Serum leptin and 25-hydroxyvitamin D levels in knees of osteoarthritis patients

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Background	:	*Osteoarthritis (OA) is a degenerative articular disease that involves progressive alterations in all joint structures resulting from aging and overuse activity. Leptin and vitamin D play a crucial role in energy metabolism; however, few reports on leptin and hydroxyvitamin D in knees of osteoarthritis (OA) has been known.*
Objective	:	*To examine serum levels of leptin and vitamin D in knee OA patients and analyze the possible relationship between serum leptin and vitamin D in OA patients.*
Methods	:	*In a cross-sectional study, 235 adult patients (212 women and 23 men, aged 65.6 ± 6.5 years) with established diagnosis of OA were recruited. Serum 25-hydroxyvitamin (25(OH)D) and serum leptin levels were measured.*
Results	:	*Serum vitamin D insufficiency (≤ 30 ng/ml) was found in 48.0% of the patients with OA; whereas serum vitamin D deficiency (≤ 20 ng/ml) was detected in 35.0% of the osteoarthritis patients. Serum leptin expression was significantly elevated in OA patients when compared with the controls (P = 0.02). There was a negative correlation between serum 25 (OH) D concentration and serum leptin in the patients with knee OA (P < 0.001) (= -0.26, P < 0.001).*

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Conclusions   :   *Vitamin D insufficiency and deficiency is highly prevalent in OA patients and associated with higher serum leptin. These findings suggest that high serum leptin could be used for predicting low vitamin D in knees of OA patients.*

Keywords   :   *Leptin, knee, osteoarthritis, vitamin D.*

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เหตุผลของการทำวิจัย : *โรคข้อเสื่อมเป็นโรคเกิดจากการเสื่อมสภาพของกระดูกอ่อนผิวข้อ ซึ่งเกี่ยวเนื่องกับผู้สูงวัยและการใช้งานมากเกิน เลปตินและวิตามินดี มีบทบาทสำคัญต่อเมตาบอลิซึมและพลังงาน อย่างไรก็ตามการศึกษา เลปตินและวิตามินดีในโรคข้อเสื่อมยังมีจำนวนน้อย*

วัตถุประสงค์ : *เพื่อตรวจวัดระดับซีรัมเลปตินและวิตามินดีในผู้ป่วยโรคข้อเสื่อม และวิเคราะห์ความสัมพันธ์ระหว่างซีรัมเลปติน วิตามินดี และตัวชี้วัด ทางคลินิกในผู้ป่วยโรคข้อเสื่อม*

วิธีการทำวิจัย : *การศึกษาภาคตัดขวางในผู้ป่วยโรคข้อเข่าเสื่อมจำนวน 235 ราย (เพศหญิง 212 ราย และเพศชาย 23 ราย อายุเฉลี่ย 65.6 ± 6.5 ปี) และกลุ่มควบคุมที่มีสุขภาพดีจำนวน 50 ราย โดยตรวจวัดระดับ ซีรัมเลปตินและวิตามินดี*

ผลการศึกษา : *ร้อยละ 48.0 ของผู้ป่วยโรคข้อเสื่อมมีภาวะพร่องวิตามินดี ($\leq$ 30 ng/ml) และร้อยละ 35.0 ของผู้ป่วยโรคข้อเสื่อมมีภาวะขาด วิตามินดี ($\leq$ 20 ng/ml) นอกจากนี้ระดับซีรัมเลปตินในผู้ป่วย โรคข้อเสื่อมสูงกว่ากลุ่มควบคุมอย่างมีนัยสำคัญ (P = 0.02) ระดับ ซีรัมเลปตินแปรผกผันกับระดับวิตามินดีในผู้ป่วยโรคข้อเข่าเสื่อม (r = -0.26, P < 0.001)*

สรุป : *ภาวะขาดวิตามินดีและพร่องวิตามินดี พบได้บ่อยในผู้ป่วยโรคข้อเข่า เสื่อม และแปรผกผันกับระดับซีรัมเลปตินที่สูงขึ้น ซึ่งชี้ให้เห็นว่าระดับ ซีรัมเลปตินที่สูงขึ้นนี้ อาจใช้เป็นตัวบ่งชี้และทำนายภาวะพร่องหรือ ขาดวิตามินดีในผู้ป่วยโรคข้อเข่าเสื่อมได้*

