CLINICS

RAPID COMMUNICATION

Residual C-peptide in patients with Type 1 diabetes and multiethnic backgrounds

Mirella Hansen de Almeida,[I,III] Joana Rodrigues Dantas,[I,II] Bianca Barone,[I,II] Fabiano Marcel Serfaty,[I,II] Rosane Kupfer,[II] Marta Albernaz,[IV] Maria Rocio Bencke,[IV] Lenita Zajdenverg,[I] Melanie Rodacki,[I] José Egídio Paulo de Oliveira[I]

[I] Clementino Fraga Filho University Hospital, Federal University of Rio de Janeiro (HUCFF), Nutrology Department, Rio de Janeiro/RJ, Brazil. [II] State Institute of Diabetes and Endocrinology Luiz Capriglione (IEDE), Rio de Janeiro/RJ, Brazil. [III] Air Force Central Hospital (HCA), Endocrinology Department, Rio de Janeiro/RJ, Brazil. [IV] Clementino Fraga Filho University Hospital, Federal University of Rio de Janeiro (HUCFF), Nuclear Medicine Department, Rio de Janeiro/RJ, Brazil.

OBJECTIVE: To evaluate serum C-peptide in 88 patients from a multiethnic population with Type-1 diabetes and variable disease durations.

METHOD: Eighty-eight patients with a mean disease duration of 8.1 ± 7.6 years were included and underwent C-peptide measurement before and after glucagon stimulation. Chi-squared and Mann Whitney U-tests were used to compare the variables between groups (all two-tailed, $\alpha = 0.05$). Spearmans correlation coefficient was used to test the association between the continuous variables. Logistic regression was used for the multivariate analysis. Twenty-eight (31.8%) individuals had significantly detectable C-peptide levels after stimuli, particularly those with a shorter disease duration ($p<0.001$).

RESULTS: Patients with detectable C-peptide levels required lower insulin doses ($p<0.009$) and had similar HbA1C results ($p = 0.182$) and fewer chronic complications ($p = 0.029$).

CONCLUSION: C-peptide detection was common in Type-1 diabetics, particularly shortly after being diagnosed. This result may have clinical implications.

KEYWORDS: Diabetes Mellitus Type-1, C-Peptide, Insulin Secretion, Disease Duration, GADA, Autoimmune Disease.

Almeida MH, Pereira JD, Barone B, Serfaty FM, Kupfer R, Albernaz M, et al. Residual C-peptide in patients with Type 1 diabetes and multiethnic backgrounds. Clinics. 2013;68(1):123-126.

E-mail: joanardantasp@ig.com.br

Tel.: 55 21 3251-3304

INTRODUCTION

Type-1 diabetes mellitus is characterized by destroyed pancreatic β cells, insulin deficiency and hyperglycemia (1,2). The progression of this process is heterogeneous and may range from months to many years (3,4). For long periods after being diagnosed, some Type-1 diabetes patients maintain a residual β-cell function (5), which may be associated with reducing the risk of complications and hypoglycemia and improving metabolic control (6-10). C-peptide is considered a reliable method for estimating the β-cell residual function (5-7), particularly after glucagon stimulation or a mixed meal. Most studies that have evaluated the C-peptide secretion in Type-1 diabetes have included Caucasians or Asians. Little is known about insulin secretion in individuals of other ethnicities with Type-1 diabetes. The aim of this study was to evaluate the β-cell function in multiethnic Type-1 diabetes patients with variable disease durations using C-peptide measurements after glucagon stimulation.

RESEARCH DESIGN AND METHODS

Volunteers who had Type-1 diabetes (11) with short (Group 1) and long (Group 2) disease durations (≤5 years and >5 years, respectively) were included in this cross-sectional study. A diabetes diagnosis was based on the American Diabetes Association criteria (11). The patients' clinical and epidemiological data were obtained from questionnaires and medical records, and all participants provided informed written consent. Blood samples from each patient were measured for fasting glucose and stimulated C-peptide (6 minutes after intravenous injections of 1 mg of glucagon), glycosylated hemoglobin (HbA1C) and anti-glutamic acid decarboxylase antibody (GADA). The tests were performed if a patient's fasting capillary glucose measured between 70 and 200 mg/dL. The participants were classified as whites and non-whites based

No potential conflict of interest was reported.

