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Beta Cell Stress in Insulin Independent Subjects Following Total Pancreatectomy and Autologous Islet Transplantation

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Abstract

In patients with chronic pancreatitis (CP), autologous islet transplantation (AIT) is often coupled with total pancreatectomy (TP) in aims to preserve patients' insulin secretory function. Despite a third of patients achieving insulin independence post-total pancreatectomy and autologous islet transplantation (TPAIT), many will require the addition of insulin therapy for maintenance of glycemic control overtime. We aimed through this study to investigate the early metabolic profile signature of insulin independent subjects post-TPAIT, specifically exploring markers of beta cell stress in this cohort. In a prospective study design, we identified 37 subjects who underwent TPAIT between 2008 and 2017. Metabolic parameters were assessed using mixed meal tolerance test data (MMTT), and the insulin-to-proinsulin index ratio, a marker of beta cell stress. Assessments between metabolic variables were evaluated using the Wilcoxon signed rank test. A significance level of 0.05 was assumed for all comparisons. At a mean (±standard deviation) follow up duration of 37.7±17 months post-TPAIT, 11 patients (30%) were insulin independent with a mean HbA1C of 5.85±0.42%. Despite adequate glycemic control in the latter cohort, we observed significantly higher median peak glucose (180.5 versus 115.0 mg/dL; p=0.031), and lower median fasting C-peptide (0.95 versus 1.5 ng/mL; p=0.008) on post-TPAIT MMTT compared to pre-TPAIT MMTT. Additionally, significantly lower insulin-to-proinsulin index AUC ratio was seen post-TPAIT compared to pre-TPAIT (p=0.022). A decline in the proinsulin processing capacity, expressed by a lower insulin-to-proinsulin index ratio was seen in insulin independent subjects post-TPAIT. Further studies exploring the pathophysiology underlying these findings should be attained.

ÖZET

Kronik pankreatitli hastalarda otolog adacik transplantasyonu genellikle total pankreatektomi de yapılır böylece insülin salgilama fonksiyonu korunmuş olur. Üçte bir hastalarda insülin bağimsiz post total pankreatektomi ve otolog adacik transplantasyonu (TPAIT) yapilmiş olmasina rağmen, glisemik kontrolü sürdürebilmek için ilave insülin tedavisi gerekmektedir. Biz bu çalişmada insülin insülin bağimsiz hastalarda TPAIT sonrasi insülinin erken metabolik profil etkisini araştirarak, bu kohortta beta hücre stresinin spesifik belirteçlerini tespit etmeyi planladik. Prospektif bir çalişma planlayarak, 2008–2017 yillari arasinda TPAIT olmuş 37 hasta belirledik. Metabolik parametreleri mikst öğün tolerans testi (MMTT) ve insülin proinsülin indeks orani (beta hücre stres belirteci) ile değerlendirdik. Metabolik değişkenler arasindaki analizler Wilcoxon

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rank testi ile yapilip değerlendirildi. 0,05 altindaki düzeyler tüm analizlerde önemli kabul edildi. TPAIT sonrasi 37,7±17 aylik bir izlem süresi ortalama (±standart sapma) planlandi. İnsülin bağimsiz 11 hastada (%30) HbA1C ortalama %5,85±0,42 saptandi. İkinci kohortta yeterli glisemik kontrol sağlanmiş olmasina rağmen, post-TPAIT MMTT, pre-TPAIT MMTT ile karşilaştirildiğinda yüksek pik glukoz (115,0 mg/dL'ye karşilik 180,5, p=0,031) ve düşük düzeyde C-peptid (1,5 ng/mL, 0,95'e karşilik, p=0,008) tespit ettik. İlaveten çok düşük düzeyde insülin-to-proinsülin indeksi saptadik (post-TPAIT MMTT ile pre-TPAIT MMTT'yi karşilaştirdik, p=0,022). Post-TPAIT insülin bağimsiz hastalarda çok düşük insülin-to-proinsülin indeks orani şeklinde proinsülin işlemleme kapasite-lerinde azalma tespit edildi. Bu bulgularin altinda yatan patofizyolojiyi araştiran çalişmalara ihti-yaç bulunmaktadır.

