTG BT. We found a difference between ¹³C-MTG BT mean values for CT/MRCP subgroups 1-4, p<0.001. ¹³C-MTG BT sensitivity and specificity for severe disease were 80% and 85%. Agreement between ¹³C-MTG BT and FE-1 was 78%. Conclusion: there was a relationship between ¹³C-MTG BT levels and severity of morphological imaging data and FE-1 results. The combination of both tests, FE-1 and ¹³C-MTG BT may contribute to better PEI detection.

KEYWORDS : Chronic pancreatitis, Breath test with mixed triglycerides ¹³C-MTG BT, FE-1, PEI

INTRODUCTION

Chronic pancreatitis (CP) is a result of progressive and irreversible damage of the pancreas. It leads to progressive loss of pancreatic exocrine and endocrine function. Current approach for the diagnosis is based on a set of imaging and functional tests. [3,5,8] Nevertheless there are direct and indirect tests to assess function, direct tests for detecting exocrine insufficiency (PEI) are standard for diagnosis [3,5,8]. Most commonly in everyday practice are indirect-Breath test with mixed triglycerides (¹³C-MTG BT) and fecal elastase-1 (FE-1). We aimed to determine ¹³C-MTG BT sensitivity and specificity and to compare it with imaging and FE-1.

MATERIALS AND METHODS

Patients and diagnosis

We have prospectively studied 45 subjects suspected for diagnosis CP March 2014 to September 2015. In five of the studied patients second imaging method was not performed and therefore they were excluded from the subsequent assessing of the data. Among CP patients 57.5%(23) were males and 42.5%(17) females; mean aged 53.38 ± 14.68 years (range 24 - 76 yrs). All participants were admitted and/or followed up in the Gastroenterology department of University Hospital "Tsaritsa Ioanna-ISUL" after obtaining of informed consent in accordance with the Declaration of Helsinki. The clinical signs and symptoms were assessed by gastroenterologist. CP diagnosis is made on accepted MANNHEIM criteria [7]. We did not included patients with previous pancreatic surgery or any other operations of the gastrointestinal tract. Criteria for alcohol abuse was SRAI 25 -900 g/day and for smoker 5 to 70 pack-years.

Pancreatic morphology

Initially all subjects underwent abdominal ultrasound and structural changes were confirmed either by Computed tomography(CT) and/or Magnetic resonance cholangiopancreatography (MRCP) using Cambridge classification[5]. Disease staging was documented by radiologist.

Breath test with mixed triglycerides

In the presence of normal enzyme activity pancreatic lipase degrades the substrate 2-octanoyl(1-¹³C)-1,3-distearoylglycerol) with releasing labeled ¹³C-fatty acids, which were absorbed and metabolized in the liver. The proportion(quotient) of ¹³CO₂/¹²CO₂ in exhaled air was measured in each breath sample by mass spectrometry. The result of the test is expressed as total 6-hour cumulative recovery rate (CRR) of

¹³CO₂. Accepted clinical cut-off for the CRR is 29%. Results less than 29% were defined as positive. Values above 29% were considered negative. The analysis of breath tests was conducted in laboratory Isomed Pharma, Spain. Test sampling was done according laboratory instructions.

Fecal Elastase-1

Fecal Elastase-1 (FE-1) was assessed by enzyme immunoassay (ELISA) with specific monoclonal antibodies (ScheBo® Biotech FE-1 ELISA Germany). We used the generally accepted cut-off 200 µg/g feces.

Statistical Methods

Statistical analysis was performed by SPSS 21.0 (Chicago, IL). The data were described by the mean, 95% confidence interval (CI) of the mean, standard deviation (SD), presented in graphical and tabular form. Alpha level of 0.05 was accepted as significant.

RESULTS

¹³C-MTG BT and patients characteristics

We have enrolled 40 subjects. The most common etiology was alcohol abuse observed in 24 patients (60%). 65% of patients were with pain symptoms(26). The demographic and clinical data and mean values from the performed ¹³C-MTG BT are presented in Table1(t-test for all comparisons)

Table 1 Patients characteristics and the ¹³C-MTG BT mean values

		N (<29%)	¹³ C-MTG BT	p*
			Mean(±SD)	
Age	<50	18(8)	27.33±15.03	0.014
	>50	22(4)	37.45±9.53	
BMI	< 20	11(6)	27.18±13.45	0.09
	> 20	29(6)	35.07±12.61	
Gender	male	23(9)	29.91±15.08	0.09
	female	17(3)	36.94±8.94	
Alcohol	no	16(3)	38.13±11.13	0.039
	yes	24(9)	29.42±13.47	

Smoking	no	19(4)	37.68±10.09	0.027
	yes	21(8)	28.57±14.32	
Diabetes	no	33(9)	33.39±13.79	0.61
	yes	7(3)	30.57±10.28	
Pain	no	14(4)	33.29±10.22	0.89
	yes	26(8)	32.69±14.70	
Diarrhea	no	30(7)	35.40±12.35	0.036
	yes	10(5)	25.40±13.28	
Weight loss	no	25(5)	35.88±10.96	0.06
	yes	15(7)	27.93±15.32	

Mean levels of ¹³C-MTG BT for the whole group were 32.9 ± 13.17% (range 3.05-57%). Positive results for ¹³C-MTG BT were observed in 30%(12) patients while 70% were negative. The mean ¹³C-MTG BT were found lower in smokers (vs non-smokers), patients with alcohol abuse (vs non-abusive), in subjects under 50 years and in those presented with diarrhea/steatorrhea symptoms, p<0.05 (Table1). Positive ¹³C-MTG BT patients had lower BMI compared with negative ones (20.92±2.97 vs 24.76 ± 4.4), p 0.009.