DOI: 10.6061/clinics/2013(01)RC02

on their phenotypes and family backgrounds. C-peptide was determined using chemiluminescence (Immulite DPC; UK). A C-peptide level ≥0.5 ng/dL was considered detectable (reference 1.1 to 5.0 ng/mL). The inter- and intra-assay coefficients of variation (CV) were 7.6% and 8.2%, respectively. GADA was determined using radio-immunoassay (*RSR-Limited*, Cardiff, UK). Values >1.0 U/mL were considered positive. The inter- and intra-assay CVs were 4.9 to 7.0% and 3.6 to 3.7%, respectively. The statistical analyses were performed using SAS 6.1 version (SAS Institute Inc., Cary, NC). Chi-squared and Mann Whitney U-tests were used to compare the variables between the groups (all two-tailed, $\alpha = 0.05$). Spearmans correlation coefficient was used to test the association between the continuous variables. Logistic regression was used for the multivariate analysis.

RESULTS

The study population comprised 88 individuals with Type-1 diabetes. The characteristics of the study group are shown in Table 1. Fasting C-peptide was detectable in 19 patients (21.6%), and these C-peptide levels were within the normal range in 7 (8%) patients. After the glucagon stimulus, C-peptide was detectable in 31.8% of the patients (n = 28, $p = 0.004$) and within the normal range in 14.8% of the patients (n = 13, $p = 0.016$). The mean C-peptide titers in the patients with detectable C-peptide levels were 1.3 ± 0.8 ng/ml (fasting) and 1.6 ± 1.2 ng/ml (after the stimulus). Given the superiority of C-peptide (after the stimulus) over the fasting measurement, the former was used for the following tests. Adverse events occurred in 35.2% of the patients (n = 30) after the glucagon infusion (e.g., transient mild nausea that was reversible within minutes in most cases, vomiting, paresthesia and malaise).

There was an association between the detectable C-peptide levels and the diabetes disease duration (detectable CP = 5 ± 7.5 vs. undetectable CP = 10 ± 6.99 years, $p<0.001$), but there was no correlation between the C-peptide titers and the Type-1 diabetes duration (r = -0.342, $p = 0.08$). In group 1, the patients with detectable C-peptide levels had a shorter Type-1 diabetes duration (2.01 ± 1.4 vs. 3.04 ± 1.5 years, $p = 0.034$). The patient's age at disease onset, ethnicity, gender, DKA at diagnosis and GADA status were not associated with the C-peptide detection. GADA was positive in 43.7% of patients. Patients with detectable C-peptide levels had a lower mean age than the other patients ($p = 0.001$), but this link disappeared after the multivariate analysis. There was a correlation between overweight (BMI≥25 kg/m^2) and undetectable C-peptide levels ($p = 0.06$).

Fasting glucose was not associated with C-peptide detection ($p = 0.16$). The patients with detectable C-peptide levels required lower insulin doses/kg than the other patients (0.61 ± 0.34 vs. 0.85 ± 0.28, $p<0.009$), but had similar HbA1c levels (8.49 ± 1.9 vs. 9.5 ± 2.6, $p = 0.182$). These results were observed in the sample as a whole and separately in each group. C-peptide detection was also linked to a lower frequency of chronic complications (3.7% vs. 21.7%, $p = 0.029$) but not to retinopathy or nephropathy individually. In group 2, other autoimmune diseases (usually thyroid diseases) occurred more frequently in the patients with detectable C-peptide levels ($p = 0.018$). This association was not observed in group 1 ($p = 0.53$). After the logistic regression analysis, the link between the detectable C-peptide levels and the lower insulin doses (in use and with the Type-1 diabetes duration) remained significant.

DISCUSSION

This study evaluated the frequency and clinical implications of C-peptide detection in Type-1 diabetes patients. This study population is particularly interesting because of the complex and mixed ethnic backgrounds of the patients. Most studies that have evaluated pancreatic function in Type-1 diabetes were performed in Caucasian and Asian patients. This study provides a unique opportunity to improve our knowledge of the progression of β-cell

Table 1 - Clinical characteristics of patients grouped by disease durations.