Keywords

Total pancreatectomy; autologous islet transplant; beta cell function; insulin independence

Keywords

Total pankreatektomi; otolog adacık transplantı; beta hücresi fonksiyonu; insüline bağımlı olunmaması

Chronic pancreatitis is a debilitating disease with progressive inflammatory changes that can lead to permanent damage in the pancreas and compromise of its exocrine and endocrine functions. It affects roughly 50 to 70 per 100,000 patients per year, and is clinically characterized by recurrent, chronic abdominal pain, often requiring repeated procedural interventions and narcotic dependence.[1] Total pancreatectomy (TP) is the final recourse after failure of medical therapy. The surgical procedure is often coupled with autotransplantation of islets of Langerhans (recovered from the resected pancreas), which is intended to prevent or ameliorate the development of surgical dia- betes.[2–4]

The outcomes of diabetes-free survival in total pancreatectomy and autologous islet transplantation (TPAIT) vary significantly across patients who undergo this procedure. Previous reports have shown that at around 3 years post-TPAIT, about one third of patients remain insulin independent, one third require some exogenous insulin (partial beta cell function), and one third are fully dependent on insulin therapy.[5]

Insulin independence has been strongly correlated with the mass of islet transplanted (expressed as the islet equivalent per kilogram of body weight [IEQ/kg]); such that higher IEQ/kg predicted more satisfactory blood glucose control and insulin independence.[6] Insulin independence following TPAIT confers great benefit for patients. Besides good glycemic control obviating the need for insulin therapy, significant improvements in quality of life are reported. However, recent accumulating evidence suggest increase in rates of attrition of insulin independence with time post-TPAIT, from as high as 46% at 5-years post-transplantation, to as low as 10% at 8-years post-transplantation.[3] Little is known regarding the metabolic signature of beta cell stress that leads to loss of insulin independence overtime.

In this study, we aimed to take a closer look at the metabolic profiles of insulin independent subjects post- TPAIT. More specifically, we aimed to analyze markers of beta cell stress in insulin independent subjects post- TPAIT, in comparison to their pre-TPAIT state and to the glycemic profiles of insulin dependent subjects. We accomplished this through analysis of mixed meal tolerance test data (MMTT) and analysis of the in- sulin-to-proinsulin index ratio, a marker of beta cell stress.

RESEARCH DESIGN AND METHODS

SUBJECTS

This was a prospective observational cohort study conducted at the Cleveland Clinic Foundation between August 2008 and February 2017. We included subjects with clinically confirmed diagnosis of chronic pancreatitis (CP) undergoing TPAIT for chronic pain or recurrent acute pancreatitis, who were not responsive to endoscopic or medical management, and who were free of alcohol dependence at the time of study. Patients were excluded if they had pancreatic carcinoma or pancreatic masses suspicious for malignancy, cirrhosis, portal hypertension, ongoing alcohol dependence, or HbA1c >8% (>64 mmol/mol) [reference range: 4.3–5.6% (23–38 mmol/mol)] pre-TPAIT. The inclusion/exclusion criteria were confirmed by a multidisciplinary team consisting of a hepatobiliary surgeon, gastroenterologist, endocrinologist, diabetes educator, social worker, nutrition therapist, psychologist and pain management specialist. The study was approved by the Cleveland Clinic Foundation Institutional Review Board.

TP AND ISLET ISOLATION AND TRANSPLANTATION

In brief, the operative procedure included pylorus-preserving total pancreatectomy with duodenectomy and usually with splenectomy to preserve pancreatic-tail per- fusion.[7] The explanted pancreas was transported on ice to an islet-cell isolation facility at the University of Pittsburgh by ground transportation. After completing biliary and duodenal anastomoses, followed by abdominal wall closure, patients awaited return of their islets in the recovery room. The harvested islet cells were heparinized and transported back then infused into the patient via the splenic vein stump and the IEQof infused cells was recorded. The total time from pancreatectomy to re-infusion of islet cells was around 8–9 hours. Post-transplant, a glycemic target between 140–180 mg/dL was maintained using an insulin infusion protocol, that was later converted to a standardized sliding scale and further titrated at clinical visits and phone encounters following discharge.

