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ARAŞTIRMA / RESEARCH

Serum apelin-36 levels in pre-diabetics and newly diagnosed diabetes mellitus patients

Pre-diyabetik ve yeni tanı almış tip 2 diyabetli hastalarda serum apelin-36 düzeyleri

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Abstract

Purpose: The aim of this study was to investigate the levels of parameters for glucose metabolism and cardiovascular risk factors and apelin-36 in patients grouped as having impaired fasting glucose(IFG), IFG and impaired glucose tolerance(IGT), newly diagnosed type 2 DM and the control group.

Materials and Methods: Fifty-three women and twenty-seven men, totally eighty subjects were enrolled in this study. The patients were classified into four groups according to their oral glucose tolerance test (OGTT) results. Group1: Normoglycemic controls(n:20), Group2: subjects with IFG(n:20), Group3: combined IFG subjects which included both IFG and IGT patients(n:20), Group4: Newly diagnosed type 2 DM patients(n:20). Levels of glucose, lipids, HbA1c, fibrinogen, insulin, cortisol, serum apelin-36 and C-peptide were analyzed.

Results: There was a statistically significant difference regarding the levels of apelin-36 between group 1 and the study groups 2, 3 and 4, respectively. Other parameters analyzed for glucose metabolism and cardiovascular risk factors such as fasting glucose, HbA1c, HOMA-IR, fibrinogen, insulin, cortisol, C-peptide, LDL-cholesterol, HDL-cholesterol, VLDL-cholesterol, triglycerides, total cholesterol were significantly higher in the study groups when compared to the control group.

Conclusion: In the future serum apelin-36 levels can be used as an indicator for presenting the insulin resistance and impairment in glucose metabolism in the early periods.

Keywords: apelin-36, pre-diabetic, diabetes mellitus, cardiovascular risk factors

Öz

Amaç: Bu çalışmada, açlık glukozu (IFG), bozulmuş glukoz toleransı (IGT) ve yeni tanı almış tip 2 DM olarak gruplandırılmış hastalarda; glukoz metabolizması ve kardiyovasküler risk faktörleri parametreleri ve apelin-36 düzeylerini araştırılması amaçlanmıştır..

Gereç ve Yöntem: Elli üç kadın ve yirmi yedi erkek, toplam seksen katılımcı bu çalışmaya dahil edildi. Orak glükoz tolerans testi (OGTT) sonuçlarına göre hastalar dört gruba ayrıldı. Grup 1: Normoglisemik kontroller (n: 20), Grup2: IFG'li hastalar (n: 20), Grup3: IFG ve IGT'li hastalar (n: 20), Grup4: Yeni tanı almış tip 2 DM hastaları (n: 20). Glikoz, lipidler, HbA1c, fibrinojen, insülin, kortizol, C-peptid ve Apelin-36 seviyeleri analiz edildi.

Bulgular: Apelin-36 düzeylerinde grup 1 ile çalışma grupları 2,3 ve 4 arasında istatistiksel olarak anlamlı bir fark vardı). Glukoz metabolizması ve kardiyovasküler risk faktörleri için analiz edilen açlık glukozu, HbA1c, HOMA-IR, fibrinojen, insülin, kortizol, C-peptid, LDL-kolesterol, HDL-kolesterol, VLDL-kolesteroll, triglisterit, total kolesterol gibi parametreler çalışma grubu ile kontrol grubu karşılaştırıldığında anlamlı bir fark bulundu

Sonuç: Gelecekte serum apelin-36 düzeylerinin erken dönemlerde glukoz metabolizma bozukluğunu ve insülin direncini göstermek için bir belirteç olarak kullanılabileceğini düşünüyoruz.

Anahtar kelimeler: apelin-36, pre-diyabetik, diabetes mellitus, kardiyovasküler risk faktörleri.

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INTRODUCTION

Pre-diabetes is a condition characterized by insulin resistance and β cell dysfunction. People with pre-diabetes are at risk for developing type 2 DM, and they have no symptoms in 4 to 7 years prior to diagnosis. Complications such as atherosclerosis have been known to develop in this period[1,2]. Atherosclerosis is the initial lesion of coronary artery disease(CAD) so the most important step to prevent the formation of atherosclerosis is pre-diabetes[3].

