Diabetes & Metabolism 45 (2019) 301–305

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Short Report

The prognostic significance of glutamic acid decarboxylase antibodies in patients with chronic pancreatitis undergoing total pancreatectomy with islet autotransplantation

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ARTICLE INFO

Article history:
Received 21 October 2017
Received in revised form 17 December 2017
Accepted 2 January 2018
Available online 11 January 2018

Keywords:
Autoimmune
Chronic pancreatitis
Diabetes mellitus
Glutamic acid decarboxylase
Islet auto-transplantation

ABSTRACT

Aim. – Islet autotransplantation (IAT) is considered a 'non-immune' model of islet transplant, with no risk for autoimmune-mediated beta cell loss, but we have previously observed de novo type 1 diabetes in one total pancreatectomy with islet autotransplantation (TPIAT) recipient. We aimed to investigate the clinical significance of glutamic acid decarboxylase antibodies (GADA), as a sensitive marker for autoimmune diabetes mellitus (DM), in patients with chronic pancreatitis undergoing TPIAT.
Methods. – We identified 9 patients undergoing TPIAT with elevated GADA pre-TPIAT (8 non-diabetic and 1 with C-peptide positive DM), otherwise demographically similar to GADA negative TPIAT recipients ($n = 341$). Metabolic and clinical measures related to islet cell function were recorded both before and after TPIAT.
Results. – None of the 9 TPIAT patients achieved insulin independence after surgery, vs. 33% of GADA negative patients ($n = 318$ with 1-yr follow-up). The two patients with the highest titters of GADA (> 250 IU/mL) both experienced islet graft failure, despite normoglycaemia pre-TPIAT and high islet mass transplanted (5276 and 9378 IEQ per kg), with elevated HbA1c levels post-TPIAT (8.3%, 9.6%). The remaining 7 seven were insulin dependent with partial graft function and HbA1c levels $< 7\%$.
Conclusion. – Insulin dependence was more frequent in 9 patients with elevated GADA prior to TPIAT than in GADA negative TPIAT recipients, with graft failure in 2 cases. We speculate that beta-cell autoimmunity may occur in a small subset of TPIAT recipients and that beta cell antibody testing prior to TPIAT may be warranted to identify individuals at higher risk for insulin dependence.

Introduction

Chronic pancreatitis is a progressive inflammatory disorder, which causes fibrosis of the pancreatic parenchyma with clinical features of disabling pain, and eventual progression to exocrine insufficiency and diabetes. Total pancreatectomy with islet auto-transplantation (TPIAT) can relieve intractable pain in patients with refractory disease. The simultaneous islet auto-transplant is performed to preserve beta cell function and reduce the risk for labile diabetes, with insulin independence in 30% at 3 years after TPIAT [1].

Type 1 diabetes mellitus (T1DM) is a complex genetic autoimmune disorder. The genes in HLA are the greatest contributor to the genetic susceptibility to T1DM, with the HLA-DR3, DR4 and DQ8 genotypes representing the highest risk [2]. Islet cell autoantibodies (ICA), antibodies to insulin (IAA), anti-glutamic acid decarboxylase (GADA), insulinoma-associated antigen-2 (IA-2), zinc transporter 8 (ZnT8) are all defined markers of beta cell autoimmunity in T1DM [3]. Glutamic acid decarboxylase antibodies (GADA) are highly sensitive markers for autoimmune type 1 diabetes mellitus (T1DM) in adults [4]. GADA has been associated with subclinical insulitis and progressive beta cell destruction, with approximately 80% of patients with T1DM positive for GADA

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https://doi.org/10.1016/j.diabet.2018.01.001
1262-3636/

[5]. In islet allo-transplantation for T1DM, increases in titers of autoantibodies including GADA have been associated with reduced longevity of the islet graft, and in islet auto-transplant we have observed one case of de novo type 1 diabetes with development of GADA *after* TPIAT [6,7]. On the other hand, GADA has been observed in other populations including type 2 DM, cystic fibrosis related diabetes, and even healthy adults [8,9].

The significance of GADA in chronic pancreatitis and TPIAT remains unknown. We have been routinely assessing GADA titters before surgery in patients undergoing TPIAT. In this case series, we investigated the diabetes outcomes for patients who were positive for GADA prior to TPIAT.

