## Research Article

# Adropin levels of biological fluids decrease in patients with proteinuria

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**Abstract:** Proteinuria can be caused, primarily by glomerular diseases or, secondarily by systemic disorders such as diabetes (DM). Adropin (ADR) is a peptide hormone that has been shown to regulate energy homeostasis by moderating glucose- mediated insulin release. The aim of this study was to measure ADR levels in blood and saliva samples taken from proteinuric patients, with and without diabetes, in whom energy homeostasis seriously differs. Samples were taken from 28 diabetic proteinuria patients, 26 non- diabetic proteinuria patients, and 26 healthy control subjects. Plasma and saliva ADR levels were measured using an ELISA kit according to the manufacturer's instructions. Statistical analysis was performed using SPSS 12 (SPSS Inc., Chicago, IL, USA). Groups were compared using the Kruskal-Wallis test, and then a Mann-Whitney post hoc test was performed. The Pearson test was used for correlation analysis. The plasma and saliva ADR levels of the non- diabetic and control groups were higher than those of the diabetic group. ADR levels of control group were also higher than non- diabetic group. There was a negative correlation between plasma adropin and urea, HbA1c levels. In this study, plasma and saliva ADR levels from the diabetic group were lower than both the non- diabetic and control groups. ADR can be a cornerstone in respect to our study, because, in states with excess proteinuria, the liver generates too many lipid products and energy homeostasis deteriorates, especially in diabetic subjects.
**Keywords:** Proteinuria, diabetic nephropathy, adropin

### INTRODUCTION

Proteinuria is generally the result of an increased permeability of glomerules to albumin and other plasma proteins. In the adult population, membranous nephropathy is the most common primary cause of nephrotic syndrome. The leading cause of end- stage renal disease (ESRD) is diabetic nephropathy (DN), and it may be the renal end point of both type 1 and type 2 diabetes and DN is one of the most common secondary causes of proteinuria [1,2].

Adropin (ADR) is a peptide hormone secreted by various tissues of the organism; it has been shown to regulate energy homeostasis [3]. ADR has been detected in the glomerulus, interstitium, and peritubular capillary endothelial cells of kidney tissues in diabetic rats. The main functions of ADR include regulating carbohydrate, lipid, and protein metabolisms by moderating glucose- mediated insulin release. Its measurement in biological fluids such as saliva will lead to further discussion of its possible clinical value, and may be a feasible option to determine its levels in the organisms [4].

Energy homeostasis, lipid parameters, and various biological processes differ in subjects with diabetic and non-diabetic proteinuria, and so, in this work, we aimed to measure levels of ADR, an important indicator molecule for energy homeostasis, in blood and saliva samples taken from proteinuric patients with and without diabetes.

### MATERIALS AND METHODS

Our study was approved by the local ethics committee. Samples were taken from 28 subjects with diabetic proteinuria, 26 non-diabetic subjects with proteinuria, and 26 healthy control subjects. Five mL samples of blood and saliva were taken from all subjects. Blood samples were taken in tubes containing 3.8 mmol EDTA. Blood and saliva samples were taken simultaneously. Both samples were promptly centrifuged at 4°C and stored at −80 °C until the assays were performed.

The plasma and saliva ADR levels were measured using an Enzyme-Linked Immuno Sorbent Assay kit (Phoenix Pharmaceuticals, Belmont, CA, USA) according to the manufacturer's instructions. ADR

measurements were validated as described previously [5]. The detection range of the kits was 0.01–100 ng/ mL. The sensitivity was 0.3 ng/ mL. The linear range was 0.3–8.2 ng/ mL. The intra- (within day) and interassay (between days) coefficients of variations were 7.4 and 9.2%, respectively. Other biochemical parameters were measured with an automated chemistry analyzer (Roche Diagnostics, Indianapolis, IN, USA) using commercially available kits.

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

**RESULTS**

The demographic characteristics and laboratory values of the groups are shown in Table 1. Plasma ADR levels in the non- diabetic and control groups were significantly higher than in the diabetic group (plasma ADR levels of diabetic group: 1, 89± 0.69 ng/ mL; non-diabetic group: 2,66± 0,89 ng/ mL; control group 2,83± 0,96 ng/ mL). Saliva ADR levels in the non-diabetic group were higher than in the diabetic group, without statistical significance, and saliva ADR levels in the control group were significantly higher than in the diabetic group (saliva ADR levels of diabetic group: 34,67± 8,26 ng/ mL; non- diabetic group: 35,46± 11,93 ng/ mL; control group 40,39± 9,35 ng/ mL) (Figure 1). There was a negative correlation between plasma ADR and urea, HbA1c levels (p< 0.01).

