OPEN ACCESS
Freely Available Online

JOURNAL OF ENDOCRINOLOGY AND HORMONES
ISSN NO: Coming Soon

Research

DOI : Coming Soon

β-Cell Function in Type 1 Diabetes may not be as low as presumed

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Abstract

Objective: We aimed to evaluate β-cell function of type 1 diabetic patients (T1DP)s based on fasting and stimulated C-peptide levels.

Material and methods: Study included 135 T1DPs and 31 healthy subjects. Fasting C-peptide levels were measured in healthy subjects and T1DPs. The Mixed-meal tolerance test (MMTT) was performed in T1DPs. Fasting and stimulated (90 minute post MMTT) C-peptide levels were measured via electrochemiluminescence assay. Two categorizations were made according to fasting (the first categorization) and at 90th minute MMTT (the second categorization) C-peptide levels. For the first categorization; the groups were classified as follows: patients with undetectable ≤0.1ng/mL(group1); minimal 0.1-0.8ng/mL(group2); and sustained ≥0.8ng/mL (group3) C-peptide levels. For the second categorization, groups were as follows: patients with undetectable ≤0.1 ng/mL(group1); minimal 0.1-0.8 ng/ml (group2); and sustained ≥0.8ng/mL (group3) in which C peptide levels were increased to ≥150% of fasting C-peptide levels at the 90th minute after MMTT .

Results: For the first category; 41.5%, 40% and 18.5% of T1DPs were in group1, group2 and group3, respectively. For the second category; 34.8%, 20.7% and 44.4% were in group1, group2 and group3, respectively.

In first categorization 58.5% and in second categorization 65.1% of T1DPs had detectable C-peptide levels. 44,4% of the T1DPs had a response to MMTT with C-peptide levels ≥0.8 ng/mL which increased to ≥150% of fasting C-peptide level at the 90th minute after MMTT as it is seen in non-diabetics.

Conclusion: The present study suggests the presence of functioning β-cells in T1DPs and 44.4 % of T1DPs have a response to MMTT as seen in non-diabetics. (ClinicalTrials.gov number: NCT02199470.)

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Running title: β-Cell function in type 1 diabetes

Key words: β-Cell function, type 1 diabetes, mixed-meal tolerance test.

Received : Sep 13, 2017 **Accepted :** Feb 02, 2018 **Published :** Feb 13, 2018

Editor: Dragan Nikolic, University of Belgrade, School of Medicine, dr Subotica 9, 11000 Belgrade, Serbia. Email: dragannikolic8@yahoo.com

Introduction

Type 1 diabetes (T1D) is currently defined as autoimmune destruction of β-cells resulting in absolute insulin deficiency [1-3]. The decrease in β-cell function in type 1 diabetic patients (T1DP)s varies individually. Why some T1DPs have functioning β-cells and some do not is probably a result of their unique immunological and genetic characteristics. Some β-cells may be able to regenerate, evade the immunological attack or the inflammation may not be high enough [1,3-6]. Type 1 diabetes is a serious disease as it causes severe short and long term complications (short term complications are ketoacidosis, hyperosmolar coma, hypoglycemia and long term complications are macrovascular complications and microvascular complications such as retinopathy, nephropathy and neuropathy) and high mortality rate [1]. Increasing endogenous insulin production in T1DPs can improve glycemic control and decrease the complication and mortality rates. Because insulin secretory capacity provide T1DPs for managing themselves intensively in a more effective manner so that to be protected from hypoglycemia and other diabetic complications[17]. However decrease in mortality and complication rate can be achieved if sufficient β-cell function is present.

β-cells were observed in pancreatic autopsy samples obtained from T1DPs and persistent C-peptide secretion following a meal stimulus, was noted in the majority of T1DPs in recent studies [1-4]. The present study aimed to determine the level of β-cell function in T1DPs based on the fasting C-peptide levels and C-peptide levels after meal stimuli.

Material and Method

Study Population

We recruited 31 healthy subjects, 135 T1DPs, aged 18-56 years, who have T1D ≤25 years. All patients had been on insulin therapy since diagnosis. Ethics committee of our hospital approved the study protocol, which was performed in accordance with the Declaration of Helsinki (24.01.2013, 30/I). Our study was registered with an approved clinical trial registry. The clinical trial registration number of this study is NCT02199470.