		Disease duration			
		Group 1 (≤5 years)	Group 2 (>5 years)	Total	*p-value*
Sex	Female n (%)	14(35.9)	34(68.8)	48(54.0)	**0.002**
	Male n (%)	25(64.1)	15(31.2)	40(46.0)	
Ethnicity	White n (%)	19(48.7)	32(64.6)	51(57.5)	0.13
	Non-white n (%)	20(51.3)	17(35.4)	37(42.5)	
Mean actual age (years)		18.6 ± 5.6	26.9 ± 9.1	23.1 ± 8.8	**<0.001**
Mean diagnostic age (years)		16.5 ± 5.7	13.3 ± 7.9	14.7 ± 7.2	**0.004**
Mean disease duration (years)		2.51 ± 1.51	13.73 ± 6.72	8.7 ± 7.6	**<0.001**
DKA at diagnosis n (%)	Yes	8(20.5)	21(41.7)	29(33.0)	**0.022**
	No	31(79.5)	27(56.3)	59(67.8)	
Mean BMI (kg/m^2 ± SD)		21.1 ± 3.5	23.6 ± 3.2	22.4 ± 3.5	**<0.001**
HbA1C (mean ± SD)		8.97 ± 2.29	8.45 ± 1.62	8.6 ± 1.95	0.22
Mean insulin dose/kg		0.72 ± 0.34	0.87 ± 0.29	0.8 ± 0.3	**0.023**
Detectable CP n (%)	Fasting (CP1)	16(41)	3(6.1)	19(21.6)	***<0.001***
	Stimulated (CP2)	23(63.9)	5(10.2)	28(31.8)	***<0.001***
Preserved CP n (%)	Fasting (CP1)	6(15.4)	1(2.0)	7(8)	***0.029***
	Stimulated (CP2)	11(28.2)	2(4.1)	13(14.8)	***0.009***
Other Auto-immune diseases n (%)*		6 (15)	7(14.5)	13(14.7)	*0.95*
GADA		24(62.5)	14(29.8)	38(43.7)	***0.004***
Total number of patients		**39**	**49**	**88**	

BMI – Body mass index, HBA1c – Glycohemoglobin, DKA- Diabetic ketoacidosis, CP – C-peptide. * Thyroid disease in 12 cases, Sjogren's syndrome and rheumatoid arthritis in one case each.

secretion in patients with Type-1 diabetes in our population because it is still unclear whether the results that were obtained in previous studies can be extrapolated to other ethnic populations, such as ours.

The tests were performed after the patients were stimulated with glucagon, which is a faster method for increasing β-cell secretion compared with the mixed meal test. However, nausea and flushing may occur more frequently as adverse events (5-7,12). This study found that adverse events occurred in 35.2% of patients. These adverse events included mild and transient nausea (in most cases), which suggested that C-peptide measurement after glucagon infusion can be well tolerated. At the beginning of this study, both tests were considered equivalent in the literature (7), and the mixed meal test was preferable (13).

We have shown that a significant proportion of patients with Type-1 diabetes had detectable C-peptide levels, and these levels were higher than those observed in the Diabetes Control and Complications Trial Research Group DCCT (8,10). We also compared the serum C-peptide levels between individuals with short and long disease durations ($\leq$5 and >5 years, respectively) of Type-1 diabetes. As expected, detectable C-peptide levels occurred more frequently in the individuals with shorter disease durations, which indicated that the progression of the β cell destruction occurred primarily during the first years of the disease (8,13-16). However, a significant proportion of the patients with long-standing Type-1 diabetes had detectable levels of C-peptide. This finding was compatible with the anatomopathological data that described the presence of insulin cells in Type-1 diabetes patients with long disease durations (5). In a group of patients with extremely favorable clinical outcomes and fewer diabetic complications, Keenan et al. have reported a surprisingly large number (18%) of Type-1 diabetes patients with disease durations >50 years and detectable C-peptide levels (17). Recently, using an ultrasensitive assay, Wang et al. have reported residual C-peptide levels in patients with long-standing Type-1 diabetes (18). However, the potential clinical implications of sustained β-cell function have been reported only for those patients with C-peptide levels above the approximate threshold that was used in our study (8).

One could question whether the patients with Type-1 diabetes and detectable C-peptide levels actually have Type-1 diabetes rather than other forms of diabetes. However, most patients needed insulin shortly after their diagnoses, were not overweight and lacked a significant family history of diabetes in more than one generation; these histories make other diagnoses, such as Type-2 diabetes and *Maturity Onset Diabetes of the Young* (MODY), unlikely. However, we cannot exclude the possibility that atypical forms of diabetes could be present in populations with mixed ethnic backgrounds, particularly those with GADA (-). Although the GADA prevalence was low in this study group (19), it is likely that its positivity has decreased over the years (20,21).

We found an association between autoimmune comorbidities and detectable C-peptide levels after stimulating patients with long disease durations, particularly in patients with thyroid diseases. Although the sample size was limited, this association has not been reported in other studies (22). Vondra et al. reported the exact opposite association, with a lower frequency of autoimmune comorbidities in patients with Type-1 diabetes and detectable C-peptide levels (23).

