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The relationship between atrial natriuretic peptide and microvascular complications of diabetes

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ABSTRACT

Objectives: In this study, we aimed to determine the relationship between the atrial natriuretic peptide and microvascular complications of diabetes.

Methods: Sixty patients with type 2 diabetes mellitus were enrolled into the study. Patients with a chronic disease other than diabetes mellitus were excluded from the study. The body-mass index, waist circumference, and hip circumference were measured and blood samples for routine biochemical tests were taken after at least 12 hours fasting. The microvascular complications of the patients were evaluated.

Results: Thirty-two of the patients had microvascular complications whereas 28 of them did not. Age, body mass index, waist and hip circumference, and atrial natriuretic peptide levels were significantly higher in the group with microvascular complications than those without complications. There was no significant difference in parameters like; waist-hip ratio, blood glucose, HbA1c, fasting insulin, fasting HOMA, sodium, potassium, magnesium, calcium and lipid levels between the two groups. When the relationship between atrial natriuretic peptide and obesity, retinopathy, neuropathy, nephropathy, duration of diabetes, HbA1c, and sex are evaluated separately, the only significant parameters related to atrial natriuretic peptide were the body-mass index, waist circumference, hip circumference measurements, and neuropathy.

Conclusions: Positive correlations between serum atrial natriuretic peptide levels and body-mass index, waist circumference, and hip circumference measurements were determined. Also, serum atrial natriuretic peptide levels were significantly higher in patients with obesity or neuropathy than those without obesity or neuropathy.

Keywords: Atrial natriuretic peptide, diabetes mellitus, microvascular complications

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Type 2 Diabetes Mellitus (DM) is the most widely seen metabolic disease. In developed countries 5-10% of the population is diabetic. In the worldwide, 90-95% of cases are type 2 diabetes, whereas 5-10% are type 1 and 2-3% are the other diabetes forms.

Hyperglycemia and insulin resistance play important role in the pathogenesis of diabetes. In the early phase of diabetes, intracellular hyperglycemia causes abnormalities in blood flow. Chronic hyperglycemia leads to microvascular and macrovascular complications.

Atrial natriuretic peptide (ANP) inhibits the transport of sodium (Na) in the medullary collecting tubules of the kidney and increases renal blood flow and glomerular filtration rate (GFR). Besides its renal effects, ANP also induces some cardiovascular,

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endocrinological and neural responses for decreasing the vascular volume and tonus [1].

There is Na retention and increased blood volume in diabetic patients. Most of these patients have high ANP or N-terminal proANP (1-98) levels in the circulation. These patients are probably in the early hyperfiltration phase of the disease or with the long-term complications. This data shows that ANP level may be important for diabetic patients with early hyperfiltration phase of the disease.

In this study, we aimed to study the relationship between ANP and microvascular complications.

METHODS

A total of 60 patients (38 females/22 males) with type 2 diabetes are enrolled in this study. The patients' age range was 30 to 70 years old. Patients with known thyroid dysfunction, hepatic failure, hearth failure, renal failure, malignancy, systemic chronic disease, collagen tissue disease, and with electrolyte imbalance, pregnancy, those having antihypertensive drug or insulin treatment were excluded from the study. Also, there was no patient taking any kind of SGLT 2 inhibitors or thiazolidinediones in our study. The study was performed according to Declaration of Helsinki and our Institutional Review Board approved the study. Informed constant was obtained from all patients.

Physical examination of all patients was undertaken. Body mass index (BMI) of patients were determined by "Tanita Body Composition Analyser". According to the World Health Organization (WHO), a BMI of greater than or equal to 30 is classified as obese. The HOMA values were calculated by using the formula: (HOMA-IR = insulin (μU/mL) × fasting glucose (mmol/L)/22.5). Waist circumference was measured from the midpoint of the distance between superior iliac crests and lowest chest ribs. Hip circumference was measured from the outermost surface of the gluteal area. Waist/hip ratios were calculated. Blood pressure of all patients was measured two times in 3 minutes by a sphygmomanometer from the left arm in sitting position after at least 5 minutes of resting. The mean value of the two measurements is recorded. Electrocardiograms of the patients are recorded and evaluated. Pulse rates are also measured. Those patients with hypertension and rhythm disturbance are excluded from the study.

