MRI appearance of the pancreas in patients with cystic fibrosis: a comparison of pancreas volume in diabetic and non-diabetic patients

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ABSTRACT. We investigated differences in the volume of the pancreas in cystic fibrosis (CF) patients with and without diabetes using MRI to study the natural history of CF-related diabetes (CFRD). We investigated 29 pancreas-insufficient adult CF patients, 13 with CFRD and 16 without diabetes. Patients with CFRD were receiving insulin therapy at the time of study. None of the non-diabetic CF patients had evidence of impaired glucose tolerance. Pancreas volume was estimated by MRI scans using T_1 weighted fat-suppression sequences and assessed by an examiner who was unaware of the patients' diabetes status. Pancreas volume of CF patients was measured and subsequently compared with that of non-CF age-matched Type 1 diabetes (T1DM) patients and healthy controls previously investigated. The two CF groups were matched for age and gender. There were no differences in spirometry values, body mass index or pancreatic exocrine function. The pancreas was visible by MRI in only 3 of 13 (23.1%) patients with CFRD and in 5 of 16 (31.3%) patients without diabetes (p -value=0.7). In total, the pancreas was not detected by MRI as an anatomical entity in 21 of 29 (72.4%) CF patients, irrespective of their diabetes status. When comparing the four study groups, the pancreas was significantly smaller in CF patients than in T1DM patients and healthy controls.

Received 27 November 2008
Revised 20 July 2009
Accepted 8 September 2009

DOI: 10.1259/bjr/24009651

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Cystic fibrosis-related diabetes (CFRD) is a unique and distinct form of diabetes mellitus (DM). The improved care and increased life expectancy of patients with CF has led to an increase in the number of patients who develop diabetes in the context of CF [1]. The prevalence of CFRD increases with age, with a prevalence rate ranging from 14% to 25% [2, 3].

The pancreas has been found to be abnormal in post-mortem studies of CF patients [4, 5]. The mutations in the CF transmembrane conductance regulator gene (CFTR), the hallmark of CF [6], lead to viscous secretions because of increased protein concentration and imbalanced chloride and bicarbonate ion secretion, coupled with reduced secretion of fluid into the pancreatic ducts [7–9]. This process, which starts during intra-uterine life [10], progresses to obstruction of pancreatic ducts, release of proteases and lipases within the obstructed ducts and

autolysis of the pancreas. In the later stages of CF, fibrosis and atrophy predominate, and from then the pancreatic tissue is replaced by fat.

Exocrine abnormalities manifest early in the lives of CF patients, with the majority needing to take pancreatic enzyme supplements to aid digestion. By contrast, CFRD more commonly appears later in life when the number of insulin-producing beta cells falls below that in normoglycaemic CF patients [5, 11].

Diabetes is a detrimental factor in patients with CF in terms of both mortality and morbidity. CFRD is associated with premature mortality, especially in female patients, with mortality rates up to six times greater than those for non-diabetic CF patients [12]. Patients who have CFRD have an accelerated decline in lung function and an increase in the rate of pulmonary exacerbations [13, 14]. Furthermore, as patients are living longer, the microvascular complications seen in patients with long-standing Type 1 and Type 2 diabetes are now being found in a proportion of patients who have had CFRD for more than 10 years [15–17].

Although CFRD is thought to be secondary to beta cell destruction by fibrous tissue replacing the exocrine pancreas, the progression of pancreatic exocrine damage in relation to the development of diabetes is not completely understood. Little is known of the natural

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This study was carried out at the Bristol Royal Infirmary, University Hospitals Bristol NHS Foundation Trust, Marlborough Street, Bristol, BS2 8HW, UK. It was funded by an unconditional grant from the Charitable Trustees for University Hospitals Bristol NHS Foundation Trust.

history of CFRD, and factors that predict the development of diabetes in CF are also unknown. CFRD may have a multifactorial pathogenesis.

Recently, Williams et al [18] showed that MRI is a reliable method of estimating pancreatic atrophy in patients with long-standing (over 10 years since diagnosis) Type 1 diabetes (T1DM). This technique offers a non-invasive and precise method of measuring pancreatic volume. To our knowledge, a similar comparison between CF patients with and without diabetes has not been made systematically. We hypothesised that there may be differences in the size and morphology of the pancreas in CF patients with and without CFRD, and that this could potentially be useful in determining the natural history of CFRD. Pancreatic assessment could possibly provide a clue to the natural history and to predictive factors for the development of CFRD.

