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Original Article

Decreased levels of plasma preptin in female patients with knee osteoarthritis

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Abstract

Aim: Osteoarthritis (OA) is a common joint disease which is caused by the effects of mechanical, genetic and biochemical factors. In studies conducted in recent years, it has been shown that osteoblasts play roles in OA pathogenesis. In this study, we investigated the relation between the level of preptin, which is known to have an effect on osteoblast proliferation and differentiation, bone metabolism, and the OA disease.

Materials and Methods: A total of 40 healthy control patients and 40 women who were diagnosed with knee OA were included in the present study. The plasma preptin levels of the individuals who were included in the study were measured with Enzyme-linked Immuno-Sorbent Assay (ELISA) Method. Blood glucose, triglyceride, High-Density Lipoprotein Cholesterol (HDL-c), Low-Density Lipoprotein Cholesterol (LDL-c), total cholesterol levels and Body Mass Indices (BMI) leukocyte, lymphocyte, neutrophil, Erythrocyte Sedimentation Rate (ESR), C-Reactive Protein (CRP) of all participants were measured.

Results: The preptin levels of the knee OA patients in plasma were significantly lower (206 ± 103 ng/L) compared to the healthy control Group (501 ± 525 ng/L) ($p<0.001$). In addition, the (CRP) levels ($p<0.02$) and the Body Mass Indices (BMI) ($p<0.001$) were also higher in the OA Group compared to the Control Group.

Conclusion: In the present study, it was determined that there is a significant relation between knee OA disease and plasma preptin levels. Preptin may have roles in the pathogenesis of the OA disease. Further studies are needed to elucidate the mechanisms through which molecular mechanisms preptin is related to knee OA.

Keywords: Osteoarthritis; knee; preptin; ELISA; plasma

INTRODUCTION

Osteoarthritis (OA) is a common joint disease which is caused by the effects of mechanical, genetic and biochemical factors (1,2). Aging is a major risk factor in OA. In aging, changes occur in the chondrocytes in the joint cartilage and in the destruction of the matrix components (3). Progressive loss of cartilage is accompanied by subchondral bone remodeling, osteophyte formation, and synovial inflammation and pain (4). About 40% of the adult population over the age of 65 years has symptomatic knee or hip OA. Knee OA is more common than hip OA (5,6). Knee OA is one of the reasons for mobility limitation and disability (7). In a study conducted in Turkey, in individuals over 50 years of age with symptomatic knee OA, the prevalence was found as 14.8%. The prevalence of knee OA was 22.5% in women and 8% in men (8).

Preptin, a peptide consisting of 34 amino acids, was discovered in 2001. Preptin was found to be secreted from isolated pancreatic beta cells with insulin and amylin (9). Although the physiological role of preptin has not been fully elucidated, it has been shown to have an anabolic effect for bone formation (10). Preptin has also been shown to have a proliferative effect in osteoblasts in vitro and to increase bone mineralization in vivo (11). In a study on humans, serum preptin levels were shown to be low in osteoporosis and osteopenia patients (12). In addition, a positive correlation was found between the level of markers of bone production and serum preptin level (13).

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The effect of preptin on bones and its relationship with bone diseases suggest that preptin may also be associated with OA disease. In this study, we investigated the relationship between knee OA disease and plasma preptin level.

MATERIAL and METHODS

Subject

The present study was conducted in accordance with the protocol approved by Bozok University Local Ethics Committee (2017-KAEK-189_2019.01.02_03); and according to the Helsinki Declaration (1975, as revised in 2000). Forty knee OA patient women between the ages of 45 and 82 applying to orthopedics clinic, and 40 healthy controls were included in the study following their informed consents were obtained. The diagnostic criteria (clinical and radiological) were based on American College of Rheumatology Criteria (1986), and on the Kellgren and Lawrence scores (14,15). The patient (study) group consisted of Grade 3 (n=12) and Grade 4 (n=28) patients who had knee plain radiographs and OA diagnosis. The Control Group consisted of those who had knee plain radiographs because of other complaints. Exclusion criteria were having previous knee injury or joint infection, secondary posttraumatic OA, systemic inflammatory or autoimmune disorders, known malignant tumor, end-stage renal or hepatic disease, diabetes, and history of corticosteroid medication. Leukocyte, neutrophil, lymphocyte, C-Reactive Protein (CRP), Erythrocyte Sedimentation Rate (ESR), blood glucose, triglyceride, Low-Density Lipoprotein Cholesterol (LDL-c), High-Density Lipoprotein Cholesterol (HDL-c), total cholesterol levels and Body Mass Indices (BMI) of all participants were measured.