Adipose tissue is not only a reservoir formed by adipocytes that store fat, but it also affects a large number of physiological processes with secreting hormones[4,5]. One of these hormones is apelin which is an adipocytokine thought to mediate endocrinological functions in fat tissue. It is also a neuropeptide and cardiovascular peptide. Apelin was found in 1998 as a member of the 7-transmembrane G-protein superfamily. G protein is an endogenous ligand of APJ (angiotensin II receptor-like 1bound receptor[6,7]. Apelin is synthesized as a pre-propeptide from 77 amino acids. Apelin-13, apelin-17 and apelin-36 are three different forms of apelin, consisting of different numbers of amino acids. The physiologically active form is thought to be apelin-36[8]. Apelin and APJ are widely exposed in the human body, and they both have functional effects on central nervous system and cardiovascular system (CVS)[8,9]. It has angiogenic role in the CVS. Apelin provides endothelium-dependent vasodilatation through nitric oxide and reduces arterial blood pressure. It shows a diuretic effect by inhibiting vasopressin. Furthermore, apelin exhibits potent and long-acting positive inotropic activity[10].

Increase in apelin levels associated with hyperinsulinemia is seen in obese subjects[11,12]. In adipose cells, apelin expression is strongly inhibited by fasting and after refeeding its expression increases in an insulin-like manner. This information indicates that the use of insulin provides direct control over apelin gene expression in adipocytes[13]. It is known that insulin increases circulating apelin concentrations while glucocorticoids reduce it[14]. Insulin acts directly on adipocytes to stimulate apelin production[15].

It has been reported that plasma apelin levels decrease in people with CAD. Therefore, it is thought that plasma apelin levels may be a diagnostic indicator for CAD[16,17]. In addition, apelin-13 provides angiogenesis and has been shown to regulate cardiac development by providing postmyocardial infarction repair with various mechanisms[18].

When all these situations are taken into consideration; while the regulation of apelin with insulin was increased; the fact that it was decreasing in the CAD prompted us to question the apelin-CAD relationship at different stages of diabetes. We aimed to evaluate the levels of apelin-36 and its relationship with conventional cardiovascular risk factors in pre-diabetics compared to newly diagnosed type 2 DM patients and healthy controls.

MATERIALS AND METHODS

In our study, from the patients whom applied to the internal medicine and endocrinology outpatient clinics, the ones with OGTT request were chosen randomly, and the test was performed due to ADA 2007 criteria. Informed consents were signed by all subjects in this study. Study group was chosen by the estimation of fasting and second hour post glucose load glucose levels, and 4 groups were formed. Group 1 was the control group (n=20) having no disease with fasting glucose levels less than 100mg/dL and Group2 (n=20) included the patients having impaired fasting glucose; that is fasting glucose levels between 100-126mg/dL. Group3 (n=20) named as combined impaired fasting glucose(IFG) which included both impaired glucose tolerance (based on the 2007 ADA diagnostic criteria) patients(IGT) and IFG, Group 4 (n=20) consisted of newly diagnosed type 2 DM patients. Sixty patients and 20 controls were included in the study (20 patient in each group). The mean±SDage of 80 subjects in total was 46±14 years, consisted of 28 males and 52 females. Our exclusion criteria were patients having metabolic diseases like hypo and hyperthyroidism, hepatic, renal, cardiac diseases, pancreatic insufficiency, and chronic infections. Also the patients using medications that affect insulin susceptibility and insulin secretion were excluded from the study. Subjects were enrolled in a nutritional program containing at least 150g of carbohydrate daily for three days prior to OGTT. Our study was planned as a single centered, randomized, open and prospective study.

Blood was drawn into gel containing Vacuette tubes (Greiner Holding AG, Kremsmunster, Austria) after an average of 10 hour night fasting in the morning at 8:00. Then the tubes were centrifuged at 1500g for 10

minutes to separate sera. All the tests were analyzed on the same day except apelin-36 and C-peptide which were kept at -40°C until analysis.