Type 1 diabetes was defined as insulin dependency from the time of diagnosis onwards according to WHO criteria [3]. Duration of diabetes ranged from 1 month to 25 years and all patients had been on insulin treatment since the diagnosis. Subjects receiving drugs or/and having diseases which influence β-cell function and insulin sensitivity were excluded from the study. Glucose intolerance (fasting glucose level and that after glucose load) was also used as an exclusion criteria in the control group.

Fasting C-peptide levels of healthy subjects and T1DPs and stimulated C-peptide levels of T1DPs were measured. Stimulated C-peptide level was defined as C-peptide level measured 90 minutes after the mixed-meal.

Two categorizations were made according to the fasting (the first categorization) and stimulated (the second categorization) C-peptide levels. For the first categorization; the groups were classified as follows: patients with undetectable ≤0.1 ng/mL (group 1); with minimal 0.1-0.8 ng/ml (group 2); and with sustained ≥0.8 ng/mL (group 3) C-peptide levels. For the second categorization, groups were as follows: patients with undetectable ≤0.1 ng/mL (group 1); with minimal 0.1-0.8 ng/ml (group 2); and with sustained ≥0.8 ng/mL (group 3) C-peptide levels which increased to ≥150% of fasting C-peptide levels at the 90th minute after MMTT.

Mixed meal tolerance test: The MMTT was administered to each patient in the morning of an overnight fast of ≥ 12 hours (86 patients 12 hours, 35 patients 12.5 hours and 14 patients 13 hours) and not smoking since 10.00 p.m. the previous day and not administered their usual morning insulin. Then, venous glucose, C-peptide, HbA1c and lipid levels were measured and MMTT was performed to all patients. After collecting fasting blood samples, a mixed meal containing 33 g of carbohydrate, 15 g of protein, and 6 g of fat (240 kcal total) [2] was ingested in less than 15 min., and then C-peptide level was measured at 90th minute after mixed- meal.

Laboratory Analysis:

The fasting glucose level was measured via the hexokinase method and the HbA1c level was measured via high performance liquid chromatography (Tosoh G7 and 2.2, Tokyo, Japan). The lipid profile was determined based on standard enzymatic colorimetric methods (using Roche Diagnostics kits) and the C-peptide level

was measured via direct electrochemiluminescence immunoassay using mouse monoclonal anti-C- peptide antibody (Immulite 2000,Siemens, Germany).

Statistical Analysis

Statistical analysis was performed using SPSS version 16.0 (Inc., Chicago, Illinois, USA). The normality of the distribution of continuous variables was determined based on the one-sample Kolmogorov–Smirnov test, and data are presented as median and interquartile range. Categorical variables are shown as frequency and group percentage. Differences in variables not normally distributed between groups were evaluated using the Kruskal Wallis test. Two-group non-parametric comparisons were made using the Mann Whitney-U test. Spearmen's rho correlation analysis was used to identify possible associations between fasting C-peptide and stimulated C-peptide levels and the other study variables. The level of statistical significance was set at $p < 0.05$.

Results

Median age of the patients (53.33% of the T1DPs were male and 46.6% were female) was 29 years (interquartile range: 22-37 years), median age at diagnosis was 23 years (interquartile range: 22-37 years), and median duration of diabetes was 52 months (interquartile range: 19-132 months).

For the first category; 56(41.5%), 54(40%), and 25(18.5%) patients of T1DPs were in group 1, group 2 and group 3, respectively. For the second category; 47 (34.8%) patients of T1DPs were in group 1, 28 (20.7%) of them in group 2 and 60 (44.4 %) of them in group 3.

The characterizations of all the groups and healthy subjects were in table 1, 2 and 3. Among participants 58.5% of the patients had a detectable fasting C-peptide level and 65.1% had a detectable stimulated C-peptide level. Forty four point four percent of the T1DPs (group 3) had a response to mixed- meal stimulus with a C-peptide levels ≥0.8 ng/mL which increased to ≥150% of the fasting C-peptide level similar to non-diabetics. Moreover, there were no difference between fasting C-peptide levels of group 3 and those of healthy subjects (p=0,5 for the comparison of two groups according to the first categorization and p=0.1 for the comparison of these two groups according to the second categorization). As shown in Table 2 and 3, according to the first and second categorizations, group 3 were the oldest at time of diagnosis (p=0.03, p=0.01 respectively) and had the shortest duration of diabetes (p<0.001, p<0.001 respectively). There were no differences among the three groups of T1DPs in gender, age, HbA1C, fasting glucose, total cholesterol, LDL-C, or triglyceride levels (p>0.05, p>0.05, p>0.05, p>0.05, p>0.05, p>0.05, p>0.05 respectively).