The primary aim of this pilot study was to compare the volume and MRI features of the pancreas in CF patients with CFRD with those in CF patients with no evidence of diabetes or impaired glucose tolerance. Secondary aims were to correlate pancreas volume with spirometry (forced expiratory volume in one second (FEV1) and forced vital capacity (FVC)), body mass index (BMI) and exocrine pancreatic function. We also aimed to compare the volume of the pancreas of CF patients with those of age-matched non-CF patients with long-standing T1DM and with healthy controls.

Methods and materials

29 pancreas-insufficient adult patients with CF, all of whom received pancreatic supplements, were enrolled in the study — 13 patients with CFRD and 16 patients without diabetes. The mean age of the patients was 26.1 years (range 17–42 years). All patients had the diagnosis of CF confirmed by clinical features, positive chloride sweat concentration tests and the presence of known CF mutations. Table 1 shows the demographic data describing CF patients with and without diabetes.

Patients were considered to be diabetic if they had previously demonstrated a 2 h plasma glucose equal to or above 11.1 mmol L^{-1} (200 mg dL^{-1}) during a standard oral glucose tolerance test (OGTT). All diabetic patients were on chronic daily insulin replacement therapy at the time of the study for sustained glucose intolerance. CF patients without diabetes had at least two normal OGTTs in the two consecutive years prior to the study and no

evidence of glucose intolerance according to World Health Organization criteria [19].

Patients' spirometry values, BMI, and exocrine pancreatic function (as assessed by patients' daily oral intake of lipase) were measured on entry to the study, during disease stability. Genotype was also recorded.

MRI scans were performed using a Siemens 1.5 T scanner. Planar images of the pancreas were obtained using two breath-hold scan sequences: T_1 weighted breath-hold with fat suppression (T1BHFS) sequence using a T_1 weighted transverse gradient-echo fat-saturated image (time of echo (TE) 2.66 ms, repetition time (TR) 159 ms, flip angle 80° , field of view (FOV) $350 \times 262 \text{ mm}$, slice thickness 6 mm); and three-dimensional (3D) volumetric interpolated breath-hold examination (VIBE) sequence, which was a T_1 volume gradient-echo sequence with fat saturation (TE 2.51 ms, TR 5.15 ms, flip angle 10° , FOV $350 \times 262 \text{ mm}$, slice thickness 2.5 mm). The MRI sequences selected were validated and found to give the best delineation of pancreatic tissue in a previous study [18]. No contrast medium was administered. All patients were scanned using both sequences.

The area of the pancreatic tissue on each slice was estimated and multiplied by the interval between the slices to obtain the pancreas volume. The area was estimated using the scanner software (Siemens) from a hand-drawn outline of the pancreas on each image. All MRI images were assessed by an examiner (MC) who was unaware of the diabetes status of each patient.

The volume of the pancreas of the CFRD patients was initially compared with that of non-diabetic CF patients, and then compared with that of previously published age-matched non-CF patients with long-standing T1DM and healthy controls [18].

This study was approved by the North Somerset & South Bristol Research Ethics Committee.

Statistical analysis

It was clear from the outset that the data describing pancreatic volume in the four study groups were not normally distributed. Pancreas volume in CF patients with and without diabetes was compared using the non-parametric Mann-Whitney *U*-test. Pancreas volumes in the four groups were compared using the non-parametric Kruskal-Wallis test.

In CF patients with and without diabetes, comparisons of age, FEV1, FVC, BMI and daily intake of lipase were

Table 1. Demographic characteristics of the four study groups — CF patients without diabetes, CFRD patients, long-standing Type 1 diabetes mellitus (DM) patients and healthy controls

	CF without diabetes	CFRD	Type 1 DM	Controls	<i>p</i> value
Number	16	13	12	12	–
Gender, male:female	7:9	6:7	12:0	12:0	–
Age (years), mean (sd)	25.8 (6.8)	26.4 (6.3)	29.2 (4.2)	27.5 (3.8)	P NS
BMI (kg m^{-2}), mean (sd)	22.1 (2.6)	23.5 (3.8)	25.6 (2.2)	25 (2.1)	P NS
FEV1 %, mean (sd)	70.9 (22.7)	67.3 (22.2)	–	–	P NS
FVC %, mean (sd)	80.7 (20.4)	78.3 (18.3)	–	–	P NS
Daily lipase (unit kg^{-1}), mean (sd)	6698 (3442)	6063 (3385)	–	–	P NS

P NS, *p*-value statistically non-significant; sd, standard deviation; BMI, body mass index; FEV1, forced expiratory volume in one second; FVC, forced vital capacity.

made using Student's two-tailed *t*-test. Differences were considered statistically significant when *p*-values were less than 0.05.