Biochemical Analysis

Blood samples were taken from patients and the control group between 09.00 - 10.00 am; and they were taken to vacutainers with Na2-EDTA (1.5 mg/mL). The complete blood count, blood glucose level, blood lipid data of the groups were received from hospital registry system. After blood collection, samples were centrifuged at 3000 rpm for 10 min for plasma isolation. The supernatant was removed rapidly and was kept frozen at -80^{0}C until the assays, which were carried out a specialist blind to patients determination of plasma preptin levels.

The Enzyme-linked Immuno-Sorbent Assay (ELISA) kit with a minimum detectable concentration of 10 ng/mL and 4000 ng/mL was employed in determining the plasma preptin levels (Bioassay Technology Laboratory, Shanghai, China). The Spectramax ELISA reader (Molecular Devices) was used to determine the optical density values of the samples and standard samples at 450 nm. The results are presented as ng/L.

Statistical Analysis

The data analysis was carried out with the SPSS 20 Package Program. The normality of the quantitative data was checked with the Shapiro-Wilk normality test; and the Independent-samples t-test was employed to compare normally-distributed data between groups. The non-normally distributed data were compared by using the Mann-Whitney U test. The correlation analysis of the data with normal distribution was carried out with the Pearson Correlation Analysis; and the correlation analysis of the data not showing normal distribution was carried out with Spearman's Correlation Analysis. To define the quantitative data, arithmetic mean ± standard deviation (SD) was employed; and $p<0.05$ was considered to be statistically significant.

RESULTS

The statistical analysis results for all the data are given in Table 1. The plasma preptin levels were low at a significant level in the OA Group (206±103 ng/L) compared to the Control Group (501±525 ng/L) ($p<0.001$). It was determined that the CRP level of the OA Group (4.41±6.79) was higher at a significant level than the Control Group (2.76±2.39) ($p<0.02$). It was also determined that the BMI values were higher at a significant level in the OA Group (32±5.3) than in the Control Group (29±3.6) ($p<0.025$). No correlations were detected between the preptin and OA disease stage and all other parameters.

Table 1. Baseline clinical and laboratory characteristics

Variables	Control (n= 40)	OA (n= 40)	P value
Age	60±6	63±9	0.9
BMI (kg/mm2)	29±3.6	32±5.3	0.001*
FBG (mg/dL)	94±11,9	96 ±12.3	0.341
Leukocyte (mm3)	7.4±2.21	7.02±1.9	0.279
Lymphocyte (mm3)	2.9±4.2	2.7±4.2	0.616
Neutrophils (mm3)	4.36±1.64	4.18±1.47	0.705
CRP (mg/dl)	2.76±2.39	4.41±6.79	0.02*
TG mmol/L	128± 60	150±80	0.736
LDL-c mmol/L	118± 39	127±25	0.300
HDL-c mmol/L	58.1±14.4	57±16.2	0.765
TC mmol/L	205±38	216±33	0.205
ESR (mm/h)	17±6.9	20±12	0.158
Preptin (ng/L)	501±525	206±103	0.001*

All values are presented as mean ± SD. *P<0.05 compared with control group. BMI indicates Body Mass Index; CRP, C-Reactive Protein; ESR, Erythrocyte Sedimentation Rate; FBG, Fasting Glucose; TG, Triglyceride; LDL-c, Low-Density Lipoprotein Cholesterol; HDL-c, High-Density Lipoprotein Cholesterol; TC, Total Cholesterol.