คำสำคัญ : *เลปติน, ข้อเข่า, โรคข้อเสื่อม, วิตามินดี.*

Osteoarthritis (OA) is the most known cause of musculoskeletal disability and pain worldwide. OA is characterized by degradation of the articular cartilage, subchondral bone cyst, osteophyte formation, joint space narrowing, and synovial inflammation.[1] The symptom of the disease increases with age and involves mainly joint pain, shortening of the surrounding of knee muscles and decreased range of motion that lead to severe pain and disability in later life.[2] There are many factors for reducing skeletal muscle mass such as sedentary lifestyle, energy intake imbalance, hormone imbalance, oxidative stress, and inflammatory cytokines.[3]

Leptin is secreted mainly by adipocytes from the white adipose tissue that functions in regulation of food intake by stimulating energy expenditure. [4] Leptin has been shown to play a role in inflammation, angiogenesis, and cartilage metabolism. Furthermore, leptin reveals a positive correlation with body mass index (BMI) that obese individuals have significantly higher circulating leptin levels in comparison to the non-obese.[5] Specifically, higher leptin levels are associated with sarcopenic visceral obesity [6] and poorer mobility-based functioning in middle-aged to elderly. [7] In OA, the potential role of leptin is supported by leptin levels in the synovial fluid that is positively related to BMI [8] and severity of OA. [9] The purpose of this study was to assess serum leptin levels and vitamin D status in knees of OA patients and in healthy controls.

Methods
Study population

Through the Eligibility criteria, 235 adult patients were recruited (112 women and 23 men, aged 65.60 ± 6.50 years) at King Chulalongkorn Memorial Hospital; they met the criteria of American College of Rheumatology including 50 healthy volunteers (47 women and 3 men; mean age 66.70 ± 6.17 years) with no clinical sign of OA.

This study has been approved by the Institutional Review Board (IRB) on Human Research of the Faculty of Medicine, Chulalongkorn University, Thailand. Written informed consent was obtained from the patients and healthy volunteers prior to their participation in the study.

Quality of life assessment

Information about age, sex, self-report pain and health-related quality of life was obtained by personal interview through questionnaires. Western Ontario and MacMaster University (WOMAC) assesses pain, stiffness, and physical disability in knees of OA patients which the scale was displayed range from 0 - 10. A total WOMAC score was created by summing the items for all three subscales that a higher score on the WOMAC indicate worse pain, stiffness, and functional limitations.

Thai version of the Short Form Health Survey (SF-12) assessed health-related quality of life including physical health composite scores (PCS), mental health composite scores (MCS) that range from 0 to 100 with higher point shown better self-reported health.

Anthropometry

The participants were measured height, weight, waist circumference (WC). Body mass index (BMI) was assessed with the following formula:

BMI = weight/height.^{2} Bioelectrical impedance analysis (BIA: Tanita BC-418, Japan) was used to estimate appendicular skeletal muscle mass (ASM). The ASM estimation was formed from the sum of the skeletal muscle mass of the arms and legs, excluding the "trunk part". Appendicular skeletal muscle mass index (ASMI) was assessed using ASM divided by squared height, whereas skeletal muscle index (SMI) was measured in terms of percentage of ASM divided by body weight (%).

Biochemical determination

Fasting early morning venous blood were collected after a fast of at least 8 hours, centrifuged, and serum samples stored at -70 $^{\circ}$C. Serum levels of leptin were determined by enzyme-linked immunosorbent assay (ELISA) using a kit from R&D Systems, Minneapolis, MN, USA. Serum levels of 25-hydroxyvitamin D (25(OH)D) were measured with chemiluminescent immunoassay (CLIA). Serum concentration of high-sensitivity C-reactive protein (hs-CRP) was measured using an autoanalyzer.

Statistical analysis

Statistical analyses were carried out using the Statistical Package for Social Sciences (SPSS software), v.22.0 for Windows. Student's t - test and Mann Whitney U test were analyzed to compare the means of two independent groups. Multiple regression analysis was performed to determine the association between serum leptin, vitamin D, and hs-CRP by taking age, gender, and BMI as the covariates. Data were summarized as mean ± SD and median (25th, 75th percentiles). A P-value less than 0.05 were considered to be statistically significant for differences and correlations.