From all patients, after 12 hours of fasting, blood samples were taken at 8-9 am for measurement of fasting blood glucose, urea, creatinine, uric acid, total cholesterol, HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), triglyceride, fasting insulin, HbA1c, sodium (Na), potassium (K), calcium (Ca), magnesium (Mg) levels. Blood samples for ANP level are taken into blood tubes containing EDTA and after rotated slowly 6-7 times the samples are put into tubes which contain 600-750 KIU aprotinin for every millimeter of blood. Then the tubes are rotated slowly with passive movements 6-7 times again. These blood samples are centrifuged at 4 C degree and 1600 cycles for 15 minutes. The isolated serum samples are preserved at – 80 C degree until the study time of the kit.

The first-morning urine samples were used to measure the microalbumin/creatinine ratios and total protein levels are measured in 24-hour urine samples. ANP levels were measured by their specific radioimmunoassays and serum samples other than ANP has been studied at the same day by routine biochemical analysis Roche Diagnostics brand Cobas at 8000 model biochemistery autoanalyzer (modul Cobas c 701) and hormon tests again the same brand (modul Cobas e 602) immunoassay analyzer with original kits (Beckman Coulter Inc, U.S.A.). Full Urine etude, at spot urine has been done with full automatic chemical and microscopic analyse system (Arkray, Japanese - Iris IQ200, USA).

All the patients are evaluated for diabetic retinopathy, diabetic peripheral neuropathy and diabetic nephropathy by related specialists. Indirect optical fundoscopy was performed by the same ophthalmologist after mydriasis and angiofluorescence when necessary. Retinopathy was classified according to the Diabetic Retinopathy Research Group. The patients were divided according to whether they had the incipient form of retinopathy (background) or the severe form (pre-proliferative or proliferative). The grade of diabetic nephropathy was evaluated (exercise, drugs and urinary infection excluded) by urinary albumin excretion rate and classified as normal (value < 20 μg/min), microalbuminuria in the range 20-200 μg/min

Table 1. Descriptive data of type 2 diabetic patients with and without microvascular complications

	At least 1 microvascular complication		*p value*
	Present (n = 32)	Absent (n = 28)	
Age (year)	54 ±9.5	47 ± 7.6	**< 0.05**
BMI (kg/m^2)	32 ±6.2	26 ± 5.6	**< 0.05**
Waist (cm)	103 ± 19	87 ± 17	**< 0.05**
Hip (cm)	114 ± 19	96 ± 18	**< 0.05**
WHR	0.89± 0.06	0.9 ± 0.04	0.851
FBG (mg/dl)	175 ±71	157 ± 56	0.612
PPG (mg/dl)	260 ±107	221 ± 64	0.576
HbA1c	9 ± 1.9	8 ± 1.9	0.912
Fasting Insulin (IU/ml)	15 ±7	12 ± 7	0.752
Fasting HOMA	6.6 ± 4.2	4.7 ± 3.3	0.244
Na (mmol/L)	140 ± 3.3	139 ± 2.5	0.656
K (mmol/dL)	4.5 ± 0.4	4.4 ± 0.3	0.811
Mg (mmol/dL)	0.8 ±0.05	0.8 ±0.06	0.912
Ca (mg/dl)	10 ± 0.5	10 ± 0.6	0.904
Total-cholesterol (mg/dl)	205 ± 48	189 ± 40	0.349
LDL-C (mg/dl)	123 ± 32	114 ± 37	0.185
HDL-C (mg/dl)	47 ± 12	45 ± 9	0.205
Triglyceride (mg/dl))	167 ± 72	154 ± 43	0.417
ANP (ng/ml)	4.99 ± 2.89	3.45 ± 2.89	**< 0.05**

Data are shown as mean±standard deviation. ANP = Atrial natriuretic peptide, BMI = Body mass index, FBG = Fasting blood glucose, HbA1c = Hemoglobin A1c, HDL-C = High-density lipoprotein cholesterol, HOMA = Homeostatic model assessment, LDL-C = Low-density lipoprotein cholesterol, NS = Not significant, PPG = Postprandial glucose, WHR = Waist-hip-ratio

(incipient nephropathy) or macroalbuminuria if > 200 µg/min (overt/clinical nephropathy). Peripheral neuropathy was diagnosed by Diabetic Neuropathy Index (DNI) and defined as a positive score of > 2 points on the DNI and evaluated by the same neurologist. Diabetic Neuropathy Score (DNS) was not evaluated and thus severity of neuropathy was not determined.