It is known that short- and long-term glycemic control may influence β-cell function in patients with Type-1 diabetes. However, previous data have indicated that residual pancreatic function may influence glycemic control (8,24,25). In this study, we excluded patients with glycemia <70 or >200 mg/dl to minimize any possible interference. Although the patients with Type-1 diabetes and detectable C-peptide levels had similar glycemic control, these patients used lower daily insulin doses, which indicated that maintaining endogenous insulin secretion might reduce the insulin requirements of patients with Type-1 diabetes.

The association between chronic complications and residual C-peptide levels was also analyzed (25,26). We found a link between C-peptide and chronic complications as a whole but not with retinopathy or nephropathy separately. It is possible that the sample size and the small number of individuals with chronic complications included in this study might have influenced our results.

To summarize, this study identified a significant number of individuals with Type-1 diabetes (of both short and long duration) with detectable C-peptide levels. It is possible that this residual β cell secretion is associated with a lower insulin requirement, a lower frequency of chronic complications and a higher frequency of other autoimmune diseases. However, prospective studies are still needed to resolve these questions.

■ ACKNOWLEDGMENTS

This study was performed at the Clementino Fraga Filho University Hospital (HUCFF) at the Federal University of Rio de Janeiro (UFRJ) and at the State Institute of diabetes and Endocrinology Luiz Capriglione (IEDE).

Thanks to Novo Nordisk Pharmaceutical of Brazil for the Glucagon® kits.

The study was supported by grants from CAPES e CNPQ.

■ AUTHOR CONTRIBUTIONS

Dantas JP and Almeida MH recruited patients, performed analyses, contributed to the statistical analyses and wrote the manuscript. Barone B recruited patients and analyzed samples. Sertafy FM recruited patients. Kupfer R contributed to the study design. Albernaz M and Bencke MR performed the radioimmunoassays. Zajdenverg L reviewed and edited the manuscript. Rodacki M contributed to the study design and interpretation of data and reviewed and edited the manuscript. Oliveira JE contributed to the discussion and reviewed the manuscript.

■ REFERENCES

1. Atkinson MA, Eisenbarth GS. Type 1 Diabetes: new perspectives on disease pathogenesis and treatment. Lancet. 2001;358(9277):221-9, http://dx.doi.org/10.1016/S0140-6736(01)05415-0.
2. Daneman D. Type 1 Diabetes. Lancet. 2006;367(9513):847-58, http://dx.doi.org/10.1016/S0140-6736(06)68341-4.
3. Screenan S, Pick AJ, Levisettim, Baldwin AC, Pugh W, Polonsky KS. Increased beta-cell proliferation and reduced mass before diabetes onset in the nonobese diabetic mouse. Diabetes. 1999;48(5):989-96, http://dx.doi.org/10.2337/diabetes.48.5.989.
4. Marchetti P, Dotta F, Zhiong L, Lupi R, Del Guerra S, Santangelo C, et al. The function of pancreatic islets isolated from type 1 diabetic patient. Diabetes Care. 2000;23(5):701-3, http://dx.doi.org/10.2337/diacare.23.5.701.
5. Tsai EB, Sherry NA, Palmer JP, Herold KC. The Rise and Fall of Insulin Secretion in type 1 diabetes mellitus. Diabetologia. 2006;49(2):261-70.
6. Palmer JP, Fleming GA, Greenbaum CJ, Herold KC, Jansa LD, Kolb H, et al. C-peptide is the appropriate outcome measure for type 1 diabetes clinical trials to preserve beta-cell function: report of an ADA workshop, 21-22 October 2001. Diabetes. 2004;53(1):250-64.

