Plasma and Saliva Irisin Levels of Patients with Diabetic Nephropathy and Non-Diabetic Proteinuria

Diyabetik Nefropatili ve Diyabetik Olmayan Proteinürili Olgularda Plazma ve Tükürük İrisin Düzeyleri

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ABSTRACT

OBJECTIVE: Primary glomerular diseases or some systemic disorders such as diabetes (DM) may cause proteinuria. Irisin (IRI) is a hormone secreted as a response to physical exercise by the skeleton muscle and thought to be protective against many metabolic disorders such as DM, obesity. Decreased levels of irisin were observed in chronic kidney disease (CKD), type 2 DM and obesity. The aim of this study was to determine IRI levels in blood and saliva of proteinuric patients with and without diabetes.

MATERIAL and METHODS: Sampling was from 15 diabetic, 15 non-diabetic proteinuria and 13 healthy control subjects. IRI concentrations were measured by using commercial ELISA kits. Statistical analysis was performed using SPSS 12. Groups were compared by the Kruskal-Wallis test and then Mann–Whitney post hoc test was performed.

RESULTS: Plasma and saliva IRI levels of non- diabetic group were higher than the diabetic group. There was a significant positive correlation between plasma IRI concentrations and HDL, albumin levels but a negative correlation between plasma IRI and LDL levels.

CONCLUSION: IRI levels were low both in plasma and saliva in diabetic subjects. IRI may be an important marker and/or therapeutic agent for disorders associated with energy expenditure and kidney diseases in the future.

KEY WORDS: Proteinuria, Diabetic nephropathy, Irisin

ÖZ

AMAÇ: Birincil glomerül hastalıkları ya da diyabet (DM) gibi bazı sistemik hastalıklar proteinüriye neden olabilir. İrisin (IRI), egzersize yanıt olarak iskelet kasından salgılanan bir hormon olup; DM ve obezite gibi metabolik hastalıklara karşı koruyucu olduğu düşünülmektedir. Kronik böbrek hastalığı (KBH), tip 2 DM ve obezite olgularında irisin düzeyleri düşük olarak bulunmuştur. Bu çalışmanın amacı, diyabetik nefropati (DN)'li ve diyabetik olmayan proteinürili olgularda plazma ve tükürük irisin düzeylerinin saptanmasıdır.

GEREÇ ve YÖNTEMLER: Çalışmada, 15 DN'li, 15 DM'lu olmayan proteinürili ve 13 sağlıklı kontrol olgusu değerlendirildi. IRI düzeyleri ticari ELİSA kitleri ile ölçüldü. İstatistik analiz, SPSS 12 paket programı ile yapıldı. Gruplar arası karşılaştırma için, Kruskal- Wallis testi ve sonra Mann–Whitney post hoc testi yapıldı.

BULGULAR: DM'lu olmayan grubun plazma ve tükürük IRI düzeyleri DN'li gruptan yüksek olarak saptandı. Plazma IRI düzeyleriyle; HDL ve albümin düzeyleri arasında pozitif, LDL düzeyleri arasında ise negatif korelasyon saptandı.

SONUÇ: DN'li olgularda hem plazma, hem de tükürük IRI düzeyleri düşüktü. IRI, gelecekte enerji harcanması ile ilgili hastalıklar ve KBH için önemli bir belirteç ve/ veya tedavi edici bir ajan olabilir.

ANAHTAR SÖZCÜKLER: Proteinüri, Diyabetik nefropati, İrisin

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INTRODUCTION

Proteinuria is mainly due to increased permeability of glomerules to albumin and some other plasma proteins. If the liver can not compensate the protein loss by the urine, tissue oedema occurs and albumin levels decrease (1-3). Glomerular diseases are the primary causes of proteinuria. For the adult population, membranous nephropathy is the most common primary cause of nephrotic syndrome. Diabetic nephropathy (DN) is one of the most common secondary causes of proteinuria. Acute kidney injury is not a common and expected complication of nephrotic syndrome (1, 4).

