İzmir Eğitim ve Araştırma Hastanesi Tıp Dergisi (Medical Journal of İzmir Hospital) 20 (3): 95-10, 2016

THE EXAMINATION OF THE RELATION BETWEEN PLASMA UROTENSIN II; HS-CRP; BODY FAT PERCENTAGE AND INSULIN RESISTANCE IN PREDIABETIC PATIENTS

PREDİYABETİK HASTALARDA SERUM UROTENSİN 2, HS-CRP, VÜCUT YAĞ YÜZDESİ VE İNSÜLİN DİRENCİ ARASINDAKİ İLİŞKİNİN İNCELENMESİ

Oktay BİLGİR[1], İbrahim DÖNER[1], Ferda BİLGİR[2], Özden YILDIRIM AKAN[1], Mehmet ÇALAN[1], Murat AKŞİT[3], Arif YÜKSEL[1]

[1] Izmir Bozyaka Training and Research Hospital, Department of Internal Medicine, Bozyaka, Izmir, Turkey.
[2] İzmir Katip Celebi University Medical School, Ataturk Training and Research Hospital, Department of Allergy and Immunology, Izmir, Turkey
[3] Izmir Bozyaka Training and Research Hospital, Department Biochemistry, Bozyaka, Izmir, Turkey.

Keywords: Prediabetes, urotensin 2, hs-CRP
Anahtar Sözcükler: Prediyabet, urotensin 2,hs-CRP

Yazının alınma tarihi: 23.09.2016 Kabul tarihi:30.09.2016 Online basım:30.09.2016

SUMMARY

Introduction: *In our study, the aim was to understand prediabetic pathogenesis or prediabetes by examining the relation between Urotensin II, hs-CRP; body fat index and insulin resistance.*

Material and Methods: *To our trial, 40 control and 40 prediabetic patients, a total of 80 patients were included. Both group's blood samples have been examined for urotensin II level, hsCRP level, insulin resistance and body lipid percentage. Their insulin resistance was calculated by using HOMA-IR and QUICKI methods.*

Results: *In our study, in prediabetic patients, plasma urotensin II level was found to be statistically higher than the patients in control group(p=0.004). A significant relation was found between plasma urotensin II level and insulin resistance indicator HOMA-IR; systolic blood pressure and diastolic blood pressure.With these findings, it was shown that a high urotensin II level could have an important role playing in prediabetic development's basic mechanism, insulin resistance and the development of insulin resistance pathophysiology*

Conclusion: *By determining urotensin II level, prediabetes development could be predicted and with proper life style changes prediabetes could be prevented. Urotensin II and/or agents against its receptor which would be developed in future would demonstrate the ability to prevent or delay prediabetes.*

ÖZ

Giriş: *Araştırmamızda ürotensin II, hs-CRP, Vücut yağ yüzdesi ve insülin direnci arasındaki ilişki incelenerek prediyabet patogenezinin anlaşılması amaçlanmıştır.*

Gereç ve yöntem: *Çalışmaya 40 prediyabetik hasta ile 40 kontrol grubu olmak üzere toplam 80 hasta dahil edildi. Her iki grubun kanları ürotensin II düzeyi, hsCRP düzeyi, insülin direnci ve vücut yağ yüzdesi açılarından çalışıldı. İnsülin direnci HOMA-IR ve QUICKI yöntemleriyle hesaplandı.*

Bulgular: *Araştırmamızda prediyabetik hastalarda serum ürotensin II düzeyi kontrol grubundaki hastalara göre istatistiksel olarak yüksek saptandı(p=0.004). Serum ürotensin II düzeyi ile insülin direnci göstergesi olan HOMA-IR, sistolik kan basıncı ve diyastolik kan basıncı arasında anlamlı bir ilişki saptandı. Bu bulgular ile prediyabetin*

oluşumundaki temel mekanizmanın insülin direnci gelişimi ve insülin direnci gelişimindeki patofizyolojide ürotensin II düzeyi yüksekliğinin önemli bir rol oynuyor olabileceği gösterilmiş oldu.