The duration of diabetes was negatively correlated with fasting and stimulated C-peptide levels (r= -0.57;p<0.001, r= -58; p<0.001 respectively). Group 1 with the longest diabetes duration (more than 92 months) did not have detectable (41.5 %) and stimulated (34.8 %) C-peptide levels. Age at diagnosis was positively correlated with fasting C-peptide levels and stimulated C-peptide levels (r=0.32;p<0.001, r=0.3;p=0.001 respectively).

Fasting and stimulated C-peptide levels of the patients whose diagnosis of diabetes before puberty were significantly lower than those of the patients whose diagnosis of diabetes after puberty (p<0.001, p<0.001).

Discussion

The majority of patients with type 1 diabetes was observed to produce insulin endogenously based on fasting and stimulated C-peptide levels. Additionally, the prevalence of a detectable fasting C-peptide level was 58.5% and the prevalence of a detectable stimulated C-peptide level was 65.1%. Furthermore, almost 44.44 % of T1DPs had a response to mixed-meal stimulus with C-peptide levels ≥0.8 ng/mL which increased to ≥150% of fasting C-peptide levels as in non-diabetics. In the present study, no difference was found between these patients including 44.44 % of T1DPs and healthy subjects in fasting C-peptide levels.

Based on the present findings, we suggest that the fasting and stimulated C-peptide levels in patients with type 1diabetes may be higher than presumed before.

Consistent with Pipeleers's [3] and Keenan's findings [4], in the present study, fasting C-peptide levels and stimulated C-peptide levels were found to be positively correlated with age at diagnosis whereas Lohr and Kloppel [5] did not observe a correlation between

Table 1. Demographic and clinical characteristics of patients and controls

Variables	Patients with type 1 diabetes (n=135)	Control Group (n=31)	P value
Age (year) Median Interquartile range	 29 21-36	 30 27-38	0.13
Gender (Male/Female)	72/63	13/18	0.56
Fasting blood glucose (mg/dL) Median Interquartile range	 175 115-263	 86 84-92	<0.001
HbA1c (%) Median Interquartile range	 8.4 7.1-10.1	 5.2 5.1-5.4	<0.001
Fasting C peptide *(nmol/L)* Median Interquartile range	 0.23 0.01-1.74	 1.35 1.05-1.66	<0.001
Total Cholesterol (mg/dL) Median Interquartile range	 168 150-194	 170 157-189	0,97
Low Density Lipoprotein Cholesterol (mg/dL) Median Interquartile range	 96 80-122	 98 73-115	0,32
High Density Lipoprotein Cholesterol (mg/dL) Median Interquartile range	 53 44-65	 57 46-73	0,09
Triglyceride (mg/dL) Median Interquartile range	 73 56-103	 73 62-86	0,8

HbA1c: Hemoglobin A1c

Table 2. Charesteristics of the groups according to the first categorization

	Group 1 (n=56)	Group 2 (n=54)	Group 3 (n=25)	P
Age *(year)* Median Interquartile range	 28 23-37	 29 23-36	 29 21-39	0.82
Gender *(n/n)* Female/Male	 31/25	 24/30	 8/17	0.14
Duration of diabetes, *(month)* Median Interquartile range	 114 59-168	 37 12-92	 18 2-28	<0.001
Fasting Blood Glucose *(mg/dL)* Median Interquartile range	 180 115-263	 171 136-251	 144 124-237	0.68
HbA1c *mmol/mol* (DCCT units %) Median Interquartile range	 63.9 (8.0) 55.2-82.5 (7.2-9.7)	 65(8.15) 50.8-85.5 (6.8-10.0)	 69.4 (8.5) 46.4-86.9 (6.4-10.1)	0.97
Fasting C peptide *(nmol/L)* Median Interquartile range	 0.10 0.10-0.10	 0.28 0.21-0.52	 1.26 0.93-1.74	<0.001
Total cholesterol *(mg/dL)* Median Interquartile range	 171 155-194	 175 151-190	 162 134-190	0.31
LDL-C *(mg/dL)* Median Interquartile range	 95 81-122	 99 83-126	 89 69-118	0.29
HDL-C *(mg/dL)* Median Interquartile range	 57 46-68	 53 44-65	 46 38-54	0.008
Triglyceride *(mg/dL)* Median Interquartile range	 75 59-105	 70 56-90	 78 64-148	0.37
Age at diagnosis *(year)* Median Interquartile range	 21 15-28	 24 17-31	 26 19-34	0.03