The leading cause of end stage renal disease (ESRD) is DN and it may be renal end point of both type 1 and type 2 diabetes (5). DN is defined as sustained albuminuria (>300 mg/ day or 200 mcg/ min) determined two times within 3-6 months (6). After the diagnosis of type 1 diabetes, nearly 20-30 % of these patients develop microalbuminuria within 15 years and less than half of them progress to DN (7). Risk factors for DN are genetic factors, poor glycemic control, hypertension, advanced age, hyperfiltration, obesity, male gender, time passed with diabetes, smoking, dyslipidemia, excess protein consumption and oral contraceptive pill use (6-11). In both type 1 and 2 DM, pathological changes of kidney tissue are same. Diffuse glomerulosclerosis, nodulary glomerulosclerosis and insudative lesions may be named as diabetic glomerulosclerosis in general. Good glycemic control, blood pressure control, restriction of protein consumption, lipid lowering therapy and weight loss are preventive and therapeutic approaches for DN (5,12,13).

IRI is a protein hormone secreted by skeletal muscle as a response to peroxisome proliferator- activated receptor C coactivator 1a (PGC-1a) activation especially after the exercise. Boström et al. discovered this myokine in 2012. Irisin regulates the energy expenditure of the body especially by regulating thermogenesis by turning white fat tissue into brown fat (14).

Both in type 2 DM and CKD irisin levels were found to be significantly lower than healthy control subjects in the previous studies (15,16).

It is not clear if irisin levels vary between diabetic nephropathic patients with proteinuria, non- diabetic proteinuric patients and healthy control subjects. Although several previous studies determined irisin in different biological fluids and demonstrated its clinical significance in various diseases, there is no previous study about the blood and saliva irisin levels in diabetic and non-diabetic proteinuric patients. Therefore, the aim of this study was to determine irisin in blood and saliva samples of proteinuric patients with and without diabetes.

MATERIAL and METHODS

Our study was approved by the local ethics commitee. Sampling was from 15 diabetic, 15 non-diabetic proteinuria and 13 healthy control subjects. Five mL samples of blood and saliva were obtained from the subjects. Blood samples were taken in tubes containing 3.8 mmol EDTA . Blood and saliva samples were taken simultaneously. Blood and saliva samples were stored at –80 °C until assays were performed.

Irisin levels were measured with a human irisin ELISA kit (Phoenix Pharmaceuticals, Belmont, California, USA). The lowest detectable concentration of irisin was 9 ng/mL. Sample absorbance at 450 nm was measured with an ELX 800 ELISA reader. Other biochemical parameters were measured with an autoanalyzer.

Statistical analysis was performed using SPSS 12 (SPSS Inc., Chicago, IL, USA). Groups were compared by the Kruskal-Wallis test and then Mann–Whitney post hoc test was performed. Pearson correlation test was used for correlation analysis. The data are expressed as arithmetic means ± standard deviation (SD). $p < 0.05$ was considered significant.

RESULTS

The demographic characteristics and laboratory values of the subjects are shown in Table I. Body mass index , urea, creatinine levels and amount of proteinuria were not significantly different between diabetic and non- diabetic groups.

Although plasma irisin levels of non- diabetic group were higher than diabetic group but without a statistical significance; irisin levels of the control group were significantly higher than both groups (plasma irisin levels were as follows; diabetic group: 39.65±10.81; non-diabetic group: 42.37±12.94; control group 64.43±11.95). Saliva irisin levels of the non-diabetic group were higher than the diabetic group without a statistical significance; irisin levels of control group were significantly higher than both groups (saliva irisin levels were as follows; diabetic group: 486.67±44.98; non-diabetic group: 519.45±83.54; control group 569.09±117.96) (Figure 1).