***Sonuç:** Sonuçta ürotensin II düzeyi ölçümü ile prediyabetin gelişiminin öngürelebileceği ve uygun yaşam tarzı değişikliği ile prediyabet oluşumunun önlenebileceği, ürotensin II ve/veya reseptörüne karşı geliştirilebilecek ajanlar ile prediyabet oluşumunun önlenebileceği veya geciktirilebileceği gösterildi.*

INTRODUCTION

Diabetes is a chronic metabolic disease, presenting itself with blood glucose level elevations, in correspondence with the dysfunction of insulin release and/or tissue response to insulin. In patients, before the development of diabetes there is a pre-diabetes stage (impaired fasting glucose and impaired glucose tolerance). In patients who developed pre-diabetes, it was shown that diabetes could develop in their future life with a 50% probability. In pre-diabetes stage, in these patients, the development of diabetes could be prevented or delayed with life style changes and pharmacological treatment (1,2).

Insulin resistance is the most important path physiological process in pre-diabetes development (3-5). For pre-diabetic patients, besides the risk of the development of diabetes, there are also cardiovascular risks(6-8). For that reason, cardiovascular risk factors should be treated aggressively and these patients should change their life style. In the previous clinical trials, hs-CRP level was observed high to be related with prediabetic patients and this has become an important reason to design this trial. Meanwhile, urotensin II, a stimulator peptid, play a role with the effects of vasculoprotective and vasculopathic at the modulation of cardiovascular function(9-15).

In our study, our aim was to examine the association between urotensin II, hs-CRP, body fat index and insulin resistance and to prevent or delay the onset of diabetes by early diagnosis and treatment. By this, besides lowering the high morbidity and mortality rates in our country and the globe, to decrease the high amount of money spent for these patients was also the target.

MATERIAL AND METHODS

In 2015 between February and March, the patients, who applied to Internal medicine policlinics and newly diagnosed as pre-diabetics, were included into this trial by being informed about this trial. Forty people for the patient group and 40 people for the control group were included into this trial voluntarily. The trial was planned as a single center, prospective trial.

The age interval of the patients included to the trial was between 20 and 73. Patients who have one of more than one of the following conditions that can affect the metabolic parameters, were excluded from the study: Pregnancy, obvious diabetes mellitus, having story of using agents affecting insulin resistance (metformin, glitazon) or with the history of using these agents for the previous 6 months, regular exercise, having a disease that can cause insulin resistance (such as hypothyroidism), cigarette and alcohol use, having a diagnosis of a malignancy, story of steroid use or still using steroids, history of using drugs affecting immune system (such as azathiopurine, cyclosporine A, cyclophosphamide, quinine, TNF-alpha blockers), acute/chronic renal failure, acute/chronic liver failure.

As control group, normoglycemic 40 healthy patients were included into the study. To assess both the study group and the control group's glycemic condition, besides the fasting and postprandial glucose, also HbA1c levels, fasting serum insulin for pancreatic β cell function assessment, serum lipid levels (total cholesterol, HDL, LDL, Triglyceride) and liver and kidney function tests (ALT, AST, urea, creatinine) as metabolic parameters were measured.

The entire study group's demographic information was registered. The weight and visceral lipid percentage measurement of the patients were performed with Omron BF510 device by working with bioelectrical impedance method. (On the device's prospectus visceral fat percentage was defined as normal in between % 1-9; defined as high if the ratio is between %10-14 and as very high if between percent 15-30).

Insulin resistance, fasting insulin and fasting glucose levels were calculated with HOMA-IR

(Homeostasis model assessment-insulin resistance) and QUICKI (Quantitative Insulin Sensitivity Check Index) formulations as below.

hs-CRP

Serum hs-CRP levels were done with sandwich ELISA method by using a commercial kit (DIAsource, Louvain-la-Neuve, Belgium). Spectrophotometric measurement was done with 450 nm wave length Thermo Scientific Multiscan Go model ELISA reader (Finland). HS-CRP concentrations were determined by using Standard curves drawn by using diluted standard absorbance. The results were defined as ng/L.