Patients and method

We identified 9 TPIAT recipients with GADA positive (2.6% of cases) prior to surgery and 341 GADA negative controls between October 2009 and December 2016. In our patients, the etiologies/putative risk factors for chronic pancreatitis were; hereditary (n: 2) (1 PRSS1 disease; 1 CFTR heterozygote), alcohol (n: 1), pancreas divisum (n: 3), sphincter of oddi dysfunction (n: 2), idiopathic (n: 1) (no risk factors identified) (1).

In all GADA positive, other antibodies screened were negative (islet cell antibodies, insulin autoantibody). Surgical candidacy for TPIAT was determined as previously described for intractable chronic or recurrent acute pancreatitis [1]. Patients were followed under an IRB approved protocol, and informed consent was obtained.

TPIAT technique and islet isolation

TPIAT and islet isolation were performed as described previously [1]. For the TPIAT procedure, a total pancreatectomy was performed, along with splenectomy, partial duodenectomy and restoration of GI and bile duct continuity by Roux-en-Y dueodenojejunostomy and choledochojejunostomy respectively. The pancreas was transported to the islet isolation lab, where the pancreas was cannulated and distended with enzyme solution by a recirculating system to maximize the enzyme delivery into the whole pancreas.

Islets were isolated using intraductal distention with an enzyme combination of intact C1 collagenase (VitaCyte, Indianapolis, IN) and neutral protease (SERVA, Heidelberg, Germany) [1], followed by mechanical digestion within a Ricordi chamber, and purification with a COBE 2991 processor (CaridianBCT, Inc., Lakewood, CO) only as needed. The patient was administered 70 to 100 U/kg of heparin before and during the islet infusion to minimize the risk of portal vein thrombosis (35 unit/kg mixed with the islets, and the remainder administered intravenously at time of infusion). The islets were infused intraportally under direct visualization prior to closure of the laparotomy. During infusion into the portal vein, portal pressures and/or portal vein flows were measured. If portal pressure persistently rose over 25 cmH_2O or the flow decreased to < 100 mL/min, the portal infusion was stopped and the rest of the islet preparation was implanted in the peritoneal cavity.

Postoperative management

Insulin was used postoperatively to keep the glucose levels between 80 and 120 mg/dL, initially as an intravenous insulin infusion, and later transitioned to subcutaneous insulin continued for at least 3 months after TPIAT [10,11]. Insulin was weaned as tolerated after TPIAT (fasting ≤ 125 mg/dL, postprandial < 180 mg/dL, and HbA1c $\leq 6.5\%$).

Metabolic outcomes and insulin independence

Patients were evaluated for insulin needs, haemoglobin A1c (HbA1c) levels, and 2-hour mixed meal tolerance test (MMTT) (6 mL/kg Bost HP to the maximum of 360 mL) for fasting and stimulated glucose and C-peptide at 3, 6 months and yearly after surgery. Metabolic and clinical measures related to islet cell function were recorded both before and after TPIAT. Patients were classified as insulin independent (II), partially insulin dependent (PD; using only basal insulin daily) or insulin dependent (ID; using multiple daily injection regimens) post-TPIAT [1]. The patients, with fasting and stimulated C-peptide levels of less than 0.6 ng/mL, are defined as having clinical islet graft failure at our institution.

Laboratory assays

GADA was analyzed by semi-quantitative enzyme linked immunosorbent assay (reference 0–5 IU/mL), insulin antibodies by semi-quantitative radioimmunoassay, and islet cell antibodies by semi-quantitative indirect fluorescent antibody. The C-peptide levels were measured by chemiluminescent immunoassay (with 'reference range' of 0.9 to 6.9 ng/mL).

Statistical analysis

Statistical analyses were performed by using JMP 11.0.0 software (SAS Institute, Cary, NC). Continuous variables were expressed as the mean ± standard deviation and median (25th, 75th percentile) and, categorical data were summarized as counts and proportions. A two-sample t-test or one-way ANOVA were used for comparison of numerical means, and Pearson's Chi^2 for differences in categorical variable frequencies. The Wilcoxon rank-sum tests were used for continuous variables that were not normally distributed. Statistical significance was accepted at $P < 0.05$.

Results

Age was similar between groups (39 [16] to 33 [24.5], $P = 0.574$). BMI, duration of pancreatitis, duration of pain, pancreas weight, pancreas/body weight, the severity of pancreatitis were also similar between groups ($P > 0.05$). No patient had autoimmune pancreatitis in this series. Preoperative fasting glucose was higher in patients with anti-GAD positivity ($P < 0.05$). Preoperative Hba1c, preoperative fasting C-peptide, preoperative stimulated C-peptide, preoperative 1st-hour glucose and preoperative 2nd-hour glucose were similar between groups ($P > 0.05$) (Table 1).

