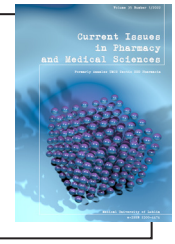
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# Correlation of apelin with microalbuminuria in type 2 diabetic patients

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**ABSTRACT**

**Introduction.** Type 2 diabetes mellitus is the root cause of diabetic nephropathy, a condition affecting the kidneys (T2DM). The number of people who have type 2 diabetes is growing.

**Aim.** To evaluate the differences in Apelin 13 levels between patients with T2DM who had normal or microalbuminuria and those who had microalbuminuria, as well as between these patients and healthy controls. The intent is to better understand its link to microalbumin, haemoglobin A1c, insulin resistance (IR), and other standard measures.

**Materials and methods.** Sixty individuals with type 2 diabetes, aged 35 to 45, were selected, and their microalbuminuria and normoalbuminuria were compared. Thirty age-matched healthy volunteers were selected to serve as controls. The concentrations of Apelin 13 and insulin in the plasma were measured with ELISA kits. The Turbilatex assay was used to calculate microalbumin concentrations. Measurements of glycosylated haemoglobin (HbA1C) were made via high-performance liquid chromatography.

**Results.** Patients with type 2 diabetes mellitus had higher levels of the protein Apelin 13 in their plasma than did healthy controls. T2DM patients with microalbuminuria were different from normoalbuminuric patients in another important way. Correlations between plasma Apelin 13 and albuminuria, HbA1c, and HOMA-IR were all positive.

**Conclusion.** Considering that plasma Apelin 13 is a critical risk factor in Type 2 diabetes mellitus and frequently arises in the early stages of nephropathy, it may be useful for the assessment of vascular issues in type 2 diabetic patients.

## INTRODUCTION

Over 415 million people have the chronic metabolic illness known as diabetes mellitus, and 5 million fatalities annually are attributed to vascular complications related to diabetes [1]. Inadequate insulin production and insulin resistance are at the heart of the pathophysiology of type 2 diabetes mellitus, which, together, lead to a severe impairment in the body's ability to regulate blood glucose levels [2-4].

Apelin, a peptide with regulatory functions, interacts with a G-protein-coupled receptor (APJ). Concentrations are high in the brain and spinal cord, as well as in adipose tissue, skeletal muscle, the digestive tract and the ovaries [5-7]. Over the past few years, researchers have paid close attention to the Apelin-APJ system because of the potential function it may play in homeostasis, fluid control, cell proliferation and energy related metabolism [8-10]. As a result of its association with decreased Apelin levels in patients with elevated LDL-cholesterol, it is also viewed as an unusual modulator of lipid metabolism. In addition, the blood Apelin level in dyslipidemic patients rises as a result of statin-induced LDL-C lowering [11,12].

Metabolic syndrome first became known in 1988 [13] to include type 2 diabetes. The most typical type of DM, type 2 (previously known as non-insulin dependent DM), is characterized by hyperglycemia, insulin resistance and relative insulin deficiency [14]. Risk factors for type 2 diabetes are genetic, environmental and behavioral [15,16]. Therefore, the purpose of this study is to examine the relationship between plasma Apelin 13 levels and microalbuminuria,

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insulin resistance, and numerous other lipid profile indicators in both healthy volunteers and patients with type 2 diabetes.

## MATERIALS AND METHODS

The NIMRA Institute of Medical Sciences in Jupudi, Andhra Pradesh, India, selected 60 type 2 diabetes patients aged 35 to 45 who were also receiving oral hypoglycemic drugs for their condition. Patients who exhibited any of the following conditions were not eligible to participate in the study: liver dysfunction, diabetes, thyroid disease, other metabolic disorders, smoking, alcoholism, tobacco chewing, abnormal urinary sediment, urinary tract infection, history of other renal disease, active or chronic persistent infection or inflammatory disorders, neoplastic disorders, myocardial infarction, stroke, or occlusive peripheral vascular disease. Thirty healthy volunteers of all sexes and similar ages were selected as a control group. Each person who took part in the study voluntarily gave their consent after it was reviewed by the Institutional Human Ethics Committee (IHEC). These observations were made in accordance with the principles outlined in the Helsinki Declaration of 1975.