DISCUSSION

As the most common joint disease, OA reduces quality of life significantly by causing movement limitation and disability. Although it is also seen in the hands, hips and the spine, it is mostly seen on the knees. The incidence of OA increases with advanced age, and is

higher in females than in males (7,8). For this reason, we conducted our study in women who were at and over the age of 45 with knee OA disease. It was reported that estrogen deprivation that occurs in elderly women is the basic reason of the destruction of cartilage that causes OA (16). Furthermore, it is also known that some other factors (genetic, obesity and mechanical effects on the joint) contribute to OA risk (17). Some theories have been put forward on OA formation one of which arguing that OA initiates with chondrocyte metabolism and cartilage destruction disorder. According to another evidence, synovitis is the primary triggering mechanism of OA process leading to cartilage damage (18). Moreover, according to recent evidence, subchondral bone might be held responsible for articular cartilage degeneration of the exaggerated bone formation (17,19). There are many studies suggesting that Osteocalcin, which is one of the biomarkers of bone formation, is associated with OA (20). There are many studies suggesting that other biomarkers that have physiological roles in bone formation are also associated with OA (17). It has been shown in previous studies that the substances that affect bone formation also have physiological roles related to regulating the energy metabolism (21).

In 2001, preptin was discovered as a peptide that was derived from proinsulin-like growth factor II (proIGF-II). The presence of proIGF-II, which is known to have mitogenic effect, in serum and many tissues in humans and animals was determined (22,23). Preptin was firstly known for its effect to increase the release of glucose-mediated insulin from the pancreas (9). The number of studies conducted on preptin is very few. In preptin-related studies, the focus is mostly on its relation with energy and bone metabolism (24). It was determined that the treatment of preptin causes osteoblast proliferation and differentiation in vitro, but it also suppresses apoptosis in osteoblasts. In the same study, the anabolic effect of preptin on bone was shown in vivo (11). Osteoblasts and chondrocytes are derived from the same stem cells (pluripotent mesenchymal stem cells) (25,26). With the evidence reported in recent studies, it has been shown that osteoblasts play roles in OA pathogenesis (27). It was shown in previous studies that there is a close relation between nuclear factor-kappaB ligand (RANKL)/RANK/osteoprotegerin (OPG) system and the subchondral bone alteration seen in OA (28,29). It was also reported in some previous studies that abnormal mineralization occurs in the subchondral bone in the pathogenesis of OA (30). In the study that was conducted by Cornish et al., it was reported that preptin has an effect that increases bone mineralization (11).

It was shown in previous studies that preptin activates the ERK/Mitogen-Activated Protein Kinase (MAPK) pathway, and increases the level of Connective Tissue Growth Factor (CTGF) (31). It was also found that there is a correlation between serum preptin and osteocalcin levels in humans (32). It was shown that preptin increases osteogenesis by activating Wnt/β-catenin signaling pathway (10). It is known that the same signaling pathway is influential in the growth and development of chondrocyte (33). This signaling pathway has relations with diseases like deformity of skeletons, dwarfism, osteoporosis, degenerative joint disorders and high-bone mass syndrome (34). In human and animal studies, the relation of Wnt pathway genes with hip OA, knee OA, and osteoporosis was reported (35). In another study, it was shown that the preptin levels are low in female osteoporosis and osteopenia patients compared to healthy women (12). Considering the findings of all previous studies, the idea that "the preptin levels are low in knee OA patients compared to healthy subjects" was our hypothesis. As a matter of fact, it was found in our study that preptin levels were lower at a significant level in female OA patients compared to healthy women. In our study, although CRP levels were found to be higher in OA group compared to the Control Group, CRP levels were within normal limits in both groups. It was reported in previous studies that CRP levels increased in OA patients. There are also several studies arguing that CRP levels are associated with OA disease stage (17,36). No correlations were detected between the grade of the disease (Grade 3 and 4) and CRP levels. In our study, the BMI levels were also higher in the OA Group compared to the Control Group. It is already known that BMI is an important risk factor for OA (37).

CONCLUSION

It was found in the present study that preptin peptide that is known to have anabolic effects on bone metabolism is associated with knee OA disease. Further studies are needed to understand the mechanisms of preptin in patients with knee OA.

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