The 9 patients with elevated GADA had a pancreatitis duration of 8.48 (7.81) years, and islet mass of 3257 (3202) islet equivalents per kilogram body weight (IEQ/kg) transplanted; Five (56%) were female. None of the patients had been diagnosed with autoimmune disease. Four had a positive family history of diabetes mellitus. Eight were non-diabetic before TPIAT (all HbA1c $< 5.7\%$). One patient had preoperative diabetes, with high C-peptide (fasting 3.9 ng/mL, stimulated 6.3 ng/mL), initially treated with metformin, later changed to glargine insulin 2 months prior to TPIAT. GADA cases were demographically similar to the GADA negative controls (Table 1), except for higher fasting glucose before TPIAT in patients with GADA positivity ($P < 0.05$).

The etiologic classification of pancreatitis, presence of preoperative prediabetes or diabetes mellitus, previous endoscopic and previous surgical interventions did not differ between groups ($P > 0.05$) (Table 1). Islet isolation parameters used commonly to monitor differences in the process and final islet product, including transplanted tissue volume, digest and final islet yield (IEQ/gm),

Table 1
Demographics and surgical characteristics of the GADA cases ($n = 9$), compared to GADA negative TPIAT cases.

	GADA (+) (n: 9)		Control (n: 341)		P
	Median or n	25th and 75th percentiles or %	Median or n	25th and 75th percentiles or %	
Age (year)	39	27.5, 43.5	33	20, 44.5	0.574
Sex (female)	5	56	244	71	0.295
BMI (kg/m^2)	26.74	17.9, 30.8	23.51	20.1, 28.2	0.586
Pancreatitis duration (year)	8.48	3.9, 11.7	4.31	2.1, 8.9	0.108
Aetiology of pancreatitis					
Hereditary	2	22.2	118	34.6	0.578
Alcohol and drugs	1	11.1	20	5.9	
Idiopathic	1	11.1	74	21.7	
Other	5	55.6	129	37.8	
Pain duration (year)	8.48	5.5, 11.9	7.48	3.9, 12.3	0.624
Preoperative HbA1C (%)	5.4	4.8, 5.7	5.3	5.1, 5.5	0.940
Preoperative fasting C-peptide (ng/mL)	1.6	1, 3.2	1.6	1.1, 2.4	0.735
Preoperative stimulated C-peptide[a] (ng/mL)	6.1	3.9, 9.3	5.6	3.9, 7.8	0.598
Preoperative fasting glucose (mg/dL)	97	90, 127.5	90	84, 98	**0.024**
Preoperative 1-hour glucose[b] (mg/dL)	105	72.7, 144.5	91	80, 114	0.513
Preoperative 2-hour glucose[b] (mg/dL)	99	78.5, 139	90	81, 102	0.237
Severity of pancreatitis (0–10)	7	5.5, 9	7	4, 9	0.590
Pancreas weight (g)	87	34.4, 96.5	64	47.2, 85	0.660

In bold: data lower than 0.05.
[a] Peak C-peptide from MMTT.
[b] 1 and 2-hour glucose from MMTT.

Table 2
Islet isolation and islet graft characteristics for GADA cases and GADA negative controls.

	GADA (+)		Control group		
	Median	25th and 75th percentiles	Median	25th and 75th percentiles	P
Digestion (%)	86.6	82.1, 92.7	87.5	81, 92.7	0.944
Switch time (min)	16	13.5, 29.5	18	14.7, 24	0.806
Digest Islet Yield (IEQ/gm)	3759	2328, 6703	4168	2862, 5770	0.770
Islet Size Index (IEQ/IPN)	0.928	0.645, 1.144	0.789	0.654, 0.982	0.354
Islet Quality Score (0–10)	8.5	8.25, 9	9	8.5, 9	0.233
Embedded tissue (%)	30	20, 55	45	25, 70	0.722
Digest pellet (cc)	10	5, 28.5	13	7, 23.2	0.999
Pellet: pancreas ratio (cc/gm)	0.194	0.113, 0.319	0.204	0.126, 0.306	0.997
Transplanted Islet Dose (IEQ/kg)	3257	2003, 5205	4018	2610, 6017	0.424
Transplanted Islet Yield (IEQ/gm)	3737	3083, 6104	3838	2613, 5524	0.790
Transplanted tissue volume (cc)	8	5, 15	10	5, 16	0.794
Transplanted tissue ratio (cc/kg)	0.107	0.074, 0.179	0.165	0.096, 0.229	0.277
Islet viability (FDA/PI)	84.9	80.6, 86.2	84.8	82, 90.8	0.247
Transplanted IEQ/cc	28470	16,580, 41,776	26177	16,923, 38,932	0.766

FDA: fluorescein diacetate; PI: propidium iodide.

and final islet dose (IEQ/kg) were similar between groups ($P > 0.05$) (Table 2).