### Biochemical analysis

Participants' blood was collected in the morning while they were fasting, and then centrifuged at 3000 g for 10 minutes. Samples were analyzed for glucose and lipid profile using an ERBA EM-360 fully automated analyzer (total cholesterol, HDL, LDL, and triglycerides). The concentrations of Apelin 13 and insulin in the plasma were determined using an enzyme-linked immunosorbent assay (ELISA). Urine samples were assessed automatically for microalbumin and creatinine, and venous blood samples were analyzed for plasma glucose (PPG) two hours after meals. The homeostasis model assessment for insulin resistance was developed using fasting plasma insulin glucose/22.5 [17].

### Statistical analysis

Statistical calculations were performed using SPSS 25.0. Results from the t-test were summarized using means and standard deviations, and differences with a p-value of 0.05 or less were considered to be significant. The Pearson correlation test was applied to examine the relationship between the variables.

## RESULTS

***Table 1.*** Baseline data of control and T2 DM patients differentiated according to microalbumin levels

| Parameters | Controls (n=30) | Normoalbuminuric T2DM (n=30) | Microalbuminuric T2DM(n=30) |
|---|---|---|---|
| Age (in years) | 45.1±3.9 | 46.7±6.3 | 48.8±4.2 |
| Body mass index (BMI - kg/m$^2$) | 24.7±1.8 | 27.5±3.2 $^{a*}$ | 27.9±3.3 $^{a*}$ |
| Waist/Hip ratio | 0.92±0.04 | 0.923±0.05 | 0.918±0.03 |
| Systolic BP (mmHg) | 114.2±6.6 | 132.8±12.8 $^{a*}$ | 135.8±11.5 $^{a*}$ |
| Diastolic BP (mm Hg) | 74.2±3.4 | 82.6±7.8 $^{a*}$ | 89.5±6.1 $^{a*,b*}$ |
| Duration DM (years) | – | 9.1±2.0 | 10.2±3.0 |

Data are expressed as mean ±SD, and P<0.05 was considered statistically significant
a – Controls versus Normoalbuminuria T2DM, Microalbuminuria T2DM
b – Normoalbuminuria T2DM versus Microalbuminuria T2DM
* p value <0.001, # p value <0.05

***Table 2.*** Clinical parameters of control and T2DM patients differentiated according to Microalbumin levels

| Parameters | Control (n=50) | Normoalbuminuria T2DM (n=50) | Microalbuminuria T2DM (n=50) |
|---|---|---|---|
| Urine Albumin Creatinine ratio (mg/gm. of creatinine) | 17.5±4.7 | 27.0±3.2$^{a*}$ | 140.3±12.4 $^{a*,b*}$ |
| FPG(mg/dl) | 91.1±5.4 | 135.6±18.9 $^{a*}$ | 152.9±15.0 $^{a*}$ |
| PPG(mg/dl) | 105.9±8.4 | 170.1±0.8 $^{a*}$ | 225±18.1 $^{a*,b*}$ |
| HbA1C (%) | 5.0±0.4 | 7.7±0.8 $^{a*}$ | 8.9±1.0 $^{a*,b*}$ |
| Insulin (µIU/mL) | 6.8±0.7 | 11.4±2.6 $^{a*}$ | 18.0±3.0 $^{a*,b*}$ |
| HOMA-IR | 1.32±0.17 | 3.8±0.9 $^{a*}$ | 6.2±1.5 $^{a*,b*}$ |
| Serum cholesterol (mg/dl) | 179.7±8.7 | 192±17.4 $^{a*}$ | 210.6±21.6 $^{a*,b\#}$ |
| Serum Triglycerides (mg/dl) | 99.1±7.0 | 140.3±31.3 $^{a*}$ | 152.4±36.0 $^{a*}$ |
| HDL cholesterol (mg/dl) | 49.3±2.3 | 42.0±2.8 $^{a*}$ | 37.5±2.5 $^{a*}$ |
| LDL cholesterol (mg/dl) | 110.2±8.9 | 129.9±12.5 $^{a*}$ | 145.2±15.5 $^{a*}$ |
| Plasma Apelin (pg/ml) | 236.8±25.5 | 322±23.6 $^{a*}$ | 383.8±29.2 $^{a*,b*}$ |

