Correlation between serum leptin, cytokines, cartilage degradation and functional impact in obese knee osteoarthritis patients

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KEYWORDS
Leptin; Matrix metalloproteinase-13 (MMP13); Cytokines; Nitric oxide (NO); Obesity; Knee osteoarthritis (KOA)

Abstract  Aim of the work: The aim of the present work was to correlate between serum level of leptin, matrix metalloproteinase-13 (MMP13), interleukin-1β (IL1β), tumour necrosis factor (TNF-α), nitric oxide (NO) and functional impact in obese patients with knee osteoarthritis (KOA).

Patients and methods: The study included 84 obese patients suffering from primary KOA. The knees were examined; pain assessed by visual analogue scale (VAS) and Lequesne’s index for functional impact calculated. Serum leptin, MMP13, IL1β, TNF-α and NO were assessed.

Results: The mean age of the patients was 54.64 ± 7.7 years. They were 72 females and 12 males (F:M = 6:1) with a mean BMI of 35.29 ± 4.8. Sixty patients had knee effusion which was bilateral in 54 (64.3%), unilateral in 6 (7.2%) while 24 (28.6%) did not have any effusion. Knee deformities were present in 60 (71.4%) patients. Female patients were only significantly higher than males regarding Lequesne’s index (15.08 ± 4.4 vs 11 ± 3.4, \( p = 0.003 \)). Patients with knee deformity had significantly higher VAS (9.12 ± 1.3 vs 6.96 ± 0.46, \( p = 0.001 \)), IL1β (621.1 ± 98.8 vs 503.9 ± 74.6 pg/ml, \( p = 0.001 \)), TNF-α (115.4 ± 29.1 vs 87.4 ± 4.4 pg/ml, \( p = 0.001 \)), NO (67.32 ± 5.7 vs 59.2 ± 2.2 μmol/L, \( p = 0.001 \)), MMP13 (33.98 ± 2.24 vs 30.1 ± 1.7 ng/ml, \( p = 0.012 \)) and leptin (13.2 ± 1.6 vs 10.4 ± 0.6, \( p = 0.004 \)) than those without. The VAS, Lequesne index, IL1β, TNF-α, NO and MMP13 were significantly higher in patients with bilateral knee effusion (\( p < 0.0001 \) for all) compared to those with unilateral effusion or without. Leptin significantly correlated with BMI, VAS, Lequesne’s index, IL1β, TNF-α, NO and MMP13 (\( p < 0.0001 \) for all).

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1. Introduction

Knee osteoarthritis (KOA) is the most common type of arthritis leading to pain and disability in the elderly population [1,2]. Obesity is a significant risk factor for KOA and also increases the risk of osteoarthritis (OA) in non-weight bearing joints such as the hands [3]. This means that the mechanical factors alone do not explain the cause effect relationship between obesity and KOA [4]. In a previous study on Egyptian patients with KOA it was found that synovial vascular endothelial growth factor (VEGF) remarkably correlated with clinical, functional impact, as well as radiological changes [5]. In other studies, osteopontin served as a biochemical marker of disease severity in KOA [6] and obesity increased the risk of renal dysfunction [7] in Egyptian patients with KOA. It has furthermore been reported that the measurements of hyaluronic acid and serum cartilage oligomeric matrix protein may be of diagnostic and prognostic value in differentiating patients with early KOA. The combination with other biochemical markers as well as with clinical and radiographic data would most likely help to improve the clinical assessment of KOA patients [8].

Recent studies suggest that systemic inflammatory mediators namely adipokines contribute to the increased risk of osteoarthritis with obesity [9-11]. These adipokines mediate synovial inflammation and up regulate cartilage matrix synthesis and degradation [9,10]. One of those adipokines is leptin which is a peptide hormone secreted primarily by adipocytes [12,13]. It regulates body weight by centrally inhibiting food intake and stimulating energy expenditure. Studies have shown high serum and synovial levels of leptin in obese patients which also correlated with percentage of body fat. This deficit in leptin action suggests that obesity may be associated with central resistance to leptin [14,15]. Leptin modulates the degradative functions of the chondrocytes through up-regulation of matrix metalloproteases. Moreover, leptin was shown to enhance the synthesis of proinflammatory mediators in human OA cartilage [15]. Thus, leptin may be a metabolic link between obesity and OA [16].

The relationship between serum levels of leptin and the severity of KOA in obese patients as reflected by the functional impairment as well as laboratory parameters has to be more clarified. The aim of this study was to correlate between serum level of leptin, matrix metalloproteinase-13 (MMP13), interleukin-1β (IL1β), tumour necrosis factor-alpha (TNFα), nitric oxide (NO) and functional impact in obese patients with knee OA.

2. Patients and methods

This cross sectional study was conducted on 84 obese patients (Body mass index BMI ≥ 30 kg/m²) [17] suffering primary knee OA according to the American College of Rheumatology (ACR) classification criteria [18]. Patients that did not receive any medication for treatment of the symptoms of knee OA at least 4 weeks prior to obtaining their blood test were enrolled in the study. Patients signed an informed consent and the ethical approval was obtained from the research committee of our institution before initiation of the study. The exclusion criteria were secondary OA due to any other causes as rheumatologic diseases such as rheumatoid arthritis, any clinical evidence of knee trauma or any orthopaedic diseases, medical diseases such as diabetes mellitus, hypercholesterolemia or cardiovascular diseases.

The following data were recorded for all patients; medical, occupational and drug history. The body mass index was calculated [19] and a detailed knee examination was performed including; alignment, presence of any deformity, bilaterality of disease, tenderness and effusion. Visual analogue scale (VAS) for knee pain was recorded [20]. Lequesne’s algofunctional index was carried out [21].

Serum leptin was detected with enzyme-linked immunosorbent assay (ELISA) (DRG International Inc., DRG Leptin sandwich ELISA; EIA-2395); the following values were observed: males 3.84 ± 1.79 ng/mL, females 7.36 ± 3.73 ng/ml. The MMP13 (Human Matrix Metalloproteinase-13; MMP-13, ELISA Cat. No.: RBMS2022R Biovendor – Laboratorı́́ų, medicı́́na a.s, Czech republic), IL1β (Abcam’s Human Interleukin-1β ELISA kit ab100562) TNF-α (RayBio® Human TNF-alpha ELISA Kit Cat#: ELH-TNFα RayBiotech, Inc.) and NO (Kit for Quantitative Determination by Human Nitric oxide ELISA Kit, CK-E30290, