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Effect of hyperparathyroidism on endothelial functions and atherosclerosis.

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Abstract

Background: Primary Hyperparathyroidism (PHPT) is associated with an increased mortality risk of cardiovascular disease, and this appears to decrease with time after parathyroidectomy. Our study aimed to determine the association between endothelial function, Carotid Artery Intima Media Thickness (CIMT), and Flow Mediated Dilatation (FMD) in hyperparathyroidism and their effects on cardiovascular disease.

Methods: The study included 20 patients each with PHPT and Secondary Hyperparathyroidism (SHPT) and 12 healthy subjects. All groups were matched with respect to age. Patients with diabetes mellitus, hypertension, and cardiovascular diseases were excluded from the study. Levels of serum calcium, Parathormone (PTH), and Daily Urinary Calcium Excretion (UCE) were calculated; furthermore, FMD and CIMT were evaluated for all subjects.

Results: Serum calcium levels were significantly higher in the PHPT group than those in the SHPT and control groups (P<0.001 and P<0.001, respectively). As expected, UCE levels of PHPT group were higher than those of both the control and SHPT groups (P<0.001, P<0.001 respectively). FMD and CIMT levels were not significantly different between the groups. There was a negative correlation between FMD and serum calcium and PTH levels in the PHPT group (P<0.05).

Keywords: Hyperparathyroidism, Endothelial functions, Calcium, Cardiovascular risk, Atherosclerosis.

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Introduction

Parathormone (PTH) is a peptide hormone, which controls ionized calcium levels in blood and extracellular fluids [1]. Primary Hyperparathyroidism (PHPT) is characterized by hypercalcemia, hypophosphatemia, and elevation in serum PTH levels. PHPT is caused by benign adenomas in 85% patients. Glandular hyperplasia and rarely carcinoma is the cause of PHPT in other patients [2]. Secondary Hyperparathyroidism (SHPT) is an adaptive process that develops in response to vitamin D deficiency [3].

Endothelium is an endocrine organ, which plays a role in the regulation of vascular tone, adhesion of platelets and leucocytes, and coagulation and fibrinolysis, leading to continuous regulation of vascular functions. Nitric oxide (NO) is the most important mediator released by endothelium [4]. Age, diabetes, smoking, and sedentary lifestyle deregulate the release of NO. This leads to increased risk of obesity, Cushing's syndrome, hypo- and hyperthyroidism, hyperparathyroidism, and cardiovascular diseases. Endothelial dysfunction is considered as a major finding of atherosclerosis and can be evaluated by measuring Flow Mediated Dilatation (FMD) and Carotid Artery Intima Media Thickness (CIMT) [5].

In some studies endothelium is defined as target tissue of Parathormone (PTH) and parathormone receptors can be on endothelium [6]. Hyperparathyroidism can lead to cardiovascular diseases, and mortality is increased in PHPT [7-11]. Endothelial dysfunction can be considered as an effective method to evaluate cardiovascular risk in patients who do not have a history of cardiovascular disease. Although the effect of PHPT on endothelial function is not fully elucidated, there are some studies assessing endothelial damage using FMD evaluation [12,13]. It is well known that PHPT is associated with a high risk of cardiovascular disease, leading to increased mortality and morbidity due to cardiovascular problems [10-12].

In this study, we aimed to evaluate endothelial functions, CIMT, and bone loss in patients with both PHPT and SHPT.

Subjects and Methods

Twenty patients each with PHPT and SHPT and 12 healthy subjects were included in our prospective study. Diagnosis of PHPT was based on clinical assessment and laboratory findings. Elevated serum calcium and PTH levels combined with hypophosphatemia, increased Urinary Calcium Excretion (UCE), and decreased tubular reabsorption of phosphate were required for diagnosing PHPT. Parathyroid adenomas were observed on both parathyroid ultrasound and 99 m Technetium scans of all the PHPT patients. SHPT was diagnosed when elevated PTH levels was observed with normal or low serum calcium level and decreased UCE. Twelve healthy age- and sex-matched subjects with normal values of biochemical parameters were used as controls. Serum calcium, phosphorus, albumin, chloride, and creatinine levels were measured in each subject. Serum intact PTH was measured from venous blood samples at a central laboratory using a solid-phase two-site chemiluminescent enzyme-labelled immunometric assay with a reference range of 15-65 pg/ml. Serum calcium, phosphorus, and creatinine levels were measured colorimetrically. Serum albumin levels were measured using immunoturbidometric assay, and serum creatinine levels were measured using an ion selective electrode. UCE levels were calculated using 24 h urine samples. Creatinine clearance (Ccr) levels were calculated using the Cockroft–Gault formula. Osteocalcin (2-22 ng/ml) and deoxypyridinium (2.3-5.4 nMDPD/mMkr) levels were measured. Bone mineral densities of the subjects were measured using Dexa, and T scores were evaluated. Blood samples were collected from all subjects after 12 h fasting, and blood glucose, insulin, and inflammation markers, including high sensitive C-reactive protein (hsCRP), Erythrocyte Sedimentation Rate (ESR), and serum homocysteine levels, were studied. ESR was determined using the Wintrobe method. hsCRP levels in serum samples were determined using high sensitivity CRP ELISA. Serum homocysteine level was estimated using fluorescence polarization immunoassay. Plasma insulin levels were measured using chemiluminescent enzyme immunoassay. The Homeostasis Model Assessment of Insulin Resistance was used for assessing insulin resistance using the following formula: (Fasting plasma glucose (mg/dl) × Fasting plasma insulin (μIU/ml))/405 [14].

We aimed to evaluate the effect of elevated parathormone or calcium levels on endothelial functions. Patients with PHPT had adenomas, requiring surgery, and evaluations were done before surgery. In the SHPT group, laboratory results correlated with SHPT, but we did not observe adenomas on ultrasonography or scintigraphy. In this group, patients with decreased parathormone levels following vitamin D replacement were included in the study. Patients with no change in parathormone levels following vitamin D replacement were not included in the study.

Endothelial functions were assessed after withholding all vasoactive medications for at least four half-lives and after 12 h fasting. Subjects did not exercise and did not consume caffeine, vitamin C, or tobacco for at least six hours before the study. Subjects underwent a complete history and physical examination, and an ECG was obtained from all subjects in order to exclude cardiac ischemia.

FMD (endothelium dependent) and Nitroglycerine Induced Dilatation (NID) (endothelium independent) were measured in all subjects using duplex Doppler ultrasound (Toshiba Applio, 7.5 MH linear probe) in the brachial artery based on the guidelines described by Corretti et al. [15]. In addition, CIMT was also evaluated. For assessing FMD, the right arm was extended at the elbow and the hand was supinated. An optimal longitudinal image of the brachial artery just above the elbow with clear anterior and posterior intimal interfaces between the lumen and vessel wall was established and kept stable. A sphygmomanometric cuff was placed distally from the elbow. A baseline image was obtained at rest. Subsequently, arterial occlusion was created by cuff inflation to 50 mmHg above the systolic blood pressure for four minutes. After deflation, the longitudinal image of the artery was recorded continuously from 30 s before to two minutes after deflation and the lumen diameter was measured at about 60 s. Lumen diameter was defined as the distance between media-adventitia interfaces of the vessel wall. After a 10 min resting interval, 0.4 mg nitroglycerine spray was administered sublingually, and vascular relaxation was measured on the 5^{th} min. FMD was defined as the per cent change in brachial artery diameter within one minute after ischemia compared to baseline. NID was defined as the per cent change in brachial artery diameter within five minutes of nitroglycerine administration.

High-resolution B-mode ultrasound was used to measure CIMT. The anterior, lateral, and posterolateral projections were used to obtain a longitudinal image of the right and left common carotid arteries. At each longitudinal projection, three CIMT evaluations were made two centimetres proximal to the bulb and site of greatest thickness. The values at each side were averaged, and the greatest value of the averaged CIMT was used as the value for each subject.

The purpose and procedure of the tests were explained to the subjects and written informed consent was obtained from each subject. The experimental protocol was designed and conducted according to the principles of the Declaration of Helsinki and was approved by the Ethical Committee of the Eskisehir Osmangazi University Medical Faculty.

