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Alterations of Vitamin D_3 Metabolism in Young Women with Various Grades of Chronic Pancreatitis

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Abstract
Background: There are still too few conclusive reports about conspicuous vitamin D-deficiency in young female patients with chronic pancreatitis, or any connection of the deficiency to the severity of the disease. Therefore the aim of this study was to examine marker of vitamin D_3 metabolism in female patients with episode of biliary pancreatitis to determine if increased severity of the disease would correlate with impaired vitamin D_3 metabolism.
Methods: Between 1996 and 2003, we investigated 53 premenopausal patients with an average age of approximately 33 years suffering from an episode of chronic pancreatitis, as well as 30 female healthy controls with an average age of 32.4 years. The severity of chronic pancreatitis in patients was determined via endocopic retrograde cholangiopancreaticography (ERCP) and assigned to 1 of 3 grades based on the Cambridge classification. Additional parameter assessed were demographics, smoking, consumption of alcohol and CD-transferrin, fasting metabolic parameters, biochemical markers of vitamin D_3 metabolism and fecal elastase1. None of the patients received hormone replacement therapy, Vitamin D or Calcium-supplementation.
Results: The serum levels of 1,25-dihydroxyvitamin D [1,25(OH_2)D] were significantly reduced compared to female healthy controls. Fecal elastase 1 correlated with this classification of severity of chronic panreatitis ($p < 0.01$). Furthermore, fecal elastase 1 of patients correlated the same way with both D-vitamins ($p < 0.01$). The level of both D_3 vitamins in patients were significantly lowered when the content of fecal elastase 1 was under 200 µg/ g compared to the others [for 1,25-$(OH_2)D_3$ $p < 0.01$; 25-OH- D_3 $p < 0.01$].
Conclusion: Premenopausal patients with chronic pancreatitis are at risk of developing decreased levels of 1,25$(OH_2)D_3$. This fact may contribute to a negative calcium balance and alteration of bone metabolism. Therefore, ERCP and fecal elastase 1 verify the severity grade of a chronic pancreatitis, and thus show a vitamin D_3 deficiency in young women, depending on the progress of disease.

Key words: Cambridge-classification, Fecal elastase 1, chronic pancreatitis, Vitamin D_3 metabolism

Introduction

Inflammation mediators are involved in the regulation of many metabolic and endocrine processes. For this reason, data on the calcium metabolism of patients with chronic pancreatitis are of particulary interest, considering constantly changing physiologic states during the course of disease. Alterations of calcium metabolism have been observed in numerous studies of small groups of male patients with chronic pancreatitis: [1, 2, 3, 4]. However, in addition to the disease process itself , social and individual factors seem to bee of importance in the pathogenesis of potentially pancreatitis-associated osteopathy, for instance, a lack of sufficient and balanced diet including vitamins and minerals or the abuse of alcohol and smoking.

Endoscopic retrograde cholangiopancreaticography (ERCP) is the most sensitive imaging procedure of diagnosing chronic pancreatitis [5]. Compared with the „gold standard", the secretin caerulein test; fecal elastase 1 is a highly sensitive and specific noninvasive pancreatic function test [6, 7]. A parallel between exocrine function and ERCP results is found in chronic pancreatitis [8] even when using fecal elastase 1 to determine exocrine pancreatic insufficiency [8, 9].

One field which has until now received little attention are the changes of vitamin D_3 serum levels subjected to the different severity grades of pancreatic exocrione insufficiency. As vitamin D deficiency and primary hyperparathyroidism are relatively common, a coexistence of these conditions must be considered. [10]. The consequences of chronic pancreatitis may be relevant to serum levels of lipid soluble vitamin D_3 because of its dependence on photosynthesis in the skin, as well as on direct intestinal resorption. It should be creates a relevant link between chronic pancreatitis and bone metabolism or if there are any other connections.

Starting from these assumptions, we performed a larger cross-sectional study of female premenopausal patients with chronic pancreatitis in which we analyzed serum samples for vitamin D_3 metabolism. In addition, we investigated which influence on markers of vitamin D_3 metabolism is exerted by fecal elastase 1 and the Cambridge classification of pancreatitis [11].

Patients and Methods

Patients:
Between 1994 and 2001 53 female patients participated in the study. They were admitted to hospital for work-up a chronic pancreatitis. All patients had typical disease history as well as diagnosis based on abdominal sonography, computer sonography (criteria: structural inhomogenity of the parenchyma, organ atrophy, expansion of the pancreatic duct, facultative existence of pseudocysts or calcification) and routine parameters. Exclusion criteria were: male sex, age under 17 or over 40 years, acute biliary obstructions, cystic fibrosis, relevant alcohol consumption; medication with influence on endocrine parameters and chronic or severe concomitant disease and diabetes mellitus. At the time of examination none of the patients had concomitant opportunistic infections; acute or chronic hepatitis with increased transaminase activities; alterations of the liver parenchyma under sonomorphological criteria; wasting symptoms; gastrointestinal disorders such as chronic diarrhoea. Fasting blood samples were obtained by puncture of a cubital vein. The serum was frozen and stored at -30 °C until analysis.

