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The Relationship Between C-Peptide Index and Proteinuria in Patients with Type 2 Diabetes Mellitus

Tip 2 Diabetes Mellitus Hastalarında C-Peptid İndeks ile Diyabetik Nefropati Arasındaki İlişki

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Abstract

Objective: Though the C-peptide index (CPI) has been a reliable marker for estimation of the beta-cell reserve, its association with microvascular complications in Type 2 diabetes mellitus (DM) patients has not been elucidated as yet. This study, therefore, aimed to investigate the relationship between C-peptide levels and CPI with microvascular complications in Type 2 DM patients. **Material and Methods:** Type 2 DM patients, over 18 years of age, whose C-peptide levels were analyzed in the endocrinology and internal medicine clinics between 2014 and 2018, having normal kidney functions (glomerular filtration rate >60 mL/min) and who are not dependent on any insulin secretagogue oral antidiabetic agent (i.e., sulfonylurea) were enrolled the study. Blood samples were collected after at least 12 h of fasting, without any drug or insulin administration. Hemogram, hemoglobin A1c (HbA1c), lipid, glucose, C-peptide parameters were analyzed from the same serum sample. The patients were classified into three groups according to the spot urine albumin/creatinine ratio. Patients with no proteinuria, patients with microalbuminuria, and patients with macroalbuminuria were defined as group 1, group 2, and group 3, respectively. **Results:** A statistically significant difference between CPI levels in the groups was observed (p<0.001). CPI levels of Groups 2 and 3 were lower than that of group 1 (p=0.007 and p<0.001). In addition, the CPI level of group 3 was significantly lower than that of group 2 (p=0.015). An inverse association between CPI level and proteinuria was thus recognized. HbA1c and proteinuria were found to be positively correlated (p<0.001). **Conclusion:** This study highlights the association between C-peptide, CPI, and diabetic nephropathy in Type 2 DM patients.

Keywords: C-peptide index; diabetes mellitus; diabetic nephropathy

Özet

Amaç: C-peptid indeksi (CPI), beta hücre rezervinin daha güvenilir bir markırı olarak kabul edilir, ancak Tip 2 diabetes mellitus (DM) hastalarında CPI'nın mikrovasküler komplikasyonlarla ilişkisi net olarak aydınlatılamamıştır. Bu nedenle, bu çalışmada, Tip 2 DM hastalarında C-peptid düzeyleri ile mikrovasküler komplikasyonların arasındaki ilişkiyi araştırmayı amaçladık. **Gereç ve Yöntemler:** 2014-2018 yılları arasında endokrinoloji ve dahiliye kliniklerinde Tip 2 DM tanısı ile takipli, C-peptid seviyeleri analiz edilen, normal böbrek fonksiyonları (glomerüler filtrasyon hızı >60 mL/dak olan) ve insülin sekretagog türevi oral antidiyabetik kullanmayan (sülfonilüre vb.) 18 yaş üstü hastalar çalışmaya dâhil edildi. Oral ilaçlarını almamış veya insulin yapmamış olan hastaların 12 saat açlık sonrası serum örnekleri alınarak hemogram, hemoglobin A1c (HbA1c), lipid, glukoz, C-peptid parametreleri çalışıldı. Çalışmaya katılan hastalar, spot idrar albumin/kreatinin oranına göre 3 gruba ayrıldı. Proteinürisi olmayanlar grup 1, mikroalbuminürisi olanlar grup 2, makroalbuminürisi olanlar grup 3 olarak adlandırıldı. **Bulgular:** CPI düzeyinde gruplar arasında istatistiksel olarak anlamlı fark bulundu (p<0,001). Grup 2, 3'ün CPI düzeyleri grup 1'den daha düşüktü (p=0,007 ve p<0,001). Ayrıca, grup 3'ün CPI düzeyi de grup 2'den düşüktü (p=0,015). CPI ile proteinüri varlığı arasında ters bir ilişki bulduk. HbA1c'nin proteinüri ile pozitif ilişkili olduğunu saptadık (p<0,001). **Sonuç:** Bu çalışma, Tip 2 DM hastalarında C-peptid, CPI ve diyabetik nefropati arasındaki ilişkiye ışık tutmaktadır.

