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ARAŞTIRMA / RESEARCH

Association between C-peptide level and microalbuminuria in patients with type 2 diabetes mellitus

Tip 2 diyabetes mellituslu hastalarda C peptid ile mikroalbuminüri ilişkisi

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Abstract

Purpose: Latest studies suggest that C-peptide may have a beneficial biological role on diabetic nephropathy. The aim of this study is to analyze whether there is an association between serum C-peptide level and microalbuminuria in type 2 diabetes mellitus (T2DM).

Materials and Methods: We enrolled 184 T2DM patents and 46 healthy subjects in this study. Clinical variables and routine biochemical tests along with serum C peptide levels measured after an overnight fasting. Serum C peptide levels between 1.1 and 4.4 accepted as normal. 24-hour-urine samples were investigated and values between 30-300mgwere recorded as microalbuminuria. Pearson correlation analysis were used to determine associations between continuous variables.

Results: C peptide levels were not significantly difference in T2DM patients compared to healthy controls. Serum C peptide levels showed positive correlation with insülin and microalbminuria with the Pearson correlation analysis. However, there was no significant association between other variables and C peptide levels.

Conclusion: A correlation was found between microalbuminuria and serum C-peptide in this present study. Findings suggest C-peptide is related with renal complications of T2DM patients.

Key words: C peptide, microalbuminuria, diabetes

Öz

Amaç: Son dönem çalışmaları C peptitin diyabetik nefropatide yararlı etkileri olduğunu öne sürmektedir. Bu çalışmada tip 2 diyabetes mellitus (T2DM) hastalarında gözlenen mikroalbuminüri ile serum C peptit düzeylerinin ilişkili olup olmadığını analiz etmeyi amaçladık.

Gereç ve Yöntem: Bu amaçla 184 tip 2 diyabet hastası ve 46 sağlıklı kontrol çalışmaya alındı. Hastaların klinik özellikleri ve rutin biyokimyasal tetkiklerine ek olarak serum C peptit seviyeleri kaydedildi. Serum C peptit normal aralığı 1.1-4.4 olarak kabul edildi. Hastalardan 24 saatlik idrar toplanarak araştırıldı ve 30-300 mg aralığı mikroalbuminuri olarak kaydedildi. Değişkenler arasındaki ilişkiyi ortaya koymak amacıyla Pearson korelasyon analizi kullanıldı.

Bulgular: Tip 2 diyabet hastaları sağlıklı control grubu ile karşılaştırıldığında C peptit düzeyleri arasında anlamlı farklılık saptanmadı. Pearson korelasyon analizinde C peptit düzeyleri, insulin ve mikroalbuminuri arasında pozitif korelasyon olduğu görüldü. Bununla birlikte C – peptit ve diğer değişkenler arasında anlamlı bir ilişki bulunmadı.

Sonuç: Bu çalışmada serum C peptit düzeyleri ile mikroalbuminüri varlığının ilişkili olduğunu gösterdik. Bulgular C peptitin T2DM hastalarında renal komplikasyonları ile ilişkili olduğunu ortaya koymaktadır.

Anahtar kelimeler: C peptit, mikroalbuminüri, diyabet

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a heterogeneous disease characterized by insulin resistance in various degrees and impaired insulin secretion and glucose production. Different genetic and metabolic defects in the insulin effect and/or secretion cause hyperglycemia in T2DM[1]. C peptide reflects pancreatic B-cell (endogenous insulin) reserve and is secreted together with insulin. C-peptide molecules,

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circulating together with insulin molecules are not caught and degraded by hepatocytes and peripheral tissue cells and are removed from the circulation mediated by kidneys in a great extent[2,3]. C-peptide may stimulate certain intracellular processes that affect renal functions in patients with type 1 DM and C-peptide deficiency. C-peptide is seen to have the capacity of decreasing urinary glomerular hyperfiltration and urinary albumin excretion in both actual type 1 DM and experimentaly induced diabetes[4,5]. The aim of this study is to analyze whether there is an association between serum C-peptide level and microalbuminuria in T2DM.