Control:
An age - matched control group consisting of 30 healthy female individuals was studied for comparison. All subjects were kept busy all a day long in departments of the german health service. No individual performed extreme physical exercise. Their values of biochemical markers were in accordance with the normal range of the laboratory.

Endoscopic investigations:
A standard endoscopic retrograde cholangiopancreatography (ERCP) was performed exclusively in patients to verify the diagnosis. Based on the ERCP results, the morphological alterations of the pancreas were classified into three grades, according to the cambridge classification of 1984 [11]: I equivocal; II mild to moderate; III severe; respectively.

Biochemical measurements:
Calcitriol ("1,25 (OH)2 Vitamin D"-kit from Immun Diagnostik, Bensheim, Germany; competitive radio receptor assay) and calcefediol ("25 (OH)2 Vitamin D"-kit from Immun Diagnostik, Bensheim, Germany; competitive protein-binding-assay). Pancreatic elastase 1 ("Pankreatic Elastase 1"-kit from ScheBo Biotech, Giessen, Germany; double-sided enzyme immuno-assay) was determined in the faeces of all participants.

Statistical analysis:
The following methods were applied for statistical analysis: Normal distribution of data was analyzed by the Kolmogorow-Smirnov normality test. Single factor variance analysis, the Scheffe'-test, the nonparametric Kruskal-Wallis-test with the subsequent Dunn test, as well as the t-test for independent random samples with and without Welch correction. The Pearson's correlation coefficient and also the nonparametric Spearman rank correlation coefficient were applied for finding any connections. Statistical analysis was performed using Statistical Package for Social Science (SPSS)[12, 13].

Results

Table 1 shows the parameter analyzed as means ± standard deviation of the respective groups concerning their Cambridge-grades and the controls.

Fecal elastase 1 and both 25 (OH)D_3 1,25 (OH)2D_3 of female patients with pancreatitis were markedly decreased compared with controls ($p < 0.01$). Fecal elastase 1 in patients correlated significantly with Calcitriol and Calcefediol (Table 4, $p < 0.05$). The vitamins 1,25 (OH)2D_3 in patients with Cambridge Grades II and III was markedly decreased compared with those with Cambridge Grade I and differed significantly within the various Cambridge groups (Table 3). There were significances in differences between the various groups concerning the 25 (OH)D_3, too.

Discussion

Few studies have addressed vitamin D_3 metabolism in chronic pancreatitis and insufficiency of exocrine pancreas. Furthermore, it became evident how relevant morphological changes of pancreas are for correct grading of disease, according to Cambridge classification of 1984 [11]. Diagnosis of chronic pancreatitis shows a parallel between exocrine function and ERCP results [2,6,7], even using fecal elastase 1 to determine pancreatic insufficiency [6, 7]. Compared with the „gold standard", the secretin caerulein test, faecal elastase 1 showed a highly significant negative correlation with morphologic changes in patients, according the Cambridge classification. Lower levels of faecal elastase 1 were expressed in higher severity grades of chronic pancreatitis. These results confirm statements made by Dominguez-Munoz et al. [7], Glasbrenner et al. [9] and Mann et al. [15] by verifying the usefulness of faecal elastase 1 in diagnosis of chronic pancreatitis.

Until now, very little has been published about deficiency of lipid soluble vitamins, especially vitamin D, in patients with chronic pancreatitis [2, 3 4, 16, 17]. The aim of the present study was to get an information about the vitamin D_3 metabolism in patients with deficiency of fecal elastase 1 in chronic pancreatitis. Both D_3-vitamins could be recognized to be lower in patients with deficiency of fecal elastase 1 more clearly for calcitriol than for calcefediol. These observations enhance statements already made by Dibble et al., Nakamura et al., Moran et al., as well as Haaber et al. [3, 4, 15, 17]. As described by these authors, the frequent presence of decreased vitamin D in patients with chronic pancreatitis is already confirmed by our own results comparing premenopausal female patients with controls. Hence, the conform statement of distinctly decreased vitamin D-levels could be associated with the severity grade of the disease according to the Cambridge classification and the faecal elastase 1. The reduction of serum concentration, as described by Scharla et al. [18], especially of calcitriol during a chronically inflammatory process, could explain its retreat due to an increasing inflammatory destruction of

Table 1. Age, faecal elastase 1, calcitriol and calcefediol (means ± standard deviation) in female patients with chronic pancreatitis and controls. $p < 0,05$ indicates a significant difference between the patient-collectives allotted by the Cambridge-classification.