Urotensin

After an average of 10 hours fasting, venous blood samples of the patient and control groups were taken into vacuum 8 mL tubes and gel separated tubes (Vacuette, Greiner Bio-One, Austria). After waiting 30 minutes for clotting, blood samples were centrifuged with 3000 turn/min for 10 minute at the room temperature. A part of the serum was used in routine biochemistry analysis. Routine biochemistry tests were worked in biochemistry autoanalyser with standard methods using a commercial kit (Olympus 2700, Olympus Optical Co. Ltd, Shizuoka-ken, Japan). The remaining serum was portioned and protected at -80 C°, till the Human Urotensin II (UII/UCN2) were analyzed. A commercial kit (Sunred, Republic of China) was used, working with sandwich ELISA method, for serum UII levels. Spectrophotometric measurement with 450 nm wave length was done with Thermo Scientific Multiscan GO model ELISA reader (Finland). MIF concentrations of the samples were determined by using standard curves drawn with diluted standard absorbance. The results were defined as pmol/L defined.

Statistical Analysis

All analyses were done with the program named Statistical Package for the Social Sciences software version 18.0 (SPSS Inc., Chicago, USA). Continuous variable distribution was done by Kolmogorov-Smimov test and all the continuous variables were found to be distributing normally (p>0, 05). Continuous variables' results were given as mean ± standard error. Demographic and laboratory data of prediabetic individuals and individuals with normal glucose tolerance were prepared with by using independent t test.

Serum urotensin II level and its relation with other demographic and laboratory data was assessed with Pearson's correlation analysis. Independent relation between serum urotensin II level and other variables was performed with multiple linear regression analysis. Urotensin II and prediabetes conjunction was examined with binary logic regression analysis. In our study, $p<0.05$ was accepted as statistically significant.

RESULTS

Demographic and laboratory analyses

The demographic and laboratory data of prediabetic individuals and the individuals in the control group having normal glucose tolerance are in table 1, in a comparison chart. Between two groups there was no statistically significant difference in age, sex, and BMI ($p>0.05$).

Serum urotensin II level (prediabetic group=4.48 ± 2.43, control group=3.09 ± 1.59, $p=0.004$) in the prediabetic group was significantly higher than the control group having normal glucose tolerance.

Fasting blood glucose, hs-CRP and HOMA-IR levels were significantly higher for the prediabetic group than the control group($p<0.001$). Serum HDL cholesterol level in prediabetic patients was found to be statistically significantly lower. Between these two groups there weren't any statistically significant difference for kidney function tests, liver function tests ($p>0.05$).

Correlation analyses and linear regression analysis

Serum Urotensin II level and its relation between clinical and laboratory parameters were calculated with Pearson's correlation quotient and is presented in Table 2. A positive and significant correlation between serum urotensin II level and the parameters as age, BMI, BFA (body fat analysis), insulin, HOMA-IR, hs-CRP, systolic blood pressure, diastolic blood pressure was demonstrated. The effects of the variables on urotensin II levels, which have a significant relation in correlation analysis, were examined

Table 1. Comparison of the demographic and the laboratory data between the two groups

Variables	Prediabetes group n=40	Control group n=40	*p*
Age, year	53.32 ± 10.22	49.47 ± 10.81	0.106
Sex, female/male	20/20	20/20	1.000
BMI, kg/m^2	29.39 ± 3.90	28.29 ± 4.67	0.259
Waist circumference, cm	100.65 ± 8.52	96.75 ± 11.00	0.080
BSA$^{\alpha}$, m2	11.25 ± 3.45	8.42 ± 2.92	<0.001
SBP, mmHg	120.00 ± 7.16	119.25 ± 7.12	0.640
DBP, mmHg	72.00 ± 5.16	70.87 ± 5.41	0.345
Glucose, mg/dl	107.35 ± 5.64	87.97 ± 6.94	<0.001**
Insulin, µlU/mL	11.70 ± 8.24	7.03 ± 2.69	0.001**
HOMA-IR	3.10 ± 2.19	1.54 ± 0.61	<0.001**
A1c, %	6.16 ± 0.21	5.50 ± 0.13	<0.001**
Total cholesterol, mg/dL	176.66 ± 32.47	203.46 ± 45.10	0.331
HDL-C, mg/dL	48.48 ± 9.03	53.45 ± 13.03	0.017*
LDL-C, mg/dL	130.50 ± 25.61	127.25 ± 37.58	0.576
Triglyceride, mg/dL	131.51 ± 40.92	138.30 ± 77.12	0.549
hs-CRP, mg/L	0.98 ± 0.66	0.49 ± 0.15	<0.001**
BUN	13.28 ± 3.43	13.13 ± 2.92	0.798
Creatinin	0.63 ± 0.21	0.60 ± 0.20	0.491
SGPT	22.68 ± 13.83	24.96 ± 9.49	0.294
Urotensin II, ng/mL	4.48 ± 2.43	3.09 ± 1.59	0.004**