METABOLIC TESTING AND DEFINITIONS OF INSULIN DEPENDENCE STATES

Preoperative metabolic assessment included HbA1c and a 5-hour mixed meal tolerance test (MMTT). After an overnight fast, patients consumed a nutritionally balanced commercial product orally over the span of five minutes (Complete Ensure Plus®; Content: protein-13 g, 17.2%, fat-11 g, 14.5%, carbohydrate −51g, 67.4%; total calories: 350 kCal). Samples for plasma glucose, insulin and C-peptide at baseline, 30, 60, 90 minutes, and hourly until 5 hours were collected. Proinsulin samples were also collected. The MMTT was also administered to all patients post-operatively once insulin requirements stabilized.

Insulin independence was defined as maintenance of adequate glycemic control (HbAlc <7%) without the requirement for exogenous insulin and with a detectable random C-peptide level >2 ng/mL [reference range: 0.83.2 ng/mL] for at least 3 months prior to inclusion into the study. Partial insulin dependence was defined as the need for insulin less frequently than one injection per day, at least for the 3 months preceding enrollment. Insulin dependence was defined as the need for insulin dosing, at least once daily, in the last 3 months preceding enrollment.

CLINICAL ASSAYS

Plasma glucose levels were measured using the enzymatic reference method with hexokinase (Roche/Hitachi Cobas c 311/501 analyzers, Roche Diagnostics, Indianapolis, IN) at Cleveland Clinic Laboratories. Plasma insulin C-peptide levels were measured using two-site sandwich chemiluminescent immunoassays (ADVIA Centur, Siemens AG, Erlangen, Germany) at Cleveland Clinic Laboratories. HbA1C was measured using turbidimetric inhibition immunoassays (Cobas Integra 400/400 plus 800 analyzers, Roche Diagnostics) at Cleveland Clinic Laboratories. Proinsulin levels were sent out to multiple laboratories including ARUP (Quantitative Chemiluminescent Immunoassay; Invitron kit; CV <=7.5%), Mayo (Immunochemiluminescent Assay using Bertholde Autolumat Plus LB953 Luminometer; CV 2.8 to 5.9%) and Esoterix (Imunochemilumi- nometric assay, ICMA, using Autolumat Plus LB953 Immunochemiluminometer; CV <20%).

STATISTICAL ANALYSES

Categorical variables were described using frequencies and percentages. Continuous variables that were normally distributed were described using means and standard deviations. Continuous Variables that were not normally distributed were described using medians and quartiles. Assessments between continuous variables were evaluated using non-parametric methods including the Wilcoxon signed rank test. Areas under the curve (AUC) for glucose, insulin and C-peptide during MMTT were calculated using the incremental AUC recommended by Wolever and compared using the Wilcoxon signed rank test.[8] Analyses were performed using the SAS software (version 9.2, Cary, NC). A significance level of 0.05 was assumed for all comparisons.

RESULTS

Thirty-seven patients underwent TPAIT at the Cleveland Clinic between August 2008 and February 2017. At a mean (±SD) follow up period of 37.7±17 months post- TPAIT, 11 patients (30%) were fully insulin-independent (termed the Full Beta Function subgroup), 6 (16%) were partially insulin dependent, and 20 patients (54%) were fully dependent on insulin therapy (termed the Poor Beta Function subgroup). Table 1 details the baseline demographics of the Full Beta Function versus the Poor Beta Function subgroups.

In assessment of the MMTT metabolic profile of the Full Beta Function subgroup pre- and post-TPAIT, significantly higher median peak glucose was seen post-TPAIT compared to pre-TPAIT (180.5 mg/dL [range: 135.0–207.5 mg/dL versus 115.0 mg/dL [range: 108.0–130.0 mg/dL]; p=0.031) (Table 2). Additionally, a trend, though not statistically significant,

was seen towards higher median AUC for glucose on post-TPAIT MMTT compared to pre-TPAIT MMTT (p=0.078). When examining other glycemic variables during MMTT, significantly lower median fasting C- peptide levels were seen post-TPAIT compared to pre-TPAIT (0.95 ng/mL [0.75–1.2 ng/mL] versus 1.5 ng/mL [1.2–2.4 ng/mL]; p=0.008). No differences were seen in insulin parameters pre- and post-TPAIT. Table 2 and Figure 1 detail the results of MMTT metabolic parameters for the Full Beta Function subgroup pre- and post-TPAIT.