There were significant positive correlations between plasma irisin concentrations and HDL, albumin levels; but negative correlation between plasma irisin and LDL levels ($p< 0.05$ for each parameter) . On the other hand there was also significant positive correlation between saliva irisin concentrations and plasma albumin levels but a negative correlation between saliva irisin and plasma LDL levels ($p< 0.05$ for each parameter).

DISCUSSION

Proteinuria is an important state that may cause several complications such as thromboembolism, infections and acute renal failure if it is presented as nephrotic syndrome (proteinuria greater than 3-3.5 g/ 24 hour or spot urine protein: creatinine ratio of > 300-350 mg/mmol, serum albumin <2.5 g/ dl, clinical evidence of peripheral oedema, severe hyperlipidemia (total cholesterol often >10 mmol/ l). Primary glomerulopathies are the most common primary causes of proteinuria and the most common secondary cause is DM (1).

Table I: Demographic characteristics and laboratory values of the subjects.

	Diabetic proteinuria (n: 15)	Non- diabetic proteinuria (n: 15)	Control subjects (n: 13)
Age	64.20±10.26	42.93±14.20	32.15±8.17
BMI (kg/m^2)	29.39±6.32	27.46±4.59	23.79±2.48
Urea (mg/dl)	94.73±50.55	62.00±41.94	28.00±5.11
Creatinine (mg/dl)	2.94±3.03	1.93±1.45	0.87±0.15
HbA1c (%)	8.60±2.07	-	-
Amount of proteinuria (gr/day)	3.38±2.89	3.87±3.01	-
HDL	34.51±6.81	34.46±7.27	51.46±4.55
LDL	161.04±51.28	168.40±60.85	99.07±19.29
albumin	3.26±0.77	3.24±0.89	4.66±0.20

BMI: Body mass index, **LDL:** Low density lipoprotein, **HDL:** High density lipoprotein.

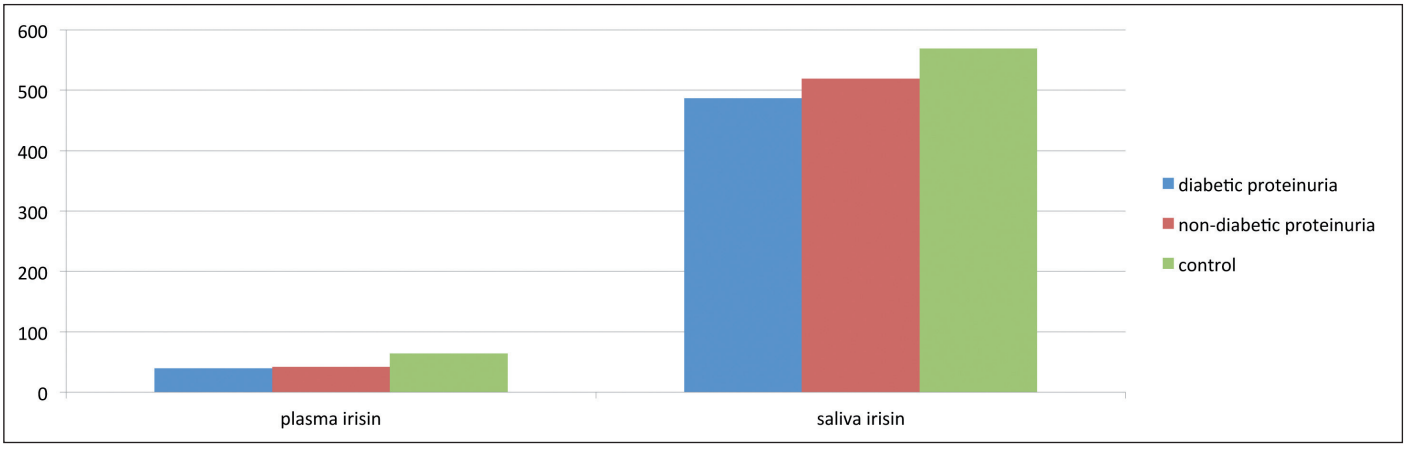

Figure 1: *Plasma and saliva irisin levels between groups (according to the mean values).*

Range of diabetic proteinuria may vary from microalbuminuria (30- 300 mg /day albuminuria) to nephrotic syndrome. With the progression of DM, nephropathy deteriorates and renal functions also decrease beside proteinuria and patients approach to chronic kidney disease (CKD) (17).