Data are expressed as mean ±SD, and P<0.05 was considered statistically significant
a – Controls versus Normoalbuminuria T2DM, Microalbuminuria T2DM
b – Normoalbuminuria T 2DM versus Microalbuminuria T2DM
* p value <0.001, # p value <0.05

***Table 3.*** Correlation between Apelin 13 with different parameters in T2DM patients

| Parameters | Correlation Coefficient (r) |
|---|---|
| FPG | 0.358 ** |
| PPG | 0.165 |
| HbA1C | 0.535 ** |
| HOMA-IR | 0.387 * |
| Cholesterol | 0.424 ** |
| TGL | 0.292 * |
| HDL | -0.191 |
| LDL | 0.375 ** |
| ACR | 0.637 ** |

* Correlation is significant at the 0.05 level (2-tailed)
** Correlation is significant at the 0.01 level (2-tailed)

## DISCUSSION

A number of studies have found that Apelin controls glucose homeostasis, insulin secretion and insulin sensitivity. As an endogenous ligand, Apelin is a peptide and a human G-protein. Apelin-13 has been discovered, according to one study, to be an anti-obesity and anti-diabetic peptide. Furthermore, it is intimately linked to hypertension and cardiovascular disorders [18-20], making it a prospective therapeutic target in metabolic illness. In our study, patients with type 2 diabetes and normal or microalbuminuria were shown to have a significantly different body mass index than healthy volunteers.

This study also found a statistically significant difference in HbA1c and HOMA-IR between normoalbuminuric and microalbuminuric patients with type 2 diabetes, and healthy volunteers. In addition, a strong positive association between HbA1c and insulin resistance was shown to exist between Apelin 13 and these parameters. Several researches have shown that apelin can cause satiety and contribute to weight loss. Because of its ability to increase insulin production and insulin sensitivity and to positively control diabetic consequences including kidney hypertrophy [21-23], Apelin has also been recognized as a potential therapeutic alternative for the treatment of diabetes.

Apelin 13 was found to have a statistically significant positive connection with total cholesterol, triglyceride, and LDL cholesterol levels. Both normoalbuminuric and microalbuminuric T2DM patients were shown to have dyslipidemias in the present study. The levels of cholesterol, triglycerides, and low-density lipoprotein (LDL) cholesterol were also correlated with apelin 13 levels. Ehan S. *et al.* [24] reported that when Apelin 13 is given to animals, their body weight decreases. Reduced food intake and better control of feelings of fullness, along with an increase in brown adipose mass and activity, are likely responsible for this effect. We discovered that Apelin 13 levels were significantly different in micro, normoalbuminuric T2DM patients compared to healthy controls, and that Apelin 13 levels positively correlated with the urine albumin creatinine ratio in T2 DM patients. The fact that Apelin 13 was evaluated in microalbuminuric T2DM patients explains both of these findings. Studies revealing that the deletion of certain APJ receptors in the pancreas impeded glucose clearance provided further evidence of the relevance of the apelinergic system in pancreatic islet function and body glucose homoeostasis. Additionally, in related experiments, treatment of cultured rat insulinoma cells with Apelin was found to result in increased beta-cell growth, indicating that Apelin may play a critical role in the proliferation of beta cells [25-27]. What is more, it has been observed that the inhibitory impact of histone acetylation prevents increasing kidney matrix size, lowers glomerular filtration rate, and decreases proteinuria. Numerous vascular problems in diabetics have been linked to this mechanism [28,29].

## CONCLUSION

Patients who have type 2 diabetes mellitus and a positive plasma Apelin 13 level have a significant increased risk of developing nephropathy at an earlier stage. Apelin level also has the potential to be used in the evaluation of vascular abnormalities that are present in persons who have type 2 diabetes.

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