In examining the insulin-to-proinsulin index AUC ratios, no significant difference was seen in the insulin- to-proinsulin index AUC ratios between the Full and Poor Beta Function subgroups pre-TPAIT (p=0.85) (data not shown). Post-TPAIT, significantly higher insulin-to-proinsulin index AUC ratio was seen in the Full Beta Function subgroup compared to the Poor Beta Function subgroup (p=0.004). Additionally, within the Full Beta Function subgroup, significantly lower insulin-to- proinsulin index AUC ratio was seen post-TPAIT compared to pre-TPAIT (p=0.022, Figure 2).

DISCUSSION

Autologous islet transplantation at the time of pancreatectomy, attempts to prevent or ameliorate the development of surgical diabetes following pancreatectomy. In contrast to allogenic islet transplantation, auto-transplantation theoretically poses a reduced risk of graft-loss from autoimmune failure and therefore obviates the need for an immunosuppressive regimen. However, increasing level of evidence suggests that in most cases, there is an imminent loss in beta cell function with time. In a systematic review by Bramis et al., which examined five studies reporting on outcomes of TPAIT, the rate of insulin independence was found to range from as high as 46% at 5-years post-transplantation, down to 10% at 8- years post-transplantation.[3] Additionally, in a systematic review and meta-analysis of 15 observational studies examining the rate of insulin independence post-TPAIT, insulin independence was reported at 8.34 per 100 person-years (95% CI: 3.32–13.37) transiently during the studies, and down to 4.62 per 100 person-years (95% CI: 1.53–7.72) at point of last follow up in the studies included.[8]

Previous studies have examined the proinsulin processing capacity as a measure of beta cell function in islet transplant recipients. Fiorina et al. showed that patients with early islet allograft failure had an increasing disproportion between the amount of proinsulin produced and the amount of proinsulin processed to insulin by the beta cells.[9] Similarly, Klimek, et al. demonstrated elevated proinsulin-to-insulin ratios in two groups of islet transplant recipients (one with allogeneic grafts as treatment for type 1 diabetes and one with autologous grafts that followed total pancreatectomy) and concluded that impaired proinsulin processing is a characteristic of transplanted islets.[10] More recently, Rickels et al. suggested that the proinsulin processing defects could be secondary to suboptimal glycemic control and therefore these could be avoided by maintaining near-normal glycemia in islet recipients.[10] This remark was based on prior published data showing that hyperglycemia increases beta-cell recruitment of immature secretory granules leading to relative hyperproinsulinemia.[11]

In our study, we examined the glycemic profiles of 11 insulin independent subjects that have undergone TPAIT, with a mean follow-up period of 38 months post-procedure. To our knowledge, this is the first study to compare pre-operative to post-operative proinsulin processing capacity in autologous islet transplant recipients. Not surprisingly, our results are aligned with the aforementioned findings by Fiorina and Klimek and support the prevailing idea that the ability to process proinsulin is distorted in transplanted islet cells.[8,9] In the Full Beta Function subgroup, we observed a significantly lower insulin-to-proinsulin index AUC ratio post-TPAIT compared to pre-TPAIT. Moreover, in this subgroup, we observed a higher peak glucose and lower fasting C-peptide following transplantation. Thus, for the first time, we have demonstrated reductions in insulin-to- proinsulin ratios following islet transplantation in subjects with normal or near-normal glycemic control. This finding suggests that a decline in the proinsulin processing capacity may occur even in insulin-independent islet recipients. Additionally, the absence of a pre-operative difference in proinsulin processing capacity between our two study subgroups (the Full Beta Function subgroup and the Poor Beta Function subgroup) suggests that a transplantation-related factor is likely the main contributor to beta-cell dysfunction post-transplant. Therefore, it appears less likely for the defects in proinsulin processing to be due to suboptimal glycemic control and/or due to islet cell impairment secondary to long-standing chronic pancreatitis. Further studies aimed at assessing the mechanisms and pathophysiology underlying these processes should be pursued.