Metabolic outcomes of the 9 TPIAT cases with elevated GADA

GADA titters, glycaemic profile and C-peptide levels before and 1 year after TPIAT, and insulin requirements at 1 year after TPIAT are shown in Table 3. All with elevated GADA remained on insulin. Three were partially insulin dependent (PD, using basal insulin once daily only); while the other 6 were insulin dependent (ID, using multiple daily injections with basal and rapid acting insulin analogs). None were insulin independent, compared with 33% [$n = 105/318$] of GADA negative TPIAT recipients ($P = 0.03$).

Two patients (25%, of 8 patients with post-transplant C-peptide) had islet graft failure, defined by stimulated C-peptide levels < 0.6 ng/mL, compared to 7.1% of GADA negative patients ($P = 0.06$). These 2 patients had the highest GADA titters before TPIAT (> 250 IU/mL), and experienced graft failure despite 5276 and 9378 IEQ/kg transplanted, a dose where II would be expected in 75% [1]. One had insulitis noted on biopsy (Fig. 1). Both had elevated HbA1c levels of 8.3 and 9.6%, compared to 6.7 (6.4) in GADA negative controls.

Discussion

Glutamic acid decarboxylase is an enzyme mainly expressed in pancreatic β cells and neuron cells that catalyses the conversion of glutamic acid to γ-aminobutyric acid (GABA). Elevated GADA is a specific immunologic marker for the diagnosis of T1D [4,5], and may be elevated before the clinical diagnosis. However, GADA positivity has been observed in some non-diabetic populations and is of unclear significance in this setting [8,12,13]. We found an overall prevalence of 2.6% of TPIAT recipients (9/350) with elevated GADA. None of the patients with GADA positivity were able to discontinue insulin after surgery, though the majority maintained islet graft function with HbA1c at or near the American Diabetes Association goal of < 7%.

The only two patients with highly positive GADA (> 250 IU/mL) progressed to islet graft failure despite high islet mass transplanted, suggestive of an autoimmune process; one of the two had insulitis present in the pancreas biopsy despite non-diabetic status before surgery. Thus, high titters of GADA may be concerning for risk of autoimmune destruction regardless of euglycaemic status before surgery. It is difficult to yet argue for TP alone in non-diabetic patients, based solely on data from 2 patients. However, these cases highlight the potential prognostic value of screening

Table 3
Metabolic and clinical measures of patients with GADA positivity both before and after TPIAT.

Case[c]	Age	Sex	GAD (IU/mL) (RR: 0.0–5.0)	IEQ/kg	Before TPIAT					After TPIAT					IS	TDI (units)
					FPG (mg/dL)	PPG (mg/dL)	FC (ng/mL)	SC (ng/mL)	HbA1c (%)	FPG (mg/dL)	PPG (mg/dL)	FC (ng/mL)	SC (ng/mL)	HbA1c (%)		
1	39	F	48.7[a]	5134	97	91	2.5	8.7	5.4	102	149	1.4	5.2	6.6	PD	4
2	34	M	26.7	1279	–	140	1.6	5.6	5.2	–	–	–	–	6.6	ID	–
3	15	M	21.5	2283	94	138	0.8	3.9	5.6	151	295	0.5	1.3	7.4	ID	26
4	46	M	>250[a]	9378	105	65	1.6	10	5.4	110	216	<0.1	<0.1	8.3	ID	48
5	29	M	>250[a]	5276	86	66	1.2	3.9	4.8	124	265	0.1	0.3	9.6	ID	–
6	41	F	16.4[b]	1724	89	99	2	6.1	4.8	139	220	0.4	0.6	6.7	ID	17
7	25	F	6.2	2847	103	105	8.7	13.9	4.9	75	72	0.4	1.7	6.3	ID	–
8	41	F	6.7	3257	91	92	0.7	2.9	5.4	105	121	0.9	2.1	6.2	PD	4
9	62	F	19.9[a]	3559	164	223	3.9	6.3	10.6	138	149	0.8	1.1	6.8	PD	18

F: female; M: male; GADA: antiglutamic acid decarboxylase antibody; FPG: fasting plasma glucose; PPG: 2nd hour plasma glucose in mixed meal tolerance test; FCP: fasting C-peptide; SCP: stimulated C-peptide; IS: insulin status; II: insulin independent; ID: insulin dependent; PD: partial insulin dependent; TDI: total daily insulin use; RR: reference range.