Table 3. Charesteristics of the groups according to the second categorization

	Group 1 (n=47)	Group 2 (n=28)	Group 3 (n=60)	P
Age *(year)* Median Interquartile range	 30 25-40	 26 21-31	 28 22-38	0.27
Gender *(n/n)* Female/Male	 28/21	 13/15	 21/33	0.17
Duration of diabetes, *(month)* Median Interquartile range	 138 75-170	 38 14-90	 24 4-56	<0.001
Fasting Blood Glucose *(mg/dL)* Median Interquartile range	 200 115-282	 193 144-260	 143 118-186	0.04
HbA1c *mmol/mol* (DCCT units%) Median Interquartile range	 63.9 (8.0) 55.2-79.2 (7.2-9.4)	 68.3(8.4) 57.4-59.6 (7.4-7.6)	 59.6(7.6) 46.4-80.3 (6.4-9.5)	0.21
90-minute C peptide *(nmol/L)* Median Interquartile range	 0.10 0.10-0.10	 0.28 0.24-0.65	 2.10 1.40-3.77	<0.001
Total cholesterol *(mg/dL)* Median Interquartile range	 169 154-195	 170 152-199	 164 138-190	0.32
LDL-C *(mg/dL)* Median Interquartile range	 94 78-123	 97 81-127	 96 79-121	0.92
HDL-C *(mg/dL)* Median Interquartile range	 55 45-68	 57 48-65	 48 42-58	0.06
Triglyceride *(mg/dL)* Median Interquartile range	 76 67-112	 60 48-85	 73 58-94	0.31
Age at diagnosis *(year)* Median Interquartile range	 19 13-26	 24 16-29	 26 19-35	0.01

age of onset of diabetes and residual β-cells. Additionally, in the present study, fasting and stimulated C-peptide levels decreased as the duration of diabetes increased. This finding is also consistent with Oram's findings [6].

Interestingly, based on a murine model Thorel et al showed that in β-cell-depleted mice, α-cells could differentiate into β-cells in mice after prolonged duration of diabetes [7]. Similarly, Chera et al observed that pancreas reconstituted new insulin–producing cells in the absence of autoimmunity in mice in which β-cells were completely ablated. They also reported that glucagon-producing α-cells could begin to produce insulin via a process of reprogramming (transdifferentiation) without proliferation and posited that these phenomena might be translatable to humans, because efficient β-cell regeneration had been determined in children with type 1 diabetes or after pancreatectomy [8,9] and glucagon/insulin bihormonal human cells were observed following epigenetic manipulation ex vivo [10], and in patients with diabetes [11,12]. Moreover, Zhou et al reported that aciner cells were capable of conversion into β-cells invivo when administered an adenovirus cocktail of some transcription factors [13]. β-cells in patients with long duration of type 1 diabetes have been reported in histological studies since 1965 [14,15]. These findings can explain the increase in the C-peptide levels after mixed-meal stimuli in T1DPs observed in the present study, and the Joslin Medalists[4] and Oram's [6] studies. The increase in the C-peptide levels after mixed -meal stimuli in the present study strongly indicates the presence of functioning β-cells, however, in the present study, there was no histological examination showing that aciner cells and α-cells were capable of conversion into β-cells. This was a limitation of the present study.

The source of the functioning β-cells observed in the present study and in two other earlier studies mentioned here in, is not clear, however there might be multiple sources. β-cell proliferation and redifferentiation and dedifferentiation of α-cells, δ cells and aciner cells into β-cells may result in functioning β-cells in T1DPs with long disease duration.