CONCLUSION

We observed a decline in the proinsulin processing capacity, manifested by a disturbance in the insulin-to- proinsulin index ratio post-TPAIT, in the fully insulin independent subjects. Additional work exploring the pathophysiology underlying these mechanisms should be attained.

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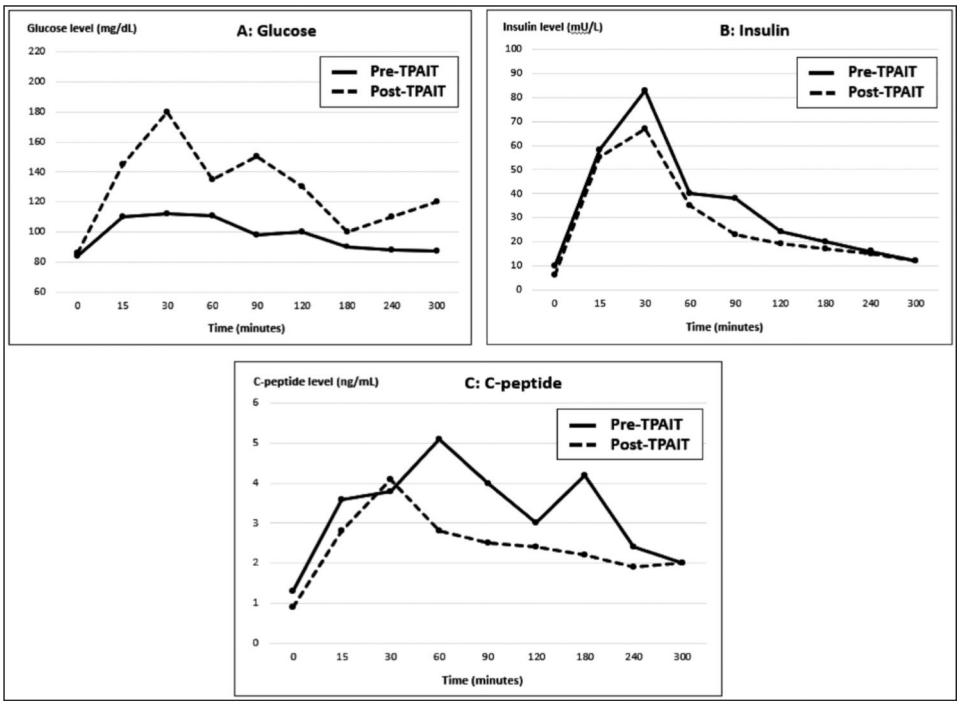

FIGURE 1:

Comparison in mean glucose (Figure 1A), insulin (Figure 1B) and C-peptide (Figure 1C) during mixed meal tolerance testing between pre- and post-TPAIT states in the Full Beta Function Subgroup.

TPAIT: T otal pancreatectomy and autologous islet transplantation.