MATERIALS AND METHODS

Study population

184 T2DM patients who had admitted to our department between January and June 2016 and 46 healthy subjects were enrolled in this study.The presence of diabetes was determined by: a previous diagnosis of T2DM; random plasma glucose levels of 200 mg/dl or higher; together with the classical features of DM, such as polyuria, polydipsia, polyphagia, weight loss; or a fasting blood glucose level of >126 mg/dl higher; or HbA1c level of 6.5%or higher. Exclusion criteria included any of the following conditions: Patients who have a pancreatic disease that affects C-peptide level, acute renal failure and chronic renal failure, acute infection and overt proteinuria. The study protocol was approved by Health Sciences University, GOP Taksim Research and Education Hospital Ethics Committee, Istanbul (42- 2016, October, 19).

Measurements

Blood samples were obtained after overnight fasting.Serum cholesterol, triglyceride, and high-density lipoprotein cholesterol (HDL-C) were measured by enzymatic colorimetric methods with commercially available kits (COBAS 311, Roche Diagnostics GmbH, Mannheim, Germany)and low-density lipoprotein cholesterol C (LDL-C) was calculated according to the Friedewald formula[6]. Serum glucose measures were determined enzymatically using the hexokinase method (Roche Diagnostics GmbH, Mannheim, Germany). Blood HbA1c was determined with a COBAS 311 analyzer using the particle-enhanced immunoturbidimetric method (Roche Diagnostics, Mannheim, Germany).Final results were expressed as a percent of HbA1c of the total hemoglobin according to the protocol of the Diabetes Control and Complications Trial/National Glycohemoglobin Standardization Program (DCCT/NGSP).The insulin and C peptide levels were determined with an electrochemiluminescence immunoassay (Roche Diagnostics,Mannheim, Germany) on an automated Roche Cobas E 411 (Roche Diagnostics).The particle-enhanced immunoturbidimetric method with a Behring Nephelometer BN-100 (Behring Diagnostic, Frankfurt, Germany) was used to measure C-reactive protein (CRP). Creatine clearance wasestimated with MDRD formula[7]. Microalbuminuria was investigated in 24-hour-urine samples, and urine albumin levels lower than 30mg/24 hours accepted as normoalbuminuric.Values between 30-300mg/24 hours were indicative of microalbuminuria, and above 300mg/24 hours revealed macroalbuminuria. The presence of abnormal albuminuria was confirmed in at least two of three consecutive samples.

Statistical analysis

We used the Utah, US statistical software, Number Cruncher Statistical System (NCSS, 2007)&Power Analysis andSample Size (PASS, 2008) for statistical analysis.While analyzing the study data, in the comparison of quantitative data, Student's test was used in the comparison of two groups that exhibited normal distribution, and Mann Whitney U test was used in the comparison of two groups that didn't exhibited normal distribution, as well as descriptive statistical methods (mean, standard deviation, median, frequency rate, minimum, maximum). Associations between continuous variables were analyzed with the Pearson correlation analysis and Spearman's correlation analysis for the variables that were distributed otherwise; p values <0.05 were considered statistically significant.

RESULTS

The clinical and biochemical characteristics of the study subjects are shown in Table 1. A total of 230 participants were included in this study.The average age of diabetic group was 49.8±13 years.The T2DM patients had significantly higher fastingplasma glucose, fasting insulin, HbA1C, microalbuminuria, andCRP levels than the control subjects ($p<$ 0.01).There were no significant differences in the

age, gender, total cholesterol, HDL, LDL, triglyceride, creatinine clearance levels between the T2DM patients and control subjects. C peptide levels were not significantly difference in T2DM patients compared to the healthy controls (3.03±1.4 ng/ml vs. 3.1±1.3 ng/ml,p>0.05).

Serum C peptide levels showed positive correlation with insülin (r= 0.216; p<0.008), and MAU (r=0.184; $p<0.045$) with the Pearson correlation analysis. However, there was no significant association between other variables and C peptide levels (Table 2).