Results

Comparison of CF patients with and without diabetes

The two groups of CF patients were matched for age and gender. 10 (62.5%) of the 16 CF patients without diabetes and 6 (46.2%) of the 13 CFRD patients were homozygous for the $\Delta F508$ mutation.

There were no statistically significant differences in spirometry values, such as FEV1 and FVC, BMI or pancreatic exocrine function between the two groups.

The pancreas was visible by MRI in just 3 (23.1%) of 13 patients with CFRD and in 5 (31.3%) of 16 patients without diabetes (*p*-value=0.7). In total, the pancreas was not detected by MRI as an anatomical entity in 21 of 29 (72.4%) patients, irrespective of their diabetes status (Figures 1 and 2).

The volume of the pancreas did not correlate with gender, age, FEV1, FVC, BMI or with the daily intake of lipase as a marker of exocrine pancreatic function.

Comparison of CF patients with and without diabetes and non-CF groups

All of the non-CF patients and healthy volunteers were male. Non-CF patients were slightly older than the CF patients, but the difference did not reach statistical significance. Similarly, although the BMI was slightly greater in the non-CF groups, this did not reach statistical significance (Table 1). Data on volume of the pancreas are detailed in Table 2.

The volume of the pancreas was significantly larger in the healthy control group than in all other groups. As

Figure 1. Magnetic resonance planar image slice obtained using the T_1 volumetric interpolated breath-hold examination (VIBE) sequence at the level of the porto-splenic junction and splenic vein from a patient with cystic fibrosis-related diabetes (CFRD). The pancreas area looks indistinct, completely substituted by fat. The pancreas is not visible as an entity.

Figure 2. Magnetic resonance planar image slice obtained using the T1BHFS sequence including the largest area of the pancreas from a cystic fibrosis patient without diabetes. The pancreas is easily visible.

previously reported, pancreatic volume was reduced in patients with long-standing T1DM in comparison with that of the healthy controls [18]. Pancreatic volume in CF patients was significantly smaller than that in both healthy controls (*p*-value <0.001) and T1DM patients (*p*-value <0.001). In the majority of cases, it was not possible to assess pancreatic volume in CF patients by MRI (Figures 3 and 4).

Discussion

MRI has been shown to be a reliable and reproducible method of detecting and measuring the size of the pancreas [18]. An important advantage of MRI is the possibility of accurate pancreatic assessment without the risks of ionising radiation associated with CT scans. MRI is also superior to ultrasound examinations for analysis of the pancreas because it allows precise differentiation between intra- and peripancreatic fat. This is not always possible when ultrasound is used: a falsely enlarged pancreatic volume may be measured if extra-pancreatic regions that have been replaced by fat are included in addition to true pancreatic tissue [20].

Post-mortem analyses and *in vivo* imaging of the pancreas of CF patients have shown that the pancreas is significantly reduced in size in patients with CF [21], and that pancreatic fatty replacement is the most commonly found pattern on CT and MRI [20, 22]. Therefore, prior to the beginning of our study, we anticipated that the volume of the pancreas of CF patients would differ from that of healthy individuals and that the volume loss would be greater in CFRD patients. In contrast to other studies [22, 23], we applied the MRI fat-suppression sequences T1BHFS and VIBE. We were therefore able to differentiate real pancreatic tissue from fatty replacement within the pancreas and to measure the volume of the pancreatic tissue alone. As a result, no measurable pancreatic tissue was the most common finding. The absence of the pancreas as an anatomical entity in the vast majority of patients, irrespective of their diabetes status, was unexpected. The severity of the pancreatic loss is probably owing to the gradual autolytic process suffered by the pancreas.

Table 2. Pancreas volume in the four study groups as assessed using the T_1 volumetric interpolated breath-hold examination (VIBE) sequence — cystic fibrosis (CF) patients without diabetes, cystic fibrosis-related diabetes (CFRD) patients, Type 1 diabetes mellitus (DM) patients and healthy controls

	CF without diabetes	CFRD	Type 1 DM	Controls
Number	16	13	12	12
Median volume (ml)	0	0	54.7	100.5
Mean volume (ml)	9.9	7.1	52.5	104.8
95% CI (ml)	0.39–19.5	–2.7–17.0	40.1–65.0	90.9–118.6

CI, confidence interval.