**Table 1: Demographic properties and labaratory values of the subjects**

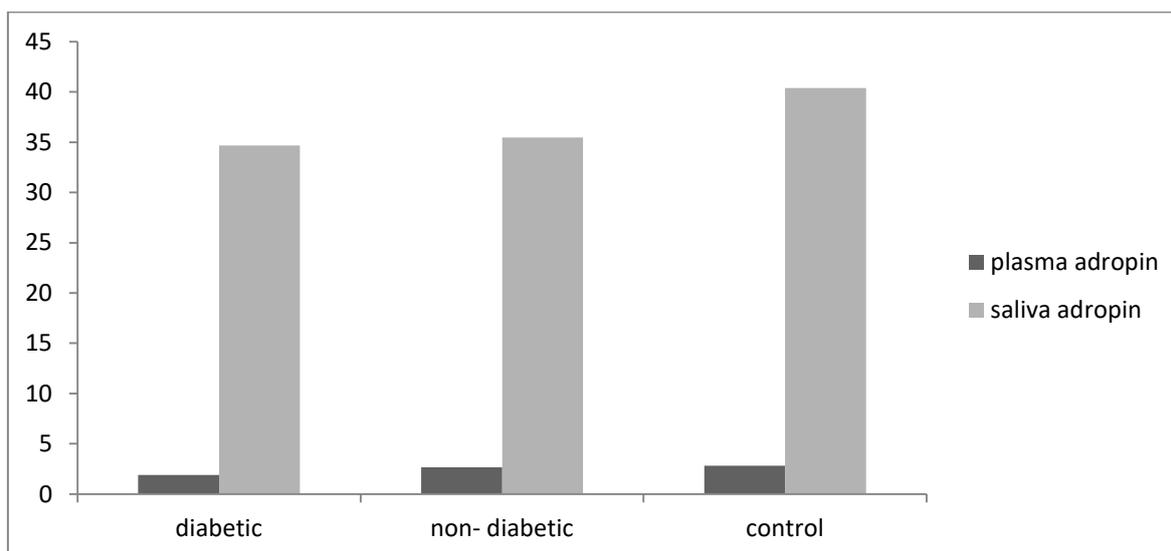|  | Diabetic proteinuria (n: 28) | Non- diabetic proteinuria (n: 26) | Control subjects (n: 26) |
|---|---|---|---|
| Age | 46,54±8,4 | 44,72±10,19 | 37,62±7,65 |
| BMI (kg/m$^2$) | 28,43±5,43 | 25,64±5,54 | 24,82±3,71 |
| Urea (mg/dl) | 84,32±36,52 | 58,32±26,74 | 32,14±7,23 |
| Creatinine (mg/dl) | 2,77±2,92 | 1,86±1,62 | 0,96±0,22 |
| HbA1c (%) | 7,87±2,36 | - | - |
| Amount of proteinuria (gr/day) | 4,02±3,11 | 3,68±2,86 | - |
| HDL | 31,44±5,07 | 33,52±7,14 | 49,49±3,32 |
| LDL | 158,33±48,54 | 163,80±36,47 | 103,14±15,33 |
| Albumin | 3,16±0,54 | 3,34±0,72 | 4,34±0,26 |

Data presented as mean±SD.



**Fig- 1: Plasma and saliva adropin levels between groups (according to the mean values)**

**DISCUSSION**

This study demonstrated that plasma and saliva ADR levels in diabetic nephropathic patients were lower than those in non-diabetic proteinuric patients and control subjects. The main pathological mechanism of proteinuria is the increased permeability of glomerules

to large molecules, especially albumin and some other plasma proteins. If the protein loss by the urine could not be compensated for by the liver, peripheric tissue edema occurred, and plasma albumin levels decreased. Proteinuria is an important clinical situation that may cause several complications, including thromboembolism, infections, and acute renal failure, if it is presented as nephrotic syndrome (proteinuria greater than 3-3.5 g/ 24 hours or spot urine protein: creatinine ratio of >300- 350 mg/ mmol, serum albumin <2.5 g/ dL, clinical evidence of peripheral edema, severe hyperlipidaemia (total cholesterol often >10 mmol/ L) is often present), although it may be in the subnephrotic range (<3- 3.5 g/day). The primary causes of proteinuria (especially in the nephrotic range >3- 3.5 g/day) are due to primary glomerular diseases. The most common primary cause of nephrotic syndrome in adults is membranous nephropathy. Also, various drugs and disorders may cause nephrotic syndrome or proteinuria; DN is one of the most common causes of this clinical situation [1].