Anahtar kelimeler: C-peptid indeks; diabetes mellitus; diyabetik nefropati

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Introduction

C-Peptide levels are generally evaluated in clinical practice as a marker for the demonstration of beta-cell reserves. Recent studies have emphasized the role of C-peptide as an active hormonal molecule (1). It plays an important role in blood glucose regulation by activation of many intracellular pathways. Inflammation and intracellular reactive oxygen molecules may cause the progression of diabetic microvascular complications. C-peptide exerts a cytoprotective effect against oxidative damage and inflammation induced by hyperglycemia (2). For instance, type 1 DM patients have a negative correlation between diabetic nephropathy and C-peptide levels (3). On the other hand, the relationship between C-peptide level and microvascular or macrovascular complications has not been clearly elucidated in non-insulin-dependent (type 2) DM patients (4-6).

Previous studies assert that C-peptide levels may be associated with microvascular complications in patients with type 2 DM. C-peptide levels can be affected by fasting. For that reason, the C-peptide index (CPI) may prove to be a useful indicator of beta-cell function. Previous studies have shown that CPI is a more reliable marker in demonstrating beta-cell reserves (7). The function of CPI as a more valuable marker for demonstration of microvascular complications is not yet clear. The authors assume that CPI may be associated with microvascular complications in type 2 DM patients. Therefore, this study was performed with an aim to determine the relationship between CPI and diabetic nephropathy in type 2 DM patients.

Material and Methods

The study is in accordance with the patient rights regulation of the Helsinki Declaration and has been approved by the Scientific Research Evaluation Commission of Ankara Numune Training and Research Hospital on 25/05/2018 (decision number 1990/2018).

Patient Selection

This retrospective study was performed at Ankara Numune Training and Research Hospital. A total of 359 type 2 DM patients who had C-peptide levels measured in the Endocrine and Internal Medicine clinics between 2014-2018 were enrolled in the study. Patients with a history of malignancy, infectious disease, chronic kidney disease and those using insulin secretagogue (such as sulfonylurea) were excluded from the study.

Parameters in the Study

Blood samples were collected from the antecubital vein after fasting for at least 12 h and without the consumption of any drug or insulin injection. All biochemical parameters were analyzed from the same serum sample. Laboratory parameters were recorded in the electronic files of each patient.

Glucose was measured using the enzymatic UV Hexokinase method in Beckman Coulter AU 5800 (Beckman Coulter Inc., USA) autoanalyzer. Spot urine protein and microalbumin were measured via the turbidimetric method using Hitachi Modular P800 (Roche Diagnostic Corp., Indiana, Indianapolis, USA) autoanalyzer. Hemogram parameters were measured via the hematology analyzer Sysmex XE 2100 (Roche Diagnostic Corp., Indiana, Indianapolis, USA). HbA1c was measured via an automated glycohemoglobin analyzer (Tosoh HLC-723 7; Tosoh co., Tokyo, Japan) using a high-performance liquid chromatography method. Plasma C-peptide was assessed by the radioimmunoassay method (Coat-count RIA kit, Diagnostic Products Corporation, Los Angeles, California, USA).

C-peptide index (CPI): Fasting C-peptide as ng/mL and Fasting blood glucose as mg/dL×100.

Patients were classified into three groups according to spot urine albumin/creatinine ratio.

The value of the albumin-creatinine ratio in the spot urine <30 mg: normal, defined as Group 1; 30 to 300 mg: microalbuminuria, defined as Group 2, >300 mg: macroalbuminuria, defined as Group 3.

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) 22.0 (IBM SPSS Inc., Chicago, IL) software. Kolmogorov-Smirnov test was utilized to determine the distribution of data. Normally distributed numerical variables are expressed as mean±standard deviation,

while non-normally distributed variables are indicated as median (25^{th}-75^{th} percentile). Continuous variables were compared using independent sample t-test, ANOVA or Mann Whitney U test, and Kruskal Wallis H test, as appropriate. A comparison of categorical variables was done with the chi-square test. P<0.05 values were considered statistically significant. Logistic regression was used to determine the odds ratio (OR) for the independent predictors. A stepwise multiple logistic regression analysis was performed to identify independent determinants of proteinuria. Since C-peptide measurements were not normally distributed, the logarithmic transformation was applied in multivariate linear regression analysis.

Results

The demographic characteristics and laboratory findings of type 2 DM patients are summarized in Table 1. A statistically significant difference was noted between the age of patients in the three groups (p=0.014); the age of patients in Groups 2 and 3 was lower than that in Group 1 (p=0.004 and p=0.035). No statistically significant difference was observed between the other groups (p>0.05).

A statistically significant difference was found between the gender of patients in the groups (p=0.018); the number of women in Groups 2 and 3 was lower than that in Group 1 (p=0.049 and p=0.031).