All statistical analysis was performed using SPSS version 21.0 and Simastat 3.5. Comparisons between two different groups were assessed by independent samples t test, and differences between variables within groups were assessed by paired samples t test. Pearson correlation analysis was used to evaluate the relationship between variables. P value <0.05 was considered statistically significant. The results were summarized as mean ± SD.

Results

Basic characteristics of the study population are shown in Table 1. Bone turnover parameters of the patients are summarized in Table 2. Bone mineral densities were evaluated,

and bone densities of the femur, neck, and lumbar region were lower in the PHPT group (P<0.05). Osteocalcin levels were higher in the PHPT group (P<0.05). ALP levels were high in the PHPT and SHPT groups.

Table 1. *Baseline characteristics and serum laboratory parameters of the study population.*

	PHPT	SHPT	Control	
	N: 20	N: 20	N: 12	P value
Age	54.4 ± 12.0	51.9 ± 12.7	52.7 ± 12.3	NS
Systolic blood pressure (mm Hg)	118.0 ± 12.8	115 ± 8.2	104 ± 11.6	<0.05 (1-3, 2-3)
Diastolic blood pressure (mm Hg)	78.0 ± 7.6	76.5 ± 7.4	68.3 ± 7.1	<0.05 (1-3, 2-3)
PTH (pg/mL)	249 (176-483)	161 (115-233)	50 (33-59)	<0.001 (1-3, 2-3)
Calcium (mg/dL)	11.3 ± 0.5	8.5 ± 0.7	9.4 ± 0.4	<0.001 (1-2, 1-3)
Phosphorus (mg/dL)	2.1 ± 0.5	3.3 ± 0.4	3.3 ± 0.4	<0.001 (1-2, 1-3)
Urine calcium	352 (269-476)	57 (38-77)	160 (140-212)	<0.05
Tubular phosphate reabsorption (%)	68.5 (64.5-76.5)	86 (82-90)	90 (81.5-93.8)	<0.001 (1-2, 1-3)
Ccr (ml/min)	101.1 ± 18.6	93.9 ± 21.0	107.5 ± 17.0	NS

PHPT: Primary Hyperparathyroidism; SHPT: Secondary Hyperparathyroidis; NS: Non Significant; PTH: Parathormone; Ccr: Creatinine Clearance.

Table 2. *Comparison of bone turnover parameters.*

	PHPT N: 20	SHPT N: 20	Control N: 12	P value
Osteocalcin (2-22)	9.1 (6.9-13.9)	4.0 (1.7-8.8)	4.2 (2.5-5.5)	<0.05 (1-2, 1-3)
Urine deoxypyridinium (2.3-5.4)	9.7 (6.8-15.6)	7.7 (5.1-12)	5.2 (4.9-6.6)	<0.05 (1-3)
L 1-4 T score	-2.8 ± 1.2	-2.2 ± 1.7	-0.9 ± 0.8	<0.05 (1-3)
Neck T score	-2.6 (−3.2-2.0)	-2.3 (-2.7-1.4)	-1.4 (-1.8-0.6)	<0.05 (1-3)
ALP	278 (215-577)	241 (184-300)	124 (109-166)	<0.05 (1-3, 2-3)

NS: Non Significant; L1-4: Lumbar Vertebrae 1-4; PHPT: Primary hyperparathyroidism; SHPT: Secondary Hyperparathyroidism; ALP: Alkalen phosphatase.

Table 3. *Comparison of cardiovascular risk parameters of the study population.*

	PHPT N: 20	SHPT N: 20	Control N: 12	P value
ESR	10.5 (7-18)	14.5 (10-23)	4 (2-8.5)	<0.05 (1-3, 2-3)
hsCRP	2.0 (0.7-3.9)	3.4 (2.0-5.7)	0.85 (0.35-2.1)	<0.05 (2-3)
Insulin	7.3 (3.5-11.1)	6.3 (2.9-8.5)	4.2 (2.1-5.1)	NS
HOMA	1.5 (0.7-2.6)	1.3 (0.5-1.7)	0.8 (0.4-0.9)	NS
Homocysteine	11.7 ± 4.5	10.2 ± 4.2	9.7 ± 3.5	NS
CIMT (mm)	0.55 (0.40-0.75)	0.45 (0.40-0.80)	0.50 (0.35-0.60)	NS
FMD (%)	8.7 ± 2.0	11.6 ± 2.2	15.3 ± 2.2	NS
NID (%)	11.9 ± 2.6	11.4 ± 2.5	10.8 ± 3.1	NS