DN is the leading cause of ESRD and may be a renal end point of both type 1 and type 2 DM [2]. DN is a clinical syndrome defined as permanent albuminuria (>300 mg/ day or 200 mcg/ min) diagnosed twice in a 3- 6 month period [6]. The number of diabetic patients worldwide is estimated to be 300 million by the year 2025, with an increasing rate. Fifteen years after a typical diagnosis of type 1 diabetes, nearly 20- 30% of patients develops microalbuminuria, but less than half of those in the microalbuminuric population progress to overt nephropathy [7]. In UKPDS study, after the diagnosis of type 2 diabetes, the prevalence of the development of microalbuminuria, macroalbuminuria, creatinine elevation, and/ or ESRD was found to be 25, 5, 0.8%, respectively [8]. Pathological changes in the kidney tissue of both type 1 and 2 diabetes patients are the same. Diabetic glomerulosclerosis is a general concept that comprises diffuse glomerulosclerosis, nodulary glomerulosclerosis, and incudative lesions. The prevention and treatment of DN consists of strict glycemic control, blood pressure control, a restriction of protein consumption, lipid lowering therapy, and weight loss [9, 10].

Adropin is a 42-amino acid peptide hormone first described by Kumar et al. in 2008. It is secreted by various tissues of the organism and has been shown to regulate energy homeostasis by its effects on adipose tissue, glucose metabolism, and energy expenditure [3].

Aydin *et al.;* detected ADR in the glomerulus, interstitium, and peritubular capillary endothelial cells of kidney tissues in diabetic rats, as well as in other tissues, and attributed these elevated ADR levels to the probable pathogenetic mechanisms of diabetes [11]. The same investigating group found increased intensities of ADR and iNOS immunoreactivity in renal tissue, especially in severe diabetic cases. They interpreted these results to mean that these substances are involved in the pathophysiology of diabetes and may be a compensatory response against disease [4]. Energy homeostasis is the keystone for the normal physiological functions of the body, and abnormalities in energy homeostasis can be the cause and/ or result of several serious disorders. In ADR knockout mice, Kumar and colleagues observed increased adiposity and described the healing role of ADR in obesity, insulin resistance, and dyslipidemia [12]. ADR levels were found to be low in gestational diabetes [13]; in the states of obesity, insulin resistance, and the aging process [14]; and in cardiac syndrome X patients [15]. On the other hand, Lian *et al.;* found increased ADR levels in heart failure patients and thought this elevation to be associated with the pathogenesis of heart failure [16].

Lovren *et al.;* found ADR to have an endothelial protective role via increased NO syntase expressions of endothelial cells as a result of complicated intracellular signaling pathways [17]. In 2008, Kumar *et al.;* noted that ADR has an effect on hepatic lipogenic genes and suggested that this may be an important regulator of lipogenesis [3].

In proteinuric patients, especially when it is in the nephrotic ranges, the dynamics of metabolic and energy processes differ strongly. Because of the loss of proteins via impaired glomerular endothelial cells; lipid generation, synthesis functions of the liver, and so on, many known and unknown metabolic processes are thought to be influenced. In this point, protection, especially of the glomerular endothelial functions, can be severely important.

## CONCLUSION

In our study, plasma and saliva ADR levels in the diabetic group were lower than those in both non-diabetic and control groups. In addition, the non-diabetic proteinuric patients also had lower adropin levels, in comparison with the control subjects. As it is clearly known that ADR regulates energy expenditure via the regulation of insulin release, ADR can be considered a cornerstone in respect to our study, because of the fact that, in nephrotic syndrome states with excess proteinuria, the liver generates too many lipid products, and energy homeostasis deteriorates in proteinuric patients, especially those who are diabetic. Further studies with large numbers of patients may be useful in this field. Because adropin replacement may be a therapeutic option in proteinuric patients with and/ or without DM in the future, that may partly heal energy homeostasis and pathogenic processes in these disorders.

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