No statistically significant difference was observed in the median DM duration and C-peptide between groups (p>0.05). A statistically significant difference was noted in the CPI level between the groups (p<0.001); the CPI level of Groups 2 and 3 was lower than that of Group 1 (p=0.007 and p<0.001). In addition, the CPI level of Group 3 was significantly lower than that of Group 2 (p=0.015).

There was a statistically significant difference between the groups in terms of HbA1c levels (p<0.001); the HbA1c levels of Groups 2 and 3 were higher than that of Group 1 (p<0.001 and p<0.001). In addition, the HbA1c level of Group 3 was higher than that of Group 2 (p=0.04).

C-Peptide and CPI were compared with proteinuria using univariate logistic regression analysis (Table 2) and it was observed that C-peptide was not statistically significant in predicting proteinuria (p=0.112) and no association between C-peptide and proteinuria was observed (Odds ratio=0.898 (95% Confidence Interval: 0.787-1.025)). Instead, a statistically significant and inverse association between the CPI and proteinuria (Odds ratio=0.707 (95% confidence interval: 0.577-0.866) and p<0.001) was expressed. The multivariate regression model which included findings related to proteinuria (CPI, age, sex, DM duration, and HbA1c) showed that CPI did not predict proteinuria in a statistically significant form (p>0.05) (Table 3).

Discussion

This study highlights three important results. First, it was found that CPI was lower

Table 1. Demographic and clinical features of groups in Type 2 DM.

	Group 1 No Proteinuria (n=214)	Group 2 Microalbuminuria (n=102)	Group 3 Macroalbuminuria (n=43)	p-value
Age (year)	55.0 (46.0-61.0)[a,b]	50.0 (39.0-57.0)[a]	46.0 (37.7-59.0)[b]	0.014[†]
Gender, n (%)				0.018[‡]
Male	86 (%40.2)[a,b]	53 (%52.0)[a]	26 (%60.4)[b]	
Female	128 (%59.8)[a,b]	49 (%48.0)[a]	17 (%39.6)[b]	
DM duration (year)	5 (2-10)	5 (2-8)	8 (4-15)	0.236[†]
C-peptide (ng/mL)	2.2 (1.5-3,4)	2.0 (1.3-3.4)	1.7 (1.1-2.7)	0.125[†]
C-peptide index	1.5 (0.8-2.5)[a,b]	1.2 (0.6-1.8)[a,c]	0.8 (0.5-1.1)[b,c]	<0.001[†]
HbA1c (%)	7.6 (6.5-10.0)[a,b]	9.3 (7.2-12.0)[a,c]	10.0 (8.8-12.3)[b,c]	<0.001[†]

Descriptive statistics; median (25^{th}-75^{th}) percentile for continuous numerical variables, and number of cases (%) for categorical variables, [†]Kruskal Wallis test, [‡]Likelihood Ratio test, [a]: The difference between Group 1 vs. Group 2 (p<0.05), [b]: The difference between Group 1 vs. Group 3 (p<0.05), [c]: The difference between Group 2 vs. Group 3 (p<0. 05).

Table 2. Univariate logistic regression analysis with C-peptide parameters.

	No Proteinuria (n=214)	Proteinuria (n=145)	p-value	Odds ratio (%95 Confidence interval)
C-peptide (ng/mL)	2.2 (1.5-3.4)	1.9 (1.2-3.0)	0.112	0.898 (0.787-1.025)
C-peptide index	1.5 (0.8-2.5)	1.0 (0.6-1.6)	<0.001	0.707 (0.577-0.866)

Table 3. Investigation of the effect of CPI measurements in Group 2 and Group 3 by multivariate logistic regression analysis.

	Odds ratio	%95 Confident Interval		Wald	p-value
		Lower bound	Upper bound		
Microalbuminuria (Group 2)					
Age	0.997	0.972	1.024	0.039	0.844
Male factor	1.142	0.612	2.130	0.175	0.676
DM duration	0.943	0.889	1.000	3.782	0.052
HbA1c	1.283	1.108	1.484	11.155	<0.001
C-peptide index	0.920	0.667	1.269	0.261	0.610
Macroalbuminuria (Group 3)					
Age	0.951	0.907	0.998	4.138	0.042
Male factor	1.333	0.487	3.644	0.313	0.576
DM duration	1.039	0.963	1.120	0.977	0.323
HbA1c	1.273	1.023	1.582	4.702	0.030
C-peptide index	0.543	0.259	1.140	2.605	0.107

and HbA1c was higher in patients with proteinuria. In the second place, a statistically significant and inverse association between the CPI and proteinuria was observed. Since CPI is altered depending on fasting, it has been assumed to be a more precious marker. At the third place, it was found that HbA1c is the most important factor in the relationship with proteinuria.