Clinical measurements

In the assessment of the glycemic control, pancreatic β-cell function, cardio metabolic status, the levels of glucose with HbA1c in fasting and postload states, fasting insulin and C-peptide and serum lipid levels (total cholesterol, HDL-C, triglycerides), cortisol, and fibrinogen levels were studied respectively. LDL-C were calculated using Friedwald formula. BMI's were calculated using weight(kg)/height(m^2). Insulin resistance was estimated by the use of HOMA-IR index which uses the formula fasting insulin(mU/L) x fasting glucose(mmol/L)/22.5. Total cholesterol, HDL-Chol, and TG levels were analyzed using CHOD-PAP enzymatic colorimetric method, glucose levels GOD-PAP enzymatic colorimetric method on Roche/Hitachi Modular P autoanalyzer (Roche Diagnostics).

Serum insulin, C-peptide, and cortisol levels were analyzed on the Roche/Hitachi Modular Analytics E170 automated analyzer using the electrochemiluminescence immunoassay (ECLIA) technique. HbA1clevels were analyzed using the Bio-Rad Variant II automated analyzer with HPLC technique (high performance liquid chromatography)(Bio-Rad Laboratories,CA). Plasma fibrinogen levels were measured with Clauss method using Dade Behring fully automatic coagulometer(Dade Behring GmbH, Marburg, Germany).

Apelin-36 measurement

Serum apelin-36 levels were analyzed by enzyme-linked immunosorbent assay (ELISA) method usingapelin-36 ELISA kit (Phoenix Pharmaceuticals, Burlingame, CA USA). Measurable range was between 1.01ng/mL to 100ng/mL and the coefficient of variation was less than 10%.

Statistical analysis

All statistical analyses were performed using SPSS 11.5 (SPSS, Inc., Chicago, IL, USA) and Microsoft Office Excel Software (Microsoft Corp., Redmond, WA). Conformity of continuous variables to Gaussian distribution was tested by Kolmogorov-Smirnov analysis and shown with mean±SD. Data without normal distribution were shown as median (25th percentile- 75th percentile)±SD. Groups having normal distributed parameters were compared using one-way ANOVA analysis. Also for the statistical analysis of groups with equal variances in post-hoc comparisons and groups with unequal variances, Tukey test and Tamhane's T2 test were used, respectively. Kruskall-Wallis test was used to compare groups with abnormal distributions; Mann-Whitney U test was performed in the binary comparisons after multiple comparisons, and the statistical significance was evaluated at the level of $p<0.05$. Chi-square test was performed to compare expected and observed values. Pearson(r) and Spearman(rs) correlation coefficients were used in evaluating inter-variable correlations. Statistical significance was evaluated at $p<0.05$ (two-tailed-bidirectional) level.

RESULTS

There was no statistically significant difference between group2, group3, and group4 for age, BMI (body mass index), waist circumference, and hip circumference (Table 1).

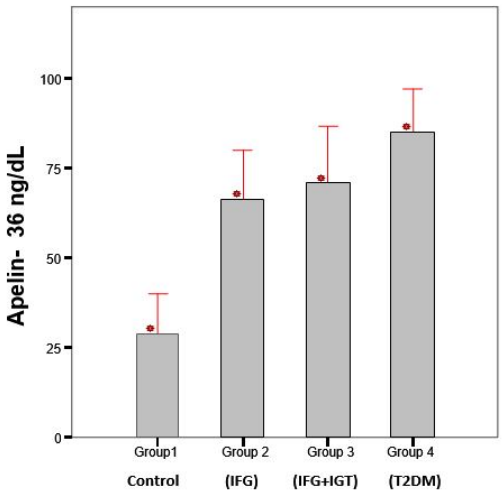

Figure 1. Change of serum apelin-36 levels in four groups (± SD)

When the correlations of apelin-36 with other parameters were examined in the control group, there was moderate positive correlation with fasting glucose (r:0.451, p=0.046), a positive poor correlation with LDL-C (r:0,262, p=0,265), a negative poor correlation with HbA1c (r:-0,272;

p=0,247) and again a negative poor correlation with waist circumference (R:-0,342; p=0,140) and negative correlation with hip circumference (r:0,395; p=0.094)

There was no correlation between the subject groups regarding serum apelin-36 levels and BMI, waist circumference, hip circumference measurements, serum insulin, total cholesterol, and cortisol levels. In group 2, there was a moderate correlation between serum apelin-36 and HbA1c (r:0.404, p=0.077).