Table 1:Clinical and biochemical characteristics of patients and controls

	Diabetic group(184) Mean±SD./n, %	**Control group(46)** Mean±SD./n, %	p
Age (Years)[a]	49.8±13	48.8±10.7	0.613
Gender			
Male	83(45%)	22(48%)	0.87
Female	101(55%)	24(52%)	
Fasting blood glucose(mg/dL)[b]	155.1±75.9	85.3±11.4	0.00**
HbA1c(%)[a]	7.6±2.2	5.4±0.5	0.00**
C-peptide(ng/ml)[b]	3.03±1.4	3.1±1.3	0.598
Insulin(uIU/ml)[b]	15.7±3.8	5.4±2.1	0.00**
Total cholesterol(mg/dL)[a]	204.9±42.2	212.2±35.9	0.305
Triglyceride(mg/dL)[a]	170.5±90.5	156.3±65.0	0.289
LDL (mg/dL)[a]	125.1±34	128.1±32.1	0.629
HDL (mg/dL)[a]	47.3±11.3	48.2±13.1	0.701
Creatine clearance (ml/min)[b]	131.8±52.6	154.0±74.4	0.169
MAU (mg/l)	79.0±13.8	16.3±5.9	0.00**
CRP(mg/l)[b]	10.1±1.7	2.5±1.0	0.00**

LDL:low-densitylipoprotein, HDL: high-density lipoprotein , MAU: microalbuminuria, CRP: C reactive protein
*p<0.05 , **p<0.001 [a]T test in independent groups [b]Mann-Whitney

Table 2. Relationship between C-peptide and other variables

Variables	**r-value**	**p-value**
Age(years)*	-0.057	0.455
Fasting blood glucose (mg/dL)**	-0.023	0.760
HbA1c(%)*	0.022	0.779
Insulin(uIU/ml)**	0.216	0.008++
Total cholesterol(mg/dL)*	-0.028	0.722
Triglyceride (mg/dL)*	0,14	0.863
LDL (mg/dL)*	-0.096	0.232
HDL (mg/dL)*	0.037	0.639
Creatine clearance (ml/min)**	-0.167	0.184
MAU(mg/dL)**	0.184	0.045+
CRP(mg/l)**	-0.166	0.288

LDL:low-densitylipoprotein, HDL: high-density lipoprotein,MAU: microalbuminuria, CRP: C reactive protein
+p<0.05 , ++p<0.01*Pearson korelasyon**Spearmans korelasyon

DISCUSSION

In this study, we investigated correlations of serum C peptide levels with clinical and biochemical parameters in T2DM patients.We especially evaluated its effect on diabetic nephropathy. Associations of serum C-peptide with creatinine clearance that is a marker of renal functions and microalbuminuria are still intriguing, a positive correlation was found between serum C-peptide and microalbuminuria but no correlation with creatinine clearance was detected in our study. Serum C-peptide has been demonstrated to play role in chronic complications of type 1 DM, especially to reverse diabetic nephropathy, with no effect on controlling blood sugar in studies performed in last decade[8,9]. Therefore, C-peptide is seen as an active peptide, supplementary to insulin that is different

from insulin in terms of its physiological effects[10]. In a study by Chowta et al, weak associations were found between renal functions and duration of diabetes and serum C-peptide. They found negative correlations between serum C-peptide level and creatinine clearance, urinary albumin excretion and urinary albumin/creatinine ratio. The study showed that creatinine clearance and urinary albumin excretion were higher in patients with low levels of C-peptide compared to patients with high levels of C-peptide[11]. Glomerular hyperfiltration frequently develops early in the course of the disease in patients with Type 1 DM. Adequate insulin treatment does not resolve this condition12,13.On the contrary, Friedman et al and Schmitz et al did not observe glomerular hyperfiltration and hypertrophy when insulin and C-peptide levels were in normal ranges or higher than normal in patients with T2DM in two different studies[14,15]. The mechanisms of beneficial effects of C-peptide on renal function in diabetes are not clear yet. Nevertheless, when the renal function is analyzed in animals in which experimental diabetes is produced, it may be thought that C-peptide has a direct effect on glomerular excretion of albumin. Johansson et al performed a double blinded study in patients with type 1 diabetes and measured glomerular hyperfiltration, microalbuminuria, GFR and proteinuria that are all markers of early stages of diabetic nephropathy. They provided a better glycemic control by administering both insulin and C-peptide in patients for 3 months and found decreased glomerular hyperfiltration and microalbuminuria both of which are induced by diabetes[16]. In another study, Johansson et al confirmed that proteinuria was decreased markedly in a group receiving C-peptide[17]. They proved in both studies that C-peptide had positive effects indistinguishable from good glycemic control. However, renal functions of diabetic patients shortly treated with C-peptide and treated with insulin remained in the ame levels[18]. All of those strongly supported the effects of C-peptide on glomerular filtration. Studies performed in experimental conditions have demonstrated that renal functions and metabolic conditions of streptozotocin induced diabetic rats treated with C-peptide were improved and even some metabolic conditions secondary to diabetic nephropathy were reversed[19,20].