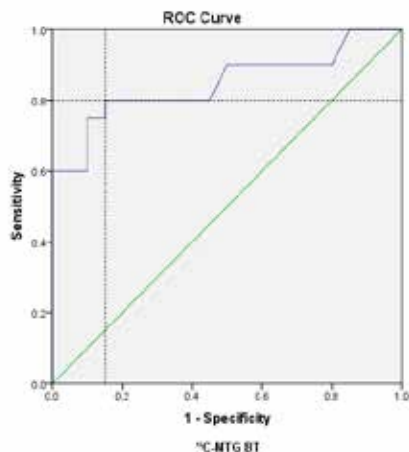
¹³C-MTG BT and pancreatic morphological changes

The results for mean ¹³C-MTG BT values within the different CT/MRCP Cambridge groups were: 1(n8) 45.13%±7.26%; 2(n10) 36.90%±5.78%; 3(n9) 31.78%±10.37%; 4(n13) 23.15%±14.84%. We found a significant difference between ¹³C-MTG BT mean values for subgroups 1- 4 by CT/MRCP, p 0.001(ANOVA). Between subgroups of mild (Cambridge 1 and 2) and severe structural changes (Cambridge 3 and 4) there was also a significant difference, p 0.001. The mean ¹³C-MTG BT were lower in patients with calcifications 28.67±12.99 (vs patients without 37.58±12.00%, p 0.031) and in patients with dilated pancreatic duct 27.47±14.59 (vs patients without 37.80±9.66, p 0.011).

Sensitivity and specificity of ¹³C-MTG BT

The relationship between ¹³C-M TG BT and the severity of the structural changes was analysed by ROC analysis (Figure 1).

Figure 1 Specificity and sensitivity of ¹³C-MTG BT according to morphological changes

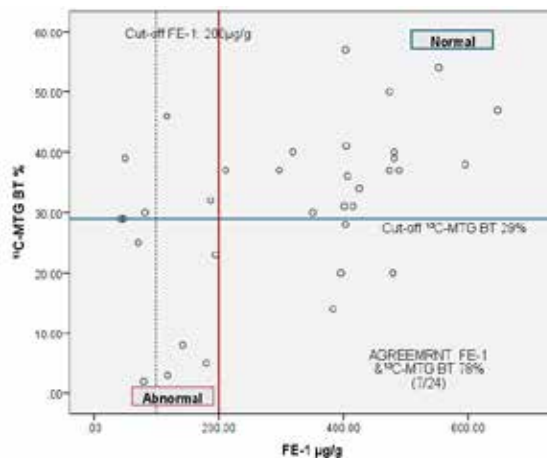


Area under the curve(AUC) is 0,848 (p <0.0001,95%CI 0.720-0.975) and determines the level of probability for PEI, corresponding with severe disease in 80% of cases - sensitivity. Specificity of the method was 85%. Based on these results we estimated positive Likelihood Ratio(LR)- 5.3 and negative LR only- 0.23, which meant that the chance of having the disease is more than 5 times more probable .

¹³C-MTG BT and Fecal elastase-1

Mean FE-1 values were 317.61±176.99 µg/g feces. Eleven patients in overall study group was with positive FE-1results with levels below the accepted clinical cut off 200µg/g.

Figure 2 ¹³C-MTG BT and FE-1 results distribution



We compared the results from ¹³C-MTG BT and fecal elastase-1 and found agreement in 78% of patients results (31 of cases figure 2). Discrepancy was found in the remaining 9 patients, 5 of them were with positive ¹³C-MTG BT. To investigate further the relation between FE-1 and ¹³C-MTG BT we performed linear regression and calculated R was 0.477, R square - 0.227, p 0.004. Multiple logistic regression revealed that combining the both tests (FE-1 and ¹³C-MTG BT) may improve PEI detection in 88.4% of cases. ¹³C-MTG BT had 43% contribution with Odd Ratio, OR 1.43 (p 0.011, 95%CI 1.086-1.872).

DISCUSSION

We have found relationship between ¹³C-MTG BT levels and alcohol abuse, smoking and presence of diarrhea/steatorrhea symptoms. There was also a relationship between low BMI and ¹³C-MTG BT. The main limiting factor in the present study is the lack of a control group and relatively short period of observation.

Current approach to the diagnosis of CP is based on the imaging. Functional tests are recommended but proper criteria of their application are not defined. [3,5,8] We found a strong relationship between CT/MRCP severity grade based on Cambridge classification and ¹³C-MTG BT levels and also with calcifications and main pancreatic duct dilatation when they are assessed separately. Calculated sensitivity was 80% and specificity was 85% of ¹³C-MTG BT based on imaging data. Published data on C-MTG BT sensitivity and specificity are based on different comparisons. Previously¹³C-MTG BT sensitivity and specificity were defined by comparison with a direct test (secretin-caerulein) in patients with mild and severe CP. According to these study sensitivity is 69-81%[6]. Another study compared ¹³C-MTG BT with Coefficient of Fat Absorption and reported sensitivity and specificity > 90% for PEI and fat malabsorption[1]. The comparison of both indirect tests ¹³C-MTG BT and FE-1 demonstrated good correlation. The low sensitivity in mild and moderate PEI are a major problem in all indirect pancreatic tests [3]. However, indirect tests are non-invasive for the patient and more suitable for routine use. The agreement between ¹³C-MTG BT and FE-1 in 78% of patients results. Although ¹³C-MTG BT is superior to FE-1 regard to it sensitivity, it is more expensive.

CONCLUSIONS

The negative results of any of the two tests should be interpreted in light of the accompanying clinical, laboratory and imaging data and when needed to carry additional functional tests. Both functional tests (¹³C-MTG BT and fecal elastase-1) could be used for better selection of patients who would need secretin enhanced MRCP.

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Disclosure statement

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