In group 3, serum apelin-36 levels were positively and moderately correlated with C-peptide (r:0.414, p=0.070), LDL-C (r: 0,434) and showed negatively good correlation with insulin/C-peptide ratio (r:-0.584, p=0.007) In group 4, apelin-36 showed positive moderate correlation with HDL-C (r:0,439, p=0,053) and negative poor correlation with VLDL, TG and fibrinogen (r:0,35, r:0,358, r:-0,358 respectively). The relationship between the parameters studied in between the groups is given (Table 2).

Table 1. Demographic characteristics of groups

Variable (mean ± SD)	Group1 (n=20)	Group2 (n=20)	Group 1-2	Group3 (n=20)	Group 1-3	Group4 (n=20)	Group 1-4
Age (Year)	41.7 ± 7.5	48.9 ± 9.3	P<0.05	51.1 ± 7.2	P<0.01	48.5 ± 7.0	P<0.05
Gender (M/F)	9/11	9/11	p>0.05	5/15	p>0.05	5/15	p>0.05
Bmi (Kg/M²)	24.0 ± 3.1	30.7 ± 4.9	P<0.0001	30.4 ± 4.1	P<0.0001	31.7 ± 2.9	P<0.0001
Waist (cm) Circumference	86.6 ± 11.3	103.5 ±16.2	P<0.0001	100.1 ± 10.4	P<0.01	101.2 ± 8.9	P<0.001
Hip (cm) Circumference	99 (92.5-105.0)	110 (103.5-117.0)	P<0.01	108 (105.0-116.0)	P<0.01	110 (104.0-120.0)	P<0.01

Table 2. Results and the group comparisons for the tests analyzed.

Variable (mean ± SD)	Group1(n=20)	Group2(n=20)	Group1-2	Group3(n=20)	Group1-3	Group4(n=20)	Group1-4
FG (mg/dL)	87.5 ± 7.7	108.8 ± 6.1	P<0.0001	111.9 ± 7.9	P<0.0001	126.2 ± 14.0	P<0.0001
Insulin (µU/mL)	6.8 ± 2.4	13.5 ± 6.5	P=0.011	14.7 ± 5.6	P=0.002	19.5 ± 9.8	P<0.0001
C-Peptid (ng/mL)	2.0 ± 0.5*	3.0 ± 1.0	P=0.008	3.1 ± 0.9	P=0.004	3.6 ± 1.4	P<0.0001
Insulin/C-Peptid	3.4 ± 1.0	4.2 ± 0.8	p>0.05	4.5 ± 1.2	P=0.004	5.1 ± 1.0	P<0.0001
HbA1c	5.4 ± 0.3	6.0 ± 0.4	P<0.0001	6.0 ± 0.4	P<0.0001	6.5 ± 0.5	P<0.0001
Total Cholesterol (mg/dL)	176.0 ± 18.0	220.0 ± 36.0	p>0.05	221.0 ± 37.7	p>0.05	201.8 ± 38.4	p>0.05
HDL-C(mg/dL)	54.0 ± 12.4	47.4 ± 9.4	P=0.038	44.3 ± 13.1	P=0.014	42.9 ± 9.1	p>0.05
LDL-C (mg/dL)	103.2 ± 17.4	141.7 ± 31.5	P<0.001	135.5 ± 33.8	P=0.006	125.0 ± 34.4	p>0.05
VLDL-C (mg/dL)	17.0 (11.2-23.0)	32.0 (19.5- 45.0)	P<0.0001	37.5 (30.0-63.0)	P<0.0001	32.5 (24.5-39.7)	P<0.0001
TG (mg/dL)	82.5 (56.7-112.2)	149.5 (89.2-222.2)	P<0.0001	186.5 (150.2-313.2)	P<0.0001	161.5 (123.7-198.5)	P<0.0001
Cortisol (µg/dL)	13.8 ± 3.7	14.3 ± 6.2	p>0.05	15.6 ± 4.0	p>0.05	16.7 ± 5.8	p>0.05
HOMA-IR	1.4 ± 0.5	3.6 ± 1.7	P=0.005	4.1 ± 1.6	P<0.0001	6.0 ± 3.1	P<0.0001
Fibrinogen (mg/dL)	322.6 ± 54.9	450.7 ± 50.5	P<0.0001	435.6 ± 102.4	P=0.011	478.6 ± 129.0	P<0.0001
Apelin-36 (ng/mL)	28.7 ± 20.3	66.5 ± 24.7	P<0.0001	70.9 ± 28.5	P<0.0001	85.2 ± 21.5	P<0.0001