Statistical Analysis

The data were analyzed in SPSS 11.5 (SPSS Inc., Chicago, IL, USA) software. Descriptive statistics for continuous variants were given as mean +/- standard deviation and for categorical variants were given as the percentage (%). Between independent groups, the significance of the difference in mean values was evaluated by Student's t-test and Mann Whitney U test. The correlation analysis of all data was evaluated by Pearson correlation test. A p value of < 0.05 is defined as significant statistically.

RESULTS

Thirty-two of the sixty diabetic patients had microvascular complications and the remaining 28 patients had no microvascular complications (Table 1). Among patients with microvascular complications 6 patients had retinopathy (all patients had background retinopathy), 16 patients had nephropathy (2 of them had macroalbuminuria whereas 14 had microalbuminuria), 18 patients had neuropathy. Two of the patients had both retinopathy and nephropathy, one patient had both retinopathy and neuropathy, and 2 patients had both nephropathy and neuropathy. Only 3 patients had all retinopathy, neuropathy, and nephropathy. Age, BMI, waist circumference, hip circumference and ANP levels were significantly higher in patients with microvascular complications than those without complications. But there was no significant difference in waist-hip ratio, blood glucose levels, HbA1c, fasting insulin, postprandial insulin,

Table 2. Variants in correlation with ANP

	BMI (kg/m^2)	Waist circumference (cm)	Hip circumference (cm)
ANP (ng/dl)	r=0.415	r=0.496	r=0.520
	***p* = 0.001**	***p* < 0.001**	***p* < 0.001**

ANP = Atrial natriuretic peptide, BMI = Body mass index

fasting HOMA, postprandial HOMA, Na, K, Mg, Ca, and lipid levels between two groups.

The correlation analysis of patiens are given in Table 2. Statistically significant positive correlations were determined between ANP level and BMI (r = 0.415, $p = 0.001$), waist circumference (r = 0.496, $p < 0.001$), and hip circumference (r = 0.520, $p < 0.001$) measurements.

When the relationships between ANP level and obesity, retinopathy, neuropathy, nephropathy, duration of diabetes, HbA1c, and sex were evaluated separately and there was a significant relationship between ANP level and obesity, and neuropathy. The relationship between ANP levels and other parameters was not significant (Table 3).

Table 3. Factors related to ANP

		n	ANP (ng/dl)	*p* value
Obesity	Present	30	5.4 ± 2.8	**< 0.001**
	Absent	30	2.8 ± 2.5	
Sex	Female	38	4.7 ± 2.8	0.163
	Male	22	3.2 ± 2.9	
Retinopathy	Present	6	3.4 ± 2.7	0.438
	Absent	54	4.2 ± 3	
Nephropathy	Present	16	4.8 ± 3.1	0.324
	Absent	44	3.9 ± 2.9	
Neuropathy	Present	18	5.4 ± 3.1	**< 0.05**
	Absent	42	3.6 ± 2.7	
Duration of diabetes	≤ 10 years	37	3.9 ± 2.9	0.611
	> 10 years	23	4.4 ± 3.1	
HbA1c	< 7	16	3.9 ± 3.1	0.303
	≥ 7	44	4.2 ± 2.9	

Data are shown as mean±standard deviation. ANP = Atrial natriuretic peptide, NS = Not significant

DISCUSSION

The best-known function of ANP is inhibiting the renin-angiotensin-aldosterone system by stimulating the wasting of salt and water via kidneys [2, 3]. The increase in plasma ANP level in response to acute hyperglycemia is important in diabetic patients. Because it was shown that Na sparing effect of insulin causes an increase in total Na pool and leads to an increase in extracellular volume which results in chronic stimulus for ANP [4]. This effect is also seen in type 1 diabetic patients [5].