7. Vendrame F, Zappaterreno A, Dotta F. Markers of beta cell function in type 1 diabetes mellitus. Minerva Med. 2004;95(2):79-84.
8. Steffes MW, Sibley S, Jackson M, Thomas W. b-cell Function and the development of diabetes-related complications in diabetes control and complication trial. Diabetes Care. 2003;26(3):832-6, http://dx.doi.org/10.2337/diacare.26.3.832.
9. Sherry NA, Tsai EB, Palmer JP, Herold KC. Natural history of beta cell function in type 1 diabetes. Diabetes. 2005;54:Suppl 2:S32-9.
10. Effect of intensive therapy on residual beta-cell function in patients with type 1 diabetes in the diabetes control and complications trial. A randomized, controlled trial. The Diabetes Control and Complications Trial Research Group. Ann Intern Med. 1998;128(7):517-53.
11. American Diabetes Association (ADA). Diagnosis and classification of diabetes mellitus (position statements). Diabetes Care. 2012;35 Suppl1:S64-71.
12. Scheen AJ, Castillo MJ, Lefebvre PJ. Assessment of residual insulin secretion in diabetic patients using the intravenous glucagon stimulatory test: methodological aspects and clinical applications. Diabetes Metab. 1996;22(6):397-446.
13. Greenbaum Cj, Mandrup-Poulsen T, Mcgee Pf, Battelino T, Haastert B, Ludvigsson J, et al. Mixed-meal tolerance test versus glucagon stimulation test for the assessment of β-cell function in therapeutic trials in type 1 diabetes. Diabetes Care. 2008;31(10):1966-71, http://dx.doi.org/10.2337/dc07-2451.
14. Wallensten M, Dahlquist G, Persson B, Landin-Olsson M, Lernmark A, Sundkvist G, et al. Factors influencing the magnitude, duration, and rate of fall of b-cell function in type 1 (insulin dependent) diabetic children followed for two years from their clinical diagnosis. Diabetologia. 1988;31(9):664-9, http://dx.doi.org/10.1007/BF00278749.
15. Effects of age, duration and treatment of insulin-dependent diabetes mellitus on residual beta-cell function: observations during eligibility testing for the Diabetes Control and Complications Trial (DCCT). The DCCT Research Group. J Clin Endocrinol Metab. 1987;65(1):30-6.
16. Steele C, Hagopian WA, Gitelman S, Masharani U, Cavaghan M, Rother KI, et al. Insulin Secretion In Type 1 Diabetes. Diabetes. 2004;53(2):426-33, http://dx.doi.org/10.2337/diabetes.53.2.426.
17. Keenan HA, Sun JK, Levine J, Doria A, Aiello LP, Eisenbarth G, et al. Residual insulin production and pancreatic cell turnover after 50 years of diabetes: Joslin Medalist Study. Diabetes. 2010;59(11):2846-53, http://dx.doi.org/10.2337/db10-0676.
18. Wang L, Lovajoy NF, Faustman DL. Persistence of Prolonged C-peptide Production in Type 1 Diabetes as Measured With an Ultrasensitive C-peptide Assay. Diabetes Care. 2012;35(3):465-70, http://dx.doi.org/10.2337/dc11-1236.
19. Pardini VC, Mourao DM, Nascimento PD, Vívolo MA, Ferreira SR, Pardini H. Frequency of islet cell autoantibodies (IA-2 And GAD) in young Brazilian type 1 Diabetes patients. Braz J Med Biol Res. 1999;32(10):1195-8, http://dx.doi.org/10.1590/S0100-879X1999001000003.
20. Rodacki M, Zajdenverg L, Albernaz MS, Bencke-Gonçalves MR, Milech A, Oliveira JE. Relationship between the prevalence of anti-glutamic acid descarboxilase autoantibodies and duration of type 1 diabetes mellitus in Brazilian patients. Braz J Med Biol Res. 2004;37(11):1645-50.
21. Winter WE, Harris N, Schatz D. Type 1 diabetes islet autoantibody markers. Diabetes Technol Ther. 2002;4(6):817-39, http://dx.doi.org/10.1089/152091502321118838.
22. Glastras SJ, Craig ME, Verge CF, Chan AK, Cusumano JM, Donaghue KC. The role of autoimmunity at diagnosis of type 1 diabetes in the development of thyroid and celiac disease and microvascular complications. Diabetes Care. 2005;28(9):2170-5, http://dx.doi.org/10.2337/diacare.28.9.2170.
23. Vondra K, Bendlová B, Sterzl I, Vrbíková J, Zamrazil V. [Diabetes mellitus in adult patients with type I diabetes shows immunological, functional and clinical differences depending on the presence of autoimmune thyroiditis]. Cas Lek Cesk. 2007;146(3):267-72.
24. Madsbad S, Krarup T, Reguer L, Faber OK, Binder C. Effect of strict blood glucose control on residual b-cell function in insulin-dependent diabetics. Diabetologia. 1981;20(5):530-4.
25. Sjöberg S, Gjötterberg M, Berglund L, Möller E, Östman J. Residual C-peptide excretion is associated with better long-term glycemic control and slower progress of retinopathy in type 1 (insulin-dependent) diabetes mellitus. J Diabetes Complications. 1991;5(1):18-22.
26. Zerbini G, Mangili R, Luzi L. Higher post-absortive C-peptide levels in type 1 diabetic patients without renal complications. Diabet Med. 1999;16(12):1048-9, http://dx.doi.org/10.1046/j.1464-5491.1999.00181.x.