Parameters	**Controls**	**Female Patients**				
		Cambridge-grade			**p**	**Total**
		I	**II**	**III**		
	(N = 30)	(N = 16)	(N = 26)	(N = 11)		(N = 53)
Age (years)	32.4 ± 5.7	31.2 ± 11.3	33.1 ± 7.4	32,8 ± 8,1	n.s.	32.3 ± 8.7
Faecal elastase 1 (mg/g)	703 ± 132.2	326 ± 81.9	161.2 ± 74.1	117.8 ± 54.0	$p < 0.05$	201.9 ± 72,3
Calcitriol (pg/ml)	63.9 ± 5.7	43.1 ± 11.2	29.4 ± 7.1	24.6 ± 10.4	$p < 0.05$	36.9 ± 9.2
Calcefediol (nmol/l)	74.1 ± 14.7	39.1 ± 13.7	27.6 ± 5.3	20.9 ± 6.1	$p < 0.05$	29.68 ± 8.0

Table 2. Correlation between Cambridge-grade and elastase 1 in faeces in female patients with chronic pancreatitis. $p < 0.05$ indicates a significant correlation.

N = 53 **Parameter**	**Cambridge-grade**
Elastase 1 in faeces	
Correlation Spearman	-0.607
p	$p < 0.05$

Table 4. Correlation between vitamin D_3 and elastase 1 in faeces in patients with chronic pancreatitis. $p < 0,05$ indicates a significant correlation.

N = 53 **Parameter**	**Vitamin D_3** Calcitriol	Calcefediol
Elastase 1 in faeces		
Correlation Pearson	0,69	0,61
p	$p < 0,05$	$p < 0,05$

Table 3. Comparison of faecal elastase 1, calcitriol and calcefediol between different patient-collectives allotted by the Cambridge-classification and controls. $p < 0,05$ indicates a significant difference between patients with chronic pancreatitis and controls.

Parameters	Error probabilities of variation in comparison between patients and controls (N = 20)			
	Collectives of patients with chronic pancreatitis			
	Total (N = 53)	**Cambridge I** (N = 16)	**Cambridge II** (N = 26)	**Cambridge III** (N = 11)
Elastase 1 in faeces	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Calcitriol	$p < 0.001$	$p = 0.001$	$p < 0.001$	$p < 0.001$
Calcefediol	$p < 0.001$	$p = 0.001$	$p < 0.001$	$p < 0.001$

the pancreas. Nevertheless, the increasing morphological pancreas alterations accompanied by exocrine functional restriction with corresponding absorption disturbance in higher severance grades of chronic pancreatitis seem to be the most important reason for decreased vitamin D in patients. This respective occurrence of decimating vitamin D-pool, represented by calcefediol, would also explain the predominant constellation of decreased calcitriol in our patient study and those of Nakamura et al. [17], almost exclusively in all those with also low calcefediol.

Also, Haaber et al. [4] described the lack of difference for calcitriol and calcefediol depending on the exocrine insufficiency as well as on the duration of the disease in patients with chronic pancreatitis. Nevertheless, all these observations are not suitable to invalidate the link of elastase 1 values in feces, severity of disease and vitamin D-deficiency in patients with chronic pancreatitis. The results from Haaber et al. [4] also loose their significance, because enzymes were substituted in patients with exocrine pancreatic insufficiency. On the basis that under normal circumstances 80-90% of experimentally applied, radio-actively labelled vitamin D_3 is absorbed by the intestines, although only 40% in patients with pancreatic insufficiency [20], exocrine pancreatic function gains significance and supports our own results with corresponding evaluation of elastase 1 in feces. Furthermore, it is

conceivable that elastase 1 plays an independent role with regard to vitamin D_3-supply in the organism. Since by passing the intestines, elestase 1 changes to a complete protein sterol complexity by loading neutral steroids [21], and vitamin D_3 is also a sterol molecule, there is a hypothetical cross-link here. Therefore, there are still queries regarding the importance of sterol linking in elastase 1 in interaction with its excretion status for vitamin D_3-supply. As vitamin D deficiency and primary hyperparathyroidism are relatively common, a coexistence of these conditions must be considered. Vitamin D deficiency may increase the severity of primary hyperparathyroidism (presence of larger adenomas, higher PTH levels, and greater bone turnover). Nevertheless, some of the biochemical features of primary hyperparathyroidism can be masked by the coexisting vitmain D deficiency leading to an inappropriate therapeutic mangement of these patients [10].

In summary, female patients with chronic pancreatitis show alterations of the vitamin D metabolism. The impaired exocrine pancreas function expressed as reduced fecal elastase 1 causes a reduced intestinal vitamin D_3-supply. The estimation of fecal elastase 1 is a well founded indirect test for determination of vitamin D supply in young female patients, too.

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