$^{\alpha}$: Body Fat Analysis

Tablo 2. Spearman Correlation analysis and Multiple Regression Analysis (r= Correlation quotient, β: Regression quotient, CI: Confidence Interval.)

	Correlation analysis		Multiple Regression analysis			
Variables	**r**	***P***	β	95% GA		*P*
Age	0.134	0.021*	0.044	-0.032	0.322	0.120
BMI	0.298	0.009*	0.287	0.011	0.785	0.014†
WC	0.318	0.014*				
BSA*	0.305	0.021*	0.121	0.014	0.852	0.027†
Insulin	0.287	0.016*				
FBG	0.201	0.032*				
HOMA-IR	0.457	0.021*	0.051	0.007	1.316	0.038†
Hs-CRP	0.176	0.031*	0.021	-0.123	1.025	0.023†
Systolic blood pressure	0.442	0.012*	0.167	-0.046	2.176	0.038†
Diastolic blood pressure	0.321	0.034*	0.145	-0.127	3.562	0.041†

with multiple regression analysis. In analysis, BMI, BSA, HOMA-IR, hs-CRP, systolic blood pressure and diastolic blood pressure were found to have independent effects on urotensin II levels.

DISCUSSION

In our study, in prediabetic patients, the serum urotensin II level was found to be higher than the control patients. There was a significant relation in between serum urotensin II level and insulin resistance indicator HOMA-IR, systolic blood pressure and diastolic blood pressure. In the individuals with high serum urotensin II level, prediabetes development risk is also found to be high. As the insulin resistance is the basic mechanism leading to prediabetes, defining the different mechanisms causing insulin resistance is needed (16-18).

In the previous clinical trials, urotensin II level was observed to be related with insulin resistance and this has become an important reason to design this trial (6-8). In our study, the significant relation demonstrated between urotensin II levels and prediabetes & insulin resistance lead us to think about the crucial role of urotensin II in pathophysiological mechanisms causing pre-diabetes and insulin resistance. By using this relation, in future, the new agents that would be developed against the urotensin II and/or urotensin II receptor could be used to prevent insulin resistance and also prediabetes development or to treat these patients.

In the trial which was performed by You Z et al(19), it was seen that urotensin II was excessively high in obese mice.; and in these mice, by using urotensin II receptor antagonist, they demonstrated improvements in plasma glucose, in blood pressure, in lipid parameters and in body weight of these mice. In similar study(20), serum urotensin II, hs-CRP, insulin level and HOMA-IR levels were found significantly higher than the control group in patients with PCOS (Polycystic ovary syndrome).

As a result, in this study, with urotensin II level measurement, it was demonstrated that, in patients with a high urotensin II level, prediabetes development could be estimated, and with life style changes, prevention of the clinically obvious diabetes could be possible. This would provide both a decrease in morbidity and mortality due to prediabetes and clinically obvious diabetes and also a decrease of money spent for treatment, nationally and internationally.