C-peptide may affect both the permeability of glomerular membrane and transport, and also the regional blood flow of the kidney. There is an association between improvement of the renal function and glomerular hyperfiltration and decreased urinary albumin excretion in response to C-peptide replacement in patients with Type 1 DM.

The effects of C-peptide in the early phases of diabetic nephropathy have also been studied in humans. The patients were administered subcutaneous C-peptide (600 mmol/day) or placebo in addition to their regular doses of regular insulin in a randomized study in 21 normotensive patients with microalbuminuria. Urinary albumin excretion was seen to be decreased compared to the baseline in patients who received C-peptide and this effect was time-dependent; however, no effects were seen on GFR[18].

A statistically significant negative correlation between C-peptide and HbA1c was detected in this present study. HbA1c will be elevated when C-peptide level is low. C-peptide level is already decreased in the stages following the initiation of T2DM in uncontrolled diabetes and HbA1c will stay high in this case. According to this present study, C-peptide is statistically significantly correlated with insulin. C-peptide and insulin precursor are secreted into the blood from the pancreatic islet cells. Both can be used to evaluate pancreatic beta cells. A marked decrease in insulin is unexpected since insulin resistance remains in the forefront in the initial phase of DM.

The level of CRP, an inflammatory marker was found to be high in diabetic patients in this present study. T2DM is characterized by an inflammation of low activity and CRP is an appropriate marker to measure this inflammatory process. Low degree inflammation has been accepted to play role in the physiopathology of T2DM. High level of CRP has seen to be an independent risk factor for development of T2DM. The risk of development of diabetes was found to be higher in patients with high CRP in Women's Health Study and West of Scotland Coronary Prevention Study[21,22]. On the other hand, CRP concentration was found to be increased in obese individuals resistant to insulin and improvement in insulin resistance and decrease in CRP levels were observed paralel to weight loss in a study performed by McLauglin et al[23].

The effect of C-peptide on lipid metabolism is still unclear. According to the preliminary results of the study performed by Rebsomen et al, one-month infusion of C-peptide had a positive effect on lipids

so that it decreased cholesterol and triglyceride levels in streptozotocin induced rats[24]. On the other hand, C-peptide was demonstrated to decrease adiponectin that is released from human adipocytes in the study by Khammar et al. Thus, the positive effects of C-peptide on lipid metabolism are partially enlightened[25]. However, we detected no statistically significant correlations between C-peptide and total cholesterol, triglyceride, LDL cholesterol and HDL cholesterol.

There are some limitations in this study. First, sample size is relatively small. Second, due to retrospective design of study, treatment data is not known. It is already known that treatment options can variate microalbuminura and lipid parameters. Our results encourages prospective and populational studies.

A correlation was found between microalbuminuria and serum C-peptide in this present study. Studies comprising long-term application of C-peptide will be required in order to establish whether C-peptide has a significant role in the prevention and treatment of diabetic nephropathy. In conclusion, the possible effects of C-peptide on patients with T2DM are underrecognized. One of them might be a positive effect on renal dysfunction in patients with T2DM who have reached the level of insulin deficiency. Accordingly, we suggest that the effects of C-peptide should be more extensively investigated in patients with T2DM.

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