IRI is a protein hormone secreted by skeletal muscle as a response to peroxisome proliferator-activated receptor C coactivator 1a (PGC-1a) actvation especially after the exercise. PGC-1 a promotes fibronectin type III domain-containing protein 5 (FNDC5) gene expression and as a result of this process muscle tissue produces irisin (14,18).

In several studies, it was demonstrated that skeleton muscle has the ability to secrete hormones that are important to regulate the metabolism called myokines. Because of its endocrine, paracrine and autocrine functions it is fit to consider muscle tissue as an endocrine organ (19,20).

It has been known for centuries that exercise is a protective factor for several diseases and especially metabolic and cardiovascular disorders. Nowadays it is possible to attribute these effects of exercise to myokines that are released during the physical activity (21) and it is possible to mention that physical inactivity may be the contributor factor for several diseases such as type 2 DM, obesity and many others (22).

Exercise increases irisin precursor FNDC5 levels in the muscle tissue, and increasing irisin levels cause increased energy expenditure, weight reduction and amelioration of insulin resistance and obesity (22).

Irisin is known to be secreted by muscle and adipose tissues, and it has been observed that increased irisin levels are correlated with decreased HbA1c levels (23), BMI(24) and in obese patients liver enzymes and triglyceride levels (25).

In new onset or old type 2 diabetic and obese patients, plasma irisin levels are lower than non-diabetic subjects probably because of impaired PGC-1a expression and/ or activity of muscle tissue (15,23,24,26).

Some proteins such as liver fibroblast growth factor-21 (27) and cardiac natriuretic peptides (28) stimulate brown adipocyte thermogenesis, and irisin can be added to the family of these proteins because of its thermogenic properties (29).

Irisin originates from skeletal muscle, and coordinates energy expenditure, homeostasis and metabolism of the body. As a response to exercise, PGC-1a expression and FNCD5 gene product expression increase and muscle tissue secretes irisin; and by means of irisin, white fat turns into brown fat tissue and thermogenesis and energy expenditure increase. Irisin is also thought to be related to insulin resistance, obesity, glucose homeostasis, and neurodegenerative disorders (29,30).

Wen M-S. et al. revealed in their current study on non-diabetic CKD patients that, irisin levels decreased in this population with positive correlation with HDL levels. Thus, this population is prone to many disorders such as cardiovascular diseases, glucose intolerance, inflammation and so many others (16).

Aydin et al. determined high irisin levels in the plasma of lactating women in comparison with non-lactating ones; and they also determined irisin in human breast milk samples in their current study (31). Human saliva glands are also known to be stained and strongly reacted with irisin (32) and depending on this reality, we also evaluated irisin levels of human saliva.

In this study, plasma and saliva irisin levels of non- diabetic group were higher than diabetic group but without a statistical significance, irisin levels of control group were significantly higher than both groups. There were significant positive correlations between plasma irisin concentrations and HDL and albumin levels, but negative correlation between plasma irisin and LDL levels. There was also significant positive correlation between saliva irisin concentrations and plasma albumin levels, but negative correlation between saliva irisin and plasma LDL levels.

The limitation of our study is the relatively small sample size so further studies may be useful in this field.

In conclusion, our findings are all compatible with previous studies about irisin. As mentioned in many previous studies, irisin could become an important marker and/or therapeutic agent for the diseases related to the energy expenditure of the body, such as diabetes, obesity, insulin resistance and maybe kidney diseases in the future.

The authors declare that there is no conflict of interest with regard to the publication of this article.

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