An important finding of the present study is that fasting and at 90th minute post MMTT C-peptide levels in the patients diagnosed as diabetes before puberty were significantly lower than in those diagnosed after puberty, which might be explained with Chera's mouse study, in which α-cells reprogramed to produce insulin from puberty to adulthood, and somatostatin- producing δ cells were able to convert to insulin-producing β-cells during puberty. Chera et al. suggested that the source of regenerated β-cells after puberty and even a long time after β-cells loss, was α-cells and that prior to puberty the source of β-cells was somatostatin-producing δ cells and reconstituted β-cells [9,16]. The present findings support their suggestion that dedifferentiation of α-cells and δ-cells into β-cells could be possible in humans.

To the best of our knowledge the present study is the first to evaluate the effect of puberty on fasting and stimulated C-peptide levels.

Moreover, the present study has been the first to have evaluated the prevalence of T1DPs having a response to mixed-meal stimulus like non-diabetics, up to now, too. The Physiologic C-peptide response to MMTT in non-diabetics is accepted as a C-peptide level ≥0.8 ng/mL which increases to ≥150% of the fasting C-peptide level [19]. The number of T1DPs with a non-diabetic-like response to mixed-meal stimulus were evaluated in only the present study, but was not evaluated in the DCCT (Diabetes Control and Complications Trial) [17], McGee'[18], Greenbaum' [19], Oram' study [6] or Joslin Medalist study[4]. It was reported that in Joslin Medalist study [4], 13 (41.93%) of 31 T1DPs who returned for MMTT and in Greenbaum's study[19], almost all the T1DPs responded with a doubling of the C-Peptide level as compared to the fasting level. However it was not evaluated that those C-peptide levels after MMTT were in normal or below the normal range in both of the aforementioned studies [4,19]. We found that 60 (44.4%) of 135 T1DPs responded to meal stimuli with C-peptide levels ≥0.8 ng/mL which increased to ≥150% of fasting C-peptide levels as non-diabetics.

Additionally, the present study administered the MMTT has had the third largest group number of T1DPs after DCCT (included 855 patients) and Greenbaum's (included 143 patients) studies up to now [4, 6,17,18,19].

There are several differences between the present study and the earlier studies cited. The present study is different from Joslin Medalist study and Greenbaum's study. Since serum C-peptide level was measured via radioimmunoassay in Medalist study [4] and measured via the assay which was unable to measure a value below the lower limit of quantification in Greenbaum's [19], whereas in the present study it was measured via a direct electrochemiluminescence immunoassay which is more sensitive than other assays used in the two aforementioned studies [4,6,19]. Oram's study [6] included type 1diabetics with the disease duration of > 5 years whereas the present study included type 1 diabetics with the disease duration of ≤ 25 years. Moreover, in the DCCT study [17] mean duration of diabetes was 2.3 ± 0.3 years and in Greenbaum's , it was ≤ 4 years less than in the present study. Detectable C-peptide levels after mixed-meal stimuli were noted in 65.1% of T1DPs in the present study, versus 73% of those in Oram's study [6], 67.4% in the Joslin Medalist Study [4], 35.43% in the DCCT [17], 87-95% in Greenbaum's study [19], and 17% in McGee's study [18] which included the patients screened via MMTT in the DCCT study 30 years later. It is interesting that the prevalence of detectable C-peptide levels after mixed- meal stimuli in the Joslin Medalist, Oram's and the present studies were higher than that in the DCCT study as type 1 diabetics in the DCCT study had the shortest duration of disease. The difference might have been result from using of a more sensitive immunoassay in the present and Oram's studies, but it is not clear why the prevalence of a detectable C-peptide level after mixed- meal stimulus in the Joslin Medalist study, was higher than that in the DCCT study .

The present study has some limitations. It is possible that some patients with non-type 1 diabetes were inadvertently included in the study although a tight type 1diabetes definition was used. Family history for MODY was recorded in each of the patients in the present study. However the patients were not genotyped for risk polymorphisms in MODY genes.

In conclusion, the present study shows that many of the type 1 diabetics had detectable fasting and stimulated C-peptide levels. Moreover, 44.4 % of T1DPs had a non-diabetic-like C-peptide level in response to mixed- meal stimulus. As, the presence of functioning β cells in type 1 diabetics can facilitate their long-term survival and reduce the incidence of macro and microvascular complications and hypoglycemia, future studies are needed to discover the source of insulin-producing cells in some T1DPs as observed in the present study. If the source can be identified, subsequent research can focus on how to enhance this source of insulin-producing cells.

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