Results

Comparison between knee OA patients and healthy control

A total of 235 knees of OA patients and 50 healthy controls participated in the study. The characteristics of the study population are shown in Table 1. No significant differences were found in demographic data between OA knees and controls regarding age and WC, but BMI of the knees of OA patients were lower than controls. However, knee OA patients had significantly higher serum leptin levels than healthy controls (26.94 ± 21.40 versus 19.78 ± 11.16 ng/ml, P = 0.02) as shown in Figure 1. Moreover, the prevalence of vitamin D insufficiency (20.0 - 30.0 ng/ml) in the knees of OA patients was 49.5%; vitamin D deficiency (≤ 20 ng/ml) was 35.0% and vitamin D sufficiency (>30.0 ng/ml) was 15.5%. There was a negative association between serum leptin and serum 25-hudroxyvitamin D levels (r = -0.26, P < 0.001) as shown in Figure 2.

Comparison between lower muscle mass and normal muscle mass in knee OA

As the criterion of low skeletal muscle index was defined below 2 SD of the mean value in the healthy young adult population, in the present study, we classified low muscle mass by using the cut-off points as SMI < 30.44 % in men and < 25.81 % in women.[10] The demographic data of knees of OA patients with lower muscle mass or normal muscle mass are presented in Table 2. The mean of age in OA patients with lower muscle mass or normal muscle mass was not different but BMI, and WC, were higher in OA patients with lower muscle mass.

Table 1. Characteristics of participants.

	Knee OA patients n = 235	Healthy controls n = 50	*P* - value
Gender (F/M)	212/23	47/3	0.61
Age (years)	65.60 ± 6.50	66.70 ± 6.16	0.11
BMI (kg/m^2)	25.79 ± 3.93	24.08 ± 3.8	0.006
WC (cm)	88 (82, 94)	82 (77, 92)	0.19
Leptin (ng/ml)	26.94 ± 21.40	19.78 ± 11.16	0.02
25 (OH)D (ng/ml)	23.52 ± 8.10	NA	

BMI: Body mass index, WC: Waist circumference, NA: Not available. Data of Age, BMI, and leptin are expressed as Mean ± SD.
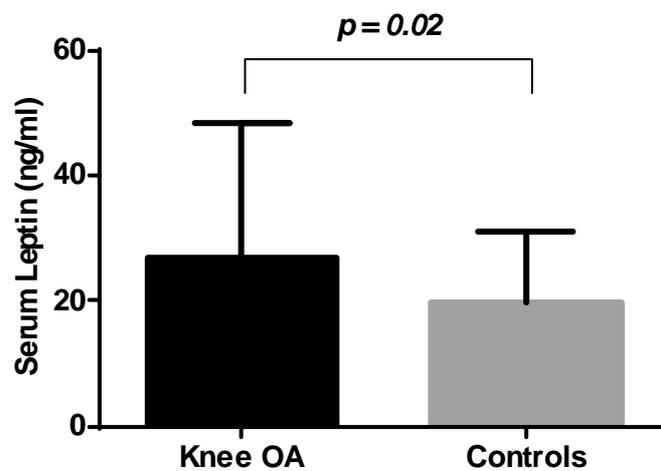
Figure 1. Serum leptin levels in knee OA and healthy controls. Data are expressed as mean ± SD.
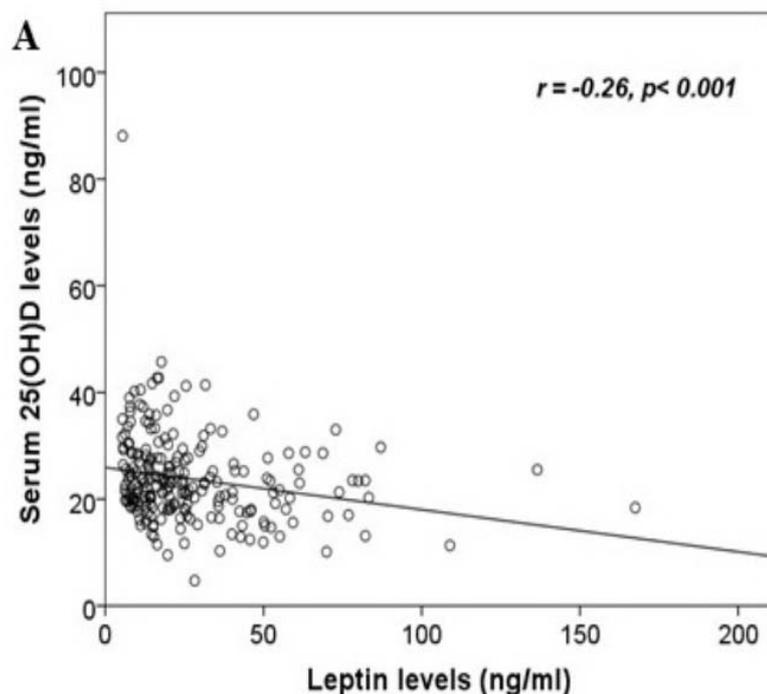
Figure 2. Aninverse correlation between serum 25(OH)D concentration and serum leptin in patients with knee OA ($P < 0.001$) ($r = -0.26$, $P < 0.001$).