We based our study on the previous study by Williams et al [18], which validated MRI for monitoring pancreatic atrophy in Type 1 DM. In that study, four sequences were used: standard T_1 weighted, standard T_2 weighed, VIBE and T1BHFS. Visualisation was best with VIBE and T1BHFS. These two sequences were proven to allow reliable measurement of pancreatic tissue in organs of reduced volume, such as the pancreas in Type 1 DM.

Contrast medium was not used in this study for two major reasons: first, the scan sequences used had previously been validated without the use of contrast media [18]; second, the use of iv contrast would significantly increase the scan time in patients with, in many cases, difficult iv access and difficulty in maintaining apnoea owing to chronic lung disease. We acknowledge that the use of contrast medium might make small amounts of pancreatic tissue more obviously visible and that the lack of contrast medium could be a limitation of the sequence selection. Preference was given to a simple, rapid and non-invasive scan sequence.

The main limitation of this study is the fact that the control group, which contained only male volunteers, cannot be properly matched with the CF study groups. This drawback does not, however, alter the fact that we observed a dramatic difference between the size of the pancreas in CF and non-CF individuals. On analysis of male participants only (Figure 4), the pancreatic volumes of all 12 healthy controls easily exceeded those of all of the CF patients. The pancreas was detected in all 12 patients with T1DM but it was visible in only 5

of the 13 male patients with CF ($p<0.001$). In addition, previous studies evaluating general population data on pancreas volume showed a gender effect [24, 25] that was small relative to the differences caused by CF seen in this study. We emphasise that the dramatic difference in pancreas volume observed would not have been affected even if the control group pancreas volumes were slightly smaller owing to the presence of female individuals. We intend to confirm that there is no gender difference in pancreatic volume between male and female healthy controls and T1DM patients.

Finally, we acknowledge that we have looked at the pancreas as an anatomical entity despite the fact that it is an organ that has both exocrine and endocrine functions. The beta cells of the pancreatic islets constitute less than 2% of pancreatic volume in healthy adults; therefore, a more detailed method of imaging is needed to allow detection of minute changes in beta cell mass or in islet structure that may be associated with loss of beta cell function and that could possibly explain why some CF patients develop CFRD.

Conclusions

To our knowledge, this is the first study to show that the pancreas as an anatomical entity cannot be visualised using modern MRI techniques in the majority of adult CF patients, irrespective of their diabetes status, indicating a severe loss of pancreatic exocrine tissue in both diabetic

Figure 3. Volume of the pancreas in the four study groups — healthy controls, long-standing Type 1 diabetes patients, cystic fibrosis (CF) patients without diabetes and cystic fibrosis-related diabetes (CFRD) patients. The pancreas was visible by magnetic resonance imaging (MRI) in only 3 (23.1%) of 13 patients with CFRD and in just 5 (31.3%) of 16 CF patients without diabetes (p -value=0.7). In total, the pancreas was not detected as an anatomical entity in 21 (72.4%) of the 29 CF patients. p -values comparing pancreas volumes between groups are shown. P DM, diabetes mellitus; NS, p -value statistically non-significant.

Figure 4. Volume of the pancreas in males only — cystic fibrosis (CF) patients, Type 1 diabetes mellitus (DM) patients and healthy controls. The p -values comparing pancreas volumes between groups are shown.

and non-diabetic CF patients. When measurable, the volume of the pancreas did not correlate with gender, age, FEV1, FVC, BMI or with the daily intake of lipase as a marker of the exocrine pancreatic function in CF patients.

The fact that the pancreas of non-diabetic CF patients was not visible by MRI emphasises that the beta cell mass embedded within the fat tissue, which is too small to be detected by MRI, represents a very small percentage of the total pancreatic tissue. This small mass may produce enough insulin in response to blood glucose levels to classify the patient as non-diabetic. Other changes, either to the islet architecture and blood supply resulting from sclerosis [26] or in beta cell apoptosis caused by islet amyloid deposition [27], may therefore be responsible for the loss of beta cell function in CF.

A longitudinal study is required to investigate the natural history of the loss of pancreatic tissue and whether non-diabetic CF patients with non-visible pancreases are more likely to develop diabetes than those with visible pancreases. Sophisticated imaging methods to identify islet cells in humans *in vivo* [28, 29] and to assess peri-islet morphology may also need to be further developed if we are to understand the determinants of CFRD.

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