CIMT: Carotid Artery Intima Media Thickness; NS: Non Significant; PHPT: Primary Hyperparathyroidism; SHPT: Secondary Hyperparathyroidism; ESR: Erythrocyte Sedimentation Rate; hsCRP: High Sensitive C-Reactive Protein; FMD: Flow Mediated Dilatation; NID: Nitroglycerine Induced Dilatation; HOMA: Homeostasis Model Assessment.

Inflammation parameters, insulin resistance, CIMT, and endothelial functions of the patients are summarized in Table 3. Comparison of insulin resistance and CIMT between the groups showed no difference. ESH levels were higher in the PHPT and SHPT groups than those of the control group. hsCRP levels were higher in the SHPT group than those of the

control group (P<0.05). No difference was observed when homocysteine and insulin levels were compared among the groups. Also, there was no difference among the groups in terms of HOMA. FMD levels were lower in the PHPT group, but it was not statistically significant. NID levels did not show any difference among the groups. A correlation analysis was performed because of low levels of FMD in the PHPT group, and there was a negative correlation between PTH and FMD. Also, there was a negative correlation when calcium levels and FMD were evaluated. There was a positive correlation between PTH levels and hsCRP, homocysteine (P<0.05), and ESH (P<0.01) levels.

Discussion

Recent studies suggest that hyperparathyroidism has many systemic effects other than bone and mineral metabolism. Increased PTH level is strongly associated with prevalent and incident cardiovascular risk factors, such as hypertension and diabetes, and also with cardiovascular diseases. There are several studies, relating adverse cardiovascular outcomes, including death, incident coronary artery disease, and myocardial infarction, with increased PTH levels [10,16,17].

According to our bone mineral density findings, bone density of the femur, neck, and lumbar region were lower the PHPT group. Osteocalcin levels were higher in patients with PHPT. This may be because of increase in bone resorption and remodelling in PHPT and inadequate remodelling to avoid osteoporosis. The difference in CIMT was not statistically significant between groups. It was thicker in the PHPT group, but it did not reach a statistically significant difference. Similar to our results, a relationship was not observed in a study between CIMT and PTH [18]. This may be a consequence of the small sample size or long period for development of atherosclerosis. Patients with PHPT underwent surgery, and patients with SHPT received medical therapy. Patients who did not receive treatment may have increased cardiovascular risk. Several studies have demonstrated the correlation between hyperparathyroidism, metabolic syndrome, and insulin resistance [19,20]. Considering their findings, HOMA and insulin levels of our patients were evaluated. Insulin levels were higher in the PHPT group, and there was a statistically significant difference among groups.

Generally, normal response to FMD is 12%, and it was 8% in the PHPT group. Although it was not statistically significant, the response was not adequate in the PHPT group. This may be because of increased endothelial damage in patients with PHPT. Also, CIMT was higher and changes in FMD were lower in the SHPT group than those in the PHPT group. This may result in higher cardiovascular risk in the PHPT.

Our study had some limitations. One of them was that the sample size in our study was small. Because we have designed a prospective study and number patients with hyperparathyroidism and without cardiovascular disease was 20. So the number of patient with SHPT was equally selected. However, power analysis was performed, and the number of the subjects was found to be enough for the study. However, a new study with longer follow-up period with higher number of subjects is required.

In conclusion, in our study there was a negative correlation between calcium levels and FMD. Also, in PHPT group there was a negative correlation between PTH and FMD. These results indicate that high levels of calcium can cause endothelial dysfunction. In patients with PHPT, high PTH levels may have the same effect. The positive correlation between PTH levels and hsCRP, homocysteine may show subclinical inflammation.

These results support the findings of above-mentioned previous studies; however, studies, including lager sample size and longer follow-up duration should be conducted for further evaluation.

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