Table 2. The demographic data of knee OA patients with lower muscle mass or normal muscle mass.

	Knee OA patients		
	Lowe rmuscle mass n = 58	Normal muscle mass n = 177	*P* - value
Gender (F/M)	56/2	156/21	0.06
Age (years)	65.49 ± 6.79	63.44 ± 7.57	0.07
BMI (kg/m^2)	28.40 (26.50, 32.30)	24.35 (22.50, 26)	<0.001
WC (cm)	94.50 (91, 99.5)	85 (80, 91)	<0.001
WOMAC			
Pain (0 - 10)	3.05 ± 2.28	2.41 ± 2.00	0.06
Stiffness (0 - 10)	3.00 ± 2.87	2.61 ± 2.39	0.34
Physical disability (0 - 10)	3.54 ± 2.26	2.90 ± 2.10	0.06
Total score (0 - 10)	3.20 ± 2.20	2.61 ± 1.89	0.08
SF-12			
PCS (0 - 100)	32.20 (29.10, 39.80)	38.00 (32.35, 46.80)	0.001
MCS (0 - 100)	50.75 (43.80, 55.10)	49.50 (41.40, 57.40)	0.70
hs-CRP (mg/dl)	2.09 (0.96, 4.42)	0.96 (0.56, 1.58)	<0.001

Table 2. (Con) The demographic data of knee OA patients with lower muscle mass or normal muscle mass.

	Knee OA patients		
	Lower muscle mass n = 58	Normal muscle mass n = 177	P - value
25 (OH)D (ng/ml)	23.29 ± 7.09	23.31 ± 7.30	0.88
Leptin (ng/ml)	35.54 (23.90, 55.08)	16.32 (10.81, 25.18)	<0.001

F: female, M: male, BMI: Body mass index, WC: Waist circumference, WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index, PCS: Physical health composite scores, MCS: Mental health composite scores, hs-CRP: high-sensitivity C-reactive protein and 25(OH)D: 25-hydroxyvitamin D.

Age, WOMAC, and 25(OH)D values are expressed as mean ± SD and assessed by two-sample t - test. Leptin, WC, SF-12, C-reactive protein, and leptin are expressed as median (25th, 75th percentiles) and analyzed by Mann-Whiney U-test.

Self-report pain and health-related quality of life, WOMAC scores were not statistically difference in the level of pain, stiffness and disability, whereas, PCS score of SF-12 was lower score in lower muscle mass group.

Biochemical markers, serum C-reactive protein and leptin levels in OA patients with lower muscle mass had significantly higher than normal muscle mass group as shown respectively. (2.09 versus 0.96 mg/dl, $P < 0.001$ and 35.54 versus 16.32 ng/ml, $P < 0.001$). Box plots of serum leptin in knee OA patients as shown in Figure 3. However, there was *no* significant *difference in* vitamin D status between groups.
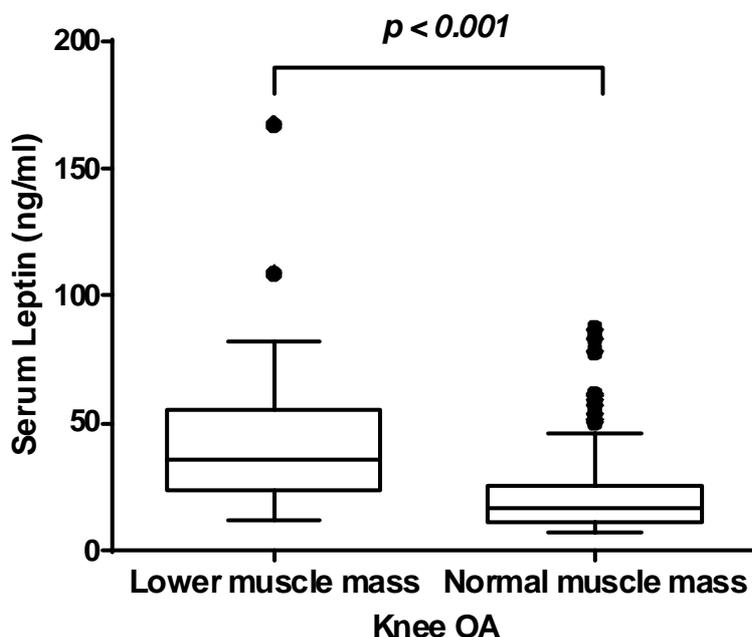