[a] Confirmed positive on repeat (repeat values of 46.5 [case 1], >250 [case 4], >250 [case 5], 11.9 [case 9]).

[b] Had one measure elevated at 16.4 and one measure of <5.[c]Anti-islet cell antibody and anti-insulin antibody were negative for all patients

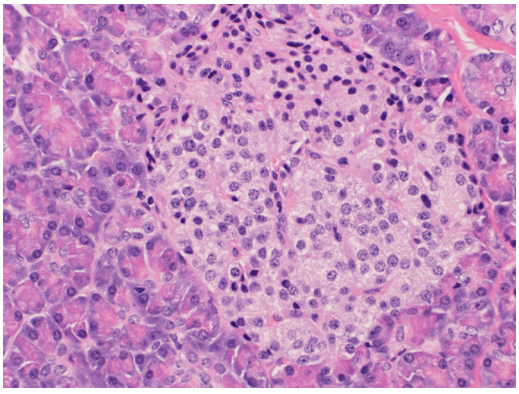

Fig. 1. Mild insulitis in a patient with GADA > 250 IU/mL (case 5) undergoing TPIAT for intractable recurrent acute pancreatitis.

for autoantibodies prior to surgery, and the need to closely counsel such patients before pancreatectomy and track islet graft function after TPIAT.

On the other hand, clinical significance of GADA positivity is controversial since GADA may be present in 0.7 to 1.7% non-diabetic individuals [8,12,13]. In addition, 9.8% (118 of 1,206) of obese youth with T2DM in one series were positive for GADA and/or insulinoma-associated protein 2 [9]. In our series, only the 2 cases with highly elevated GADA have experienced islet graft failure suggestive of autoimmune beta cell destruction, while the others with low GADA positivity remained on insulin but with C-peptide levels indicating partial islet graft function, suggesting that the titter of GADA may be important. We hypothesize that GADA is a marker of underlying autoimmunity in our population, although an alternative explanation is that this a reaction to release of beta cell antigen from islet destruction that occurs with repeated episodes of acute pancreatitis and progressive pancreatic parenchyma destruction from chronic pancreatitis.

Our standard TPIAT screening protocol has included islet cell antibodies, insulin antibody, and GADA as these were the clinically available assays at the time these assessments were added to our standard laboratory evaluation in 2009. Since that time, other beta cell autoantibodies prevalent in type 1 diabetics have become clinically available, specifically anti-tyrosine phosphatase (IA-2) and more recently zinc transporter 8 (ZnT8). In this series, we obtained IA-2 in 4 of the 9 cases, including the 2 with the most highly elevated GADA values, and IA-2 was in the normal reference range in all 4. More systematic measurement of IA-2 and ZnT8 antibodies in patients who screen positive for GADA will be important in better characterizing the significance of the elevated GADA as a potential marker of beta cell autoimmunity.

We do not know whether any GADA positive patients, in particular the 2 with high GADA, may have progressed to eventual T1DM even in the absence of surgery. However, the short interval between surgery and complete islet graft failure, which was < 1 year in both cases, is concerning that the intraportal infusion of the islet tissue may have accelerated beta cell loss. While more data are needed, this raises the question as to whether short-term immunomodulation might be an option in these selected patients.

In conclusion, we speculate that beta-cell autoimmunity may occur in a small subset of patients with chronic pancreatitis undergoing TPIAT and that beta-cell antibody testing prior to TPIAT may be warranted to identify individuals at higher risk for insulin dependence.

Disclosure of interest

The authors declare that they have no competing interest.

Acknowledgements

We are incredibly grateful to the islet manufacturing and clinical care teams that are instrumental to the success of the TPIAT program at our institution, with special thanks to Muhamad Abdulla, David Heller, Tom Gilmore, Zach Swanson, Jacob Ricks, Louise Berry, and Marie Cook. Muhammed Kizilgul was supported by a grant from The Scientific and Technical Research Council of Turkey (TUBITAK).

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