FG: Fasting Blood Glucose, HDL-C (High Density Lipoprotein-Cholesterol), LDL-C (Low Density Lipoprotein-Cholesterol), VLDL (Very Low Density Lipoprotein-Cholesterol), TG (Triglyceride), HOMA-IR (Homeostasis Model Assessment-Insulin Resistance)

DISCUSSION

The International Committee of Experts on Diabetes, reported that individuals with HbA1c levels in the range of 5.7-6.4% (39-46mmol/mol) are at high risk for diabetes and should be included in routine protection programs. Changes in the amount of adipokines secreted by fat tissue are thought to be directly related and play a role in the pathogenesis of hyperlipidemia, insulin resistance, type 2 diabetes and CAD[5,19].

In our study we evaluated the levels of apelin-36 as one of the new adipokines and found that apelin-36 levels were statistically significantly higher in patient groups compared to the control group. There was a statistically significant difference in apelin-36 levels between Group1 and study groups2, 3, and 4 ($p=0.005$, $p<0.0001$, $p<0.0001$, respectively). These findings suggest that apelin-36, an adipokine released from the fat tissue, may have an endocrine role in the development of type 2 DM.

In a study conducted by Boucher et al. in 2005, plasma insulin and apelin levels of obese non-diabetic patients were found to be significantly higher than the control group and they suggested that insulin affects the blood concentration of apelin-36[14]. In a study conducted in 2010 by Ziora et al. showed the decrease of apelin-36 and apelin-12 levels in the anorexia nervosa patients compared to the control group and they suggested that this decrease is due to the reduction of fat tissue in anorexia nervosa patients. They intensified this finding by showing the increase of these peptides in obese patients where there was an excessive increase in fat tissue[20]. In our study, we found no correlation between apelin-36 levels and fasting blood glucose, HOMA-IR, BMI, and LDL-C. However, we observed that serum apelin-36 levels increased in parallel with the increase in BMI when compared to the control group. Unlike our findings, Li et al. found that serum apelin levels correlated positively with fasting blood glucose, HOMA-IR, BMI, and LDL-C. in their study[21].

We found that the HbA1c levels in our study were significantly higher when compared to the control group. We also found positive correlations between HbA1c and serum apelin-36 levels in group 2. We can conclude that apelin-36 levels could reflect the changes in glucose metabolism in the pre-diabetic period. In our study, %50.1 of impaired glucose tolerance patients were under 50 years of age, and %29.8 of them were between 20 and 39 years of age. These young patients have long, high risky periods of life[22].

Tai ES et al. performed a study in a large patient group to evaluate the changes of IFG prevalence, cardiovascular disease, and diabetes risk after setting the IFG base level at 100mg/dL by ADA 2004 criteria. In their study, 63.5% of the patients in the pre-diabetic IFG group had hypertension, central obesity, triglyceride elevation[23].

Atherosclerosis progresses more rapidly in diabetics. In "Baltimore Longitudinal Study on Aging", 937 non-diabetic individuals were followed for 9.5 years. In this study it was found that, IGT or IFG + IGT had more atherogenic risk factors compared to those with normal glucose tolerance and a marked increase in coronary heart disease. Overall mortality was found to increase with elevation of fasting blood glucose from 110mg/dL to 120mg/dL[24]. We also found the LDL-C values of group2 and group3 significantly higher when compared to the control group and also found that HDL-C levels significantly lower in group3 and group4 compared to the control group. We also found that TG levels were significantly higher in all groups than in the control group. We interpreted this as a warning that effective protective measures should be taken before developing CAD in the pre-diabetic period.