Figure 3. Box plots of serum leptin in knee OA patients with lower muscle mass or normal muscle mass.

Discussion

Leptin is a pro-inflammatory cytokine that is associated with cardiovascular disease [11], diabetes [12] and OA knees. [1, 13] Leptin concentrations are dependent on body fat mass [14] and relate to BMI. In OA patients, synovial fluid leptin levels are positively related with BMI. [8] In our study, we found that leptin levels in OA patients were higher than in healthy controls. There were significantly positively associated with visceral obesity and C-reactive protein (hs-CRP) that shown as chronic low grade inflammation, whereas leptin levels were negatively related with vitamin D status in knee OA patients. In fact, leptin levels were found to be higher in the knees of OA patients with sarcopenia and visceral obesity or lower vitamin D status.

Furthermore, our data showed that serum leptin levels in OA patients were positively related with BMI and WC. In agreement with our results, Waters DL, et al. [15] investigated the relationship between leptin levels and body composition phenotypes, elderly persons with lower appendicular skeletal muscle mass were significantly higher leptin levels than normal muscle mass group. Kohara K, et al. [6] found that a positive relationship between serum leptin levels and visceral obesity and a negative correlation with thigh muscle cross-sectional area in middle-aged to elderly.

Moreover, we investigated that knees of OA patients with low muscle mass were visceral obesity or a higher BMI, increased serum levels of both leptin and hs-CRP, and poor quality of life than normal muscle mass group, while vitamin D level did not different between groups. Accordingly, we performed following adjustment with age, sex, and BMI. After adjustment,

leptin levels were independently associated with pro-inflammatory biomarker and low vitamin D status. Leptin and hs-CRP are pro-inflammatory cytokines related to physical disability.

Since, vitamin D is recognized as a key factor in muscle metabolic processes by the protein synthesis of muscle fibers and regulating muscle contractility.[16] Previous studies showed a low vitamin D level correlated with decreased muscle strength, difficulty in physical function, increased risk of falls, fracture, and hospitalization leading to frailty in the elderly. [17 - 19] Our data, lower and normal muscle mass group shown no difference between 25(OH)D levels. We found that the *mean 25(OH) D* serum levels between groups have shown vitamin D insufficiency. Heidari B, et al. [20] reported that a high prevalence rate of knees in OA patients with vitamin D deficiency aged younger than 60 years that was associated with early symptoms of OA and the progress of knee cartilage damage. Sanghi D, et al. [21] found that approximately 63% of primary knee OA patients had vitamin D insufficiency (25(OH) D ≤ 30 ng/ml).

In addition, the present study showed that leptin concentrations are negatively associated with 25(OH)D. These observations are in agreement with Maetani M, et al. [22] reported that low vitamin D levels were associated with increased leptin levels in adult female. Parikh S, et al. [23] demonstrated that 25(OH)D concentrations were negatively significantly correlated with leptin levels in adolescents, on the one hand. On the other hand, Al-Daghri NM, et al. [24] found that vitamin D was positively associated with serum leptin in male Saudi adult. The study from in vitro and *in vitro* were supported leptin may also involve vitamin D metabolism. *In vitro*, 1, 25(OH)$_2$D$_3$ represent

a direct inhibitory effect on leptin secretion from human adipose tissue culture. [25] In mice model, leptin particularly suppressed mRNA accumulation and activity of renal 1α-hydroxylase (CYP27B1) through leptin receptor that leading to a critical converting vitamin D to its bioactive form 1, 25(OH)$_2$D$_3$. [26] These findings suggest that serum leptin level may be associated with vitamin D status in OA.

Acknowledgements

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