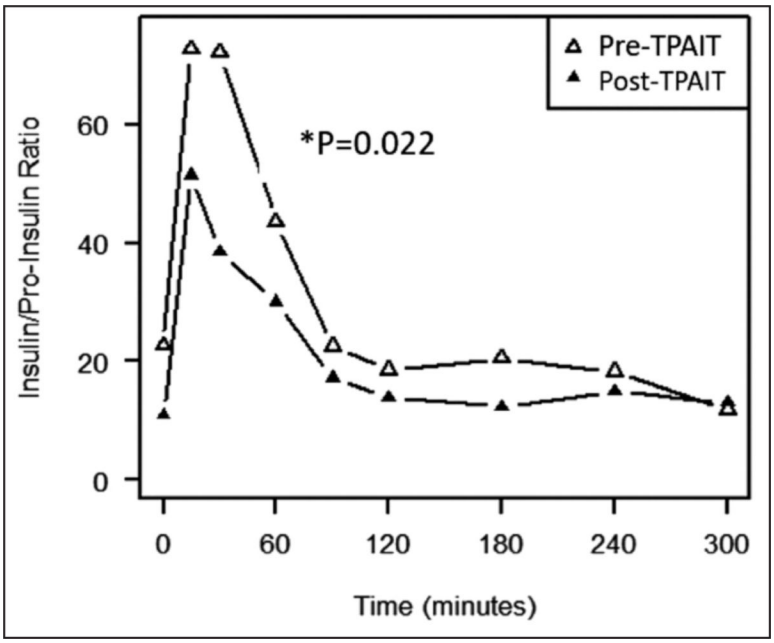

FIGURE 2:

Comparison between insulin-to-proinsulin index ratios pre-TPAIT to insulin-proinsulin index ratios post-TPAIT during MMTT for the Full Beta Function Subgroup (n=11). P-value shown represents the difference in the area under the curve between insulin-to-proinsulin index ratios pre-TPAIT to insulin-proinsulin index ratios post-TPAIT.

TPAIT: total pancreatectomy and autologous islet transplantation; MMTT: mixed meal tolerance testing; n: number of subjects.

TABLE 1:

Baseline characteristics of the Full and Poor Beta Function Subgroups.

Characteristic	Full Beta Function Subgroup (n=11)	Poor Beta Function Subgroup (n=20)
Female, n (%)	8 (73%)	8 (67%)
Mean age, years (±SD)	35±13	40±16
Non-Hispanic White, n (%)	11 (100%)	17 (85%)
Non-DM subjects (including pre-DM), n (%)	11 (100%)	17 (85%)
Pre-DM subjects, n (%)	3 (27%)	9 (45%)
Mean HbA1c pre-transplant, % (±SD)	5.5±0.4	5.8±0.4
Mean BMI, kg/m^2(±SD)	27±9.5	24.6±5.9
Mean duration of pancreatitis symptoms, mo (±SD)	70±47	93±82
Prior surgeries/procedures, n (%)	11 (100%)	1 (5%)
Mean IEQ/kg received during TPAIT (±SD)	6050±2343	3904±1763
Mean HbA1c at last follow up*, % (±SD)	5.85±0.42	8.2±1.96

TPAIT: total pancreatectomy and autologous islet transplantation; n=number of subjects; SD: standard deviation; DM: diabetes mellitus; mo: month; IEQ/kg: islet equivalents per kg of body weight.

*Last follow up for the cohorts combined was at a mean (±SD) of 37.7±17 months.

TABLE 2:

Fasting, peak and I AUC for the glycemic variables: glucose, insulin and C-peptide during pre- and post-TPAIT MMTTs in the Full Beta Function Subgroup (n=11).

Glycemic Variable	Pre-TPAIT MMTT	Post-TPAIT MMTT	p-value*
Fasting glucose (mg/dL)	85.5 [78.5–101.0]	86.5 [81.5–94.5]	0.71
Peak glucose (mg/dL)	115.0 [108.0–130.0]	180.5 [135.0–207.5]	0.031
Glucose AUC	2683.5 [1821.0–3751.8]	7949.6 [3790.6–12453.8]	0.078
Fasting insulin (mU/L)	9.2 [8.0–16.1]	5.9 [4.3–10.1]	0.31
Peak insulin (mU/L)	71.1 [45.5–89.6]	67.3 [41.8–131.2]	0.31
Insulin AUC	5513.4 [2756.2–7084.9]	4115.6 [3377.5–8930.4]	0.64
Fasting C-peptide (ng/mL)	1.5 [1.2–2.4]	0.95 [0.75– 1.2]	0.008
Peak C-peptide (ng/mL)	5.1 [4.0–7.4]	4.3 [2.8–7.7]	0.38
C-Peptide AUC	518.3 [217.5–794.4]	352.9 [304.1–621.8]	0.55

MMTT: mixed meal tolerance testing; TPAIT: total pancreatectomy and autologous islet transplantation; n: number of subjects; AUC: area under the curve. Data presented as Median [Interquartile range].

*Wilcoxon Signed Rank test.