C-peptide levels, a peripheral indicator of insulin secretion, is a parameter that accurately indicates the secretion rate even in unstable conditions[25]. We found a significant positive correlation between apelin-36 and C-peptide levels in group3. These findings suggest that apelin-36 is involved in the development of insulin resistance and type 2 diabetes. Cavallo et al. in a large-scale study which was conducted in 2012, compared patients with Type-1 diabetes, Type-2 diabetes, and non-diabetic control group and found that patients with Type 2 diabetes had higher circulating apelin levels independent of metabolic changes and the presence of obesity[26].

There are different results in the apelin related studies. For example, just similar to our findings Li et al. also indicated that serum apelin levels increased in diabetics or glucose intolerant patients[21]. Unlike our findings, Erdem et al. found that serum apelin levels were low in newly diagnosed type 2 diabetic patients[27]. In the following years, these findings need to be verified with new studies and higher number of cases.

Danesh et al. have shown in their meta-analysis of

epidemiological studies that levels of plasma fibrinogen is a major risk factor and independent marker for cardiovascular diseases and nonvascular mortality in health individuals[28]. In our study we found that plasma fibrinogen levels were significantly higher in all groups when compared to the control group. The Northwick Park Heart Study indicated that one standard deviation of rise in plasma fibrinogen levels increased the likelihood of an ischemic heart disease by 84% in one year[29]. In addition to this we found moderate negative correlation between plasma fibrinogen levels and serum apelin-36 levels in group4. We have interpreted these low levels of apelin-36 as a finding of risk factor for CAD.

Ashley et al. studied on the mouse model in which they investigated the hemodynamic effects of apelin. They found that myocardial contractility increased after acute and chronic (over 2 weeks) administration of apelin. This study is remarkable as the first study to investigate the chronic effects of apelin on CVS and indicated that apelin-36 could be used as a treatment agent in heart failure[30].

Chong et al. and Ellinor et al. found that serum apelin levels decreased in patients with chronic heart failure and patients with isolated atrial fibrillation, respectively[31,32]. Simpkin et al. investigated the cardio-protective effects of apelin, and they proposed that apelin could be given at pharmacological doses following infarction[33].

Taşçı et al. assessed serum apelin levels before and after treatment in patients with isolated hypercholesterolemia and found that serum apelin levels increased when LDL-C dropped to the target value depending on treatment[34]. When LDL-C levels were around the targeted values, apelin might also be normal to have a cardio-protective role. We think that the mechanisms related to this regulation should be supported by further studies, because they are not fully understood yet. Gourdy et al. found that the administration of apelin-13 in overweight healthy men increased insulin sensitivity. This study suggests that the apelin/APJ pathway could be an alternative option to control insulin resistance in type 2 diabetics[35].

Our limitations in this study were the selection of narrow patient and control groups which we think that they limited the comparison of absolute results. We conclude that apelin-36 may affect glucose metabolism and it may be a useful marker in combination with other parameters to prevent complications of diabetes in the pre-diabetes state. Also it may be an indicator for presenting the insulin resistance and impairment in glucose metabolism in the early periods. It may also have a probable potential role to use in the treatment of type 2 DM in the future. As a result, diabetes and its complications can be prevented by early detection of pre-diabetic cases.

Yazar Katkıları: Çalışma konsepti/Tasarımı: ZMK, İD, ES; Veri toplama: ZMK, İD, ÖS; Veri analizi ve yorumlama: ZMK, ES, İD; Yazı taslağı: ZMK, ES; İçeriğin eleştirel incelenmesi: ZMK, ES; Son onay ve sorumluluk: ZMK, ES, İD, ÖS; Teknik ve malzeme desteği: ZMK, İD; Süpervizyon: ZMK, ÖS, ES; Fon sağlama (mevcut ise): yok.
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