REFERENCES

1. Basevi V, Di Mario S, Morciano C, Nonino F, Magrini N., Comment on: American Diabetes Association. Standards of medical care in diabetes-2011. Diabetes Care 2011;34 (Suppl.1):S11-S61.
2. Armato J, DeFronzo RA, Abdul-Ghani M, Ruby R. Successful Treatment of Prediabetes in Clinical Practice: Targeting Insulin Resistance and Beta Cell Dysfunction. Endocr Pract 2012; 18(3):342-50.
3. DeFronzo, R.A. and M. Abdul-Ghani, Assessment and treatment of cardiovascular risk in prediabetes: impaired glucose tolerance and impaired fasting glucose. Am J Cardiol 108(3 Suppl): p.3B-24B.
4. Buysschaert M., Michael B. Definition of Prediabetes. Medical Clinics of North America 2011;95(2): 289-97.
5. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998;15(7):539–53.
6. Watanabe T, Kanome T, Miyazaki A, Katagiri T. Human urotensin II as a link between hypertension and coronary artery disease. Hypertens Res 2006; 29(6):375-87.
7. Iglewski M, Grant SR. Urotensin II-induced signaling involved in proliferation of vascular smooth muscle cells Vasc Health Risk Manag. 2010; 6: 723-34.
8. Rakowski E, Hassan GS, Dhanak D, Ohlstein EH, Douglas SA, Giaid A.. A role for urotensin II in restenosis following balloon angioplasty: use of a selective UT receptor blocker. J Mol Cell Cardiol 2005;39(5):785-91.
9. Tracy RP, Lemaitre RN, Psaty BM, Ives DG, Evans RW, Cushman M, Meilahn EN, Kuller LH. Relationship of C-Reactive Protein to Risk of Cardiovascular Disease in the Elderly Results From the Cardiovascular Health Study and the Rural Health Promotion Project. Arterioscler Tromb Vasc Biol 1997; 17: 1121-7.
10. Rifai N, Tracy RP, Ridker PM. Clinical Efficacy of an Automated High-Sensitivity C-Reactive Protein Assay. Clin Chem 1999; 45(12): 2136-41.
11. Ridker PM, Cook N. Clinical Usefulness of Very High and Very Low Levels of C-Reactive Protein Across the Full Range of Framingham Risk Scores. Circulation 2004; 109(16): 1955-9.
12. Rifai N, Ridker PM. High-sensitivity C-reactive protein: a novel and promising marker of coronary heart disease. Clin Chem 2001; 47(3): 403-11.
13. Rothkrantz-Kos S, Schmitz MP, Bekers O, Menheere PP, van Dieijen-Visser MP. High-Sensitivity C-Reactive Protein Methods Examined. Clin Chem 2002; 48(2): 359-62.
14. Shiesh SC, Chou TC, Lin XZ, Kao PC. Determination of C-reactive protein with an ultra-sensitivity immunochemiluminometric assay. J Immunol Methods 2006; 311(1-2): 87-95.
15. Macy EM, Hayes TE, Tracy RP. Variability in the measurement of C-reactive protein in healthy subjects: implications for reference intervals and epidemiological applications. Clin Chem 1997; 43(1): 52-8.
16. Watson AM, Olukman M, Koulis C, Tu Y, Samijono D, Yuen D, at all. Urotensin II receptor antagonism confers vasoprotective effects in diabetesassociated atherosclerosis: studies in humans and in a mouse model ofdiabetes. Diabetologia. 2013 May;56(5):1155-65.

17. Sidharta PN, Rave K, Heinemann L, Chiossi E, Krähenbühl S, Dingemanse J.Effect of the urotensin-II receptor antagonist palosuran on secretion of and sensitivity to insulin in patients with Type 2 diabetes mellitus.Br J Clin Pharmacol. 2009; 68(4):502-10.

18. Pang XX, Bai Q, Wu F, Chen GJ, Zhang AH, Tang CS.Urotensin II Induces ER Stress and EMT and Increase Extracellular Matrix Production in Renal Tubular Epithelial Cell in Early Diabetic Mice. Kidney Blood Press Res. 2016;41(4):434-49.

19. You Z, Al Kindi H, Abdul-Karim A, Barrette PO, Schwertani A. Blocking the urotensin II receptor pathway ameliorates the metabolic syndrome and improves cardiac function in obese mice. FASEB J, 2014; 28(3):1210-20.

20. Yilmaz Ö, Calan O, Kume T, Calan M. The relationship of urotensin II with insulin resistance and hs-CRP in patients having PCOS. Gynecol Endocrinol 2013;29(11):970-3.

Corresponding author

Assoc. Prof. Dr. Oktay Bilgir, MD,
Izmir Bozyaka Training and Research Hospital,
Dept. of Internal Medicine, 35380 Bozyaka, Izmir; Turkey.
e-mail: oktaybilgir@gmail.com
Tel.:+902322505050/5242;
fax: +902322614444