C-peptide levels may also have direct molecular effects, which act as protective factors against diabetic microvascular and macrovascular complications. In light of recent studies, C-peptide has been shown to inhibit the formation of endothelial cell reactive oxygen species (8,9). The C-peptide also downregulates adhesion molecules on leukocytic cells. Furthermore, it prevents atherosclerotic plaque formation (10).

Besides, since glucose itself is a great stimulant for pancreatic cells, insulin secretion is increased by higher glucose levels. For that reason, the C-peptide level may also be higher than normal (11). Therefore, for the assessment of beta-cell function, the level of C-peptide should be adjusted by glucose (7). CPI is obtained by dividing the C-peptide level by fasting blood glucose. A recent study pointed out that CPI and beta-cell function were more closely associated with insulin requirements, though its association with microvascular complications could not be clarified completely (12,13).

Type 2 is the most common type of diabetes in adults (14). In the past, insulin resistance was accepted as the main problem in the pathogenesis of type 2 DM. However, Butler et al. established that beta-cell reserve decreases in both, obese and non-obese type 2 DM patients (15). Nowadays, the lower beta-cell reserve is considered to play a role in the etiopathogenesis of both, type 1 and type 2 diabetes (16). Therefore, evaluation of beta-cell function is important in either type of diabetes.

Several studies have investigated the relationship between C-peptide levels and diabetic complications in type 2 DM patients. The low C-peptide level has been associated with the progression of diabetic microangiopathy (4). Shin et al. showed that C-peptide measurement using glucagon

stimulation test has a negative correlation with albuminuria and diabetes duration (6). Several recent studies have reported that there is an important link between low C-peptide levels and diabetic nephropathy (5). Moreover, C-peptide has been shown to exert protective effects against renal impairment, which was demonstrated in patients with combined renal-pancreatic islet transplantation. The survival rate of renal allograft was found to be increased in patients with successful islet cell transplantation (17).

Lower levels of C-peptide and decreased beta-cell reserve have been correlated to greater levels of glucose variability (18). Moreover, glucose variability is known to be associated with diabetic complications (19). It is, therefore, possible that C-peptide may be a predictor for diabetic complications.

In fact, it has long been known that the HbA1c level is a manifestation of blood glucose regulation. It can be assumed that patients with higher HbA1c levels also have more glucose toxicity than patients with lower HbA1c levels. For that reason, higher HbA1c levels have an increased risk of nephropathy.

On the other hand, some studies have shown that there is no relationship between the C-peptide level and diabetic microvasculopathy. Klein et al. demonstrated that there was no link between C-peptide level and the incidence or progression of diabetic retinopathy (20). Chowta et al. found that serum C-peptide level has a weak correlation with microalbuminuria and creatinine clearance (21).

Conclusion

In conclusion, a reverse relationship between CPI and nephropathy was observed. However, HbA1c level is a more valuable marker than others, for the prediction of diabetic microvascular complications like nephropathy. However, further studies with longer observation periods are needed to address this issue.

The limitations of the study include the retrospective design and the fact that it was performed in a single center. Also, the results could not be generalized to the common population. There is a limitation in cross-sectional studies especially in terms of causality, and randomized controlled studies are required to overcome this weakness. Patients' history relating to drugs, duration of diabetes, treatment regimens, history of chronic diseases were recorded from the file and there may be missing or incorrect data.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee that Ankara Numune Training and Research Hospital Scientific Studies Evaluation Commission on the date of 25/05/2018 with the decision number 1990/2018 and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Bilal Katipoğlu, Dilek Berker; Design: Bilal Katipoğlu, Mustafa Çomoğlu, İhsan Ateş; Control/Supervision: Nisbet Yılmaz, Dilek Berker, Mustafa Çomoğlu; Data Collection and/or Processing: Bilal Katipoğlu, Mustafa Çomoğlu, İhsan Ateş, Nisbet Yılmaz; Analysis and/or Interpretation: Bilal Katipoğlu, İhsan Ateş, Dilek Berker; Literature Review: Bilal Katipoğlu, Mustafa Çomoğlu; Writing the Article: Bilal Katipoğlu, İhsan Ateş; Critical Review: Nisbet Yılmaz, Dilek Berker; References and Fundings: Nisbet Yılmaz, Dilek Berker; Materials: Bilal Katipoğlu, Mustafa Çomoğlu.

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