Neuropathy, retinopathy, and nephropathy are known as microvascular complications of diabetes. Some studies have evaluated the relationship between these complications and ANP level. Moro and Berlan [6] have investigated the peripheral and central effects of natriuretic peptides and they have reported that these peptides have important functions in hypertrophy and regulation of cardiomyocyte. They have shown in that study that plasma ANP level was increased in patients with unstable angina, acute

myocardial infarction, congestive heart failure, diabetic microangiopathy and isolated diastolic dysfunction [6]. Also, a previous study has shown a relation between microvascular complications of diabetes and serum vitamin D levels [7].

In our study, we found that plasma ANP levels in diabetic patients with neuropathy or nephropathy were higher than those without neuropathy or nephropathy; but ANP levels were significantly higher only in patients with neuropathy. There was no relation between ANP levels and retinopathy. This result could be caused by the limited number of patients included in this study. Because of this restricted number of patients, we were unable to compare ANP levels in the subgroups of patients consisted of microvascular complications with different severity. In addition to that, there were significant correlations between ANP level and BMI, waist circumference, and hip circumference measurements.

Jacobs *et al*. [8] claimed that the excess increase in urinary protein excretion - during 1-hour ANP infusion to type 1 diabetic patients with microalbuminuria - was not only related with glomerular pressure changes but also with the decrease of tubular protein reabsorption. In another study, McKenna *et al*. [9] investigated the physiopathological markers of the albuminuric effects of increased ANP concentrations in type 1 diabetic patients. They found a close relationship between plasma ANP level and acute or chronic hyperglycemia. In that study, they showed that ANP concentrations in type 1 diabetic patients (especially with chronic poor glycemic control) were high in subgroups with systemic hypertension and microalbuminuria [9]. In another study evaluating the relationship between ANP levels and nephropathy, natriuretic peptide levels of type 2 diabetic rats were significantly higher than nondiabetic rats [10]. In that study, the filtration rate in type 2 diabetic rats was decreased 48-79% when compared to nondiabetic ones and the investigators speculated that the increase in natriuretic peptide level was caused by the ineffective metabolize of the natriuretic peptides in kidneys [10].

In a study evaluating the relationship between ANP level and retinopathy, the investigators looked for ANP levels in vitreous fluids and epiretinal membranes of diabetic patients [11]. They found that the ANP levels in vitreous fluids of diabetic patients with retinopathy were significantly higher than in patients without retinopathy. In addition to that, the ANP levels of patients without retinopathy were also higher than the control group [11]. In our study, there was no difference in serum ANP levels between the patients with and without retinopathy.

Salas-Ramirez *et al*. [12] studied the effect of increased plasma volume on ANP in diabetic patients with autonomous neuropathy and showed that there was no relationship between ANP levels and autonomic test changes. They concluded that salty water infusion would not lead to increase in ANP levels independent of autonomous neuropathy [12]. In our study, the plasma ANP levels of diabetic patients with neuropathy were significantly higher than in diabetic patients without neuropathy.

In our study, we detected that plasma ANP levels in diabetic patients with microvascular complications were higher than in patients without microvascular complications. This was independent of glycemic regulation. Also, there were significant correlations between ANP level and BMI, waist circumference and hip circumference measurements. When we evaluated the microvascular complications separately ANP level was only significantly high in patients with nephropathy. According to the literature, ANP plays an important role in diabetes physiopathogenesis. We need studies with greater patient size investigating the role of ANP in early diagnosis and treatment.

Limitations

We had a limited number of study group and thus we were unable to compare ANP levels in the subgroups of patients consisted of microvascular complications with different severity. We could not exclude the silent hearth failure as we did not perform an echocardiographic evaluation to the patients. None of our patients were taking alcohol but physical activity and smoking habit were not assessed in statistical analysis.

CONCLUSION

In this study, we found positive correlations between serum atrial natriuretic peptide levels and body-mass index, waist circumference, and hip circumference measurements in patients with type 2

diabetes mellitus. Also, serum atrial natriuretic peptide levels were significantly higher in patients with obesity or neuropathy than those without obesity or neuropathy.

Authorship declaration

All authors listed meet the authorship criteria according to the latest guidelines of the International Committee of Medical Journal Editors, and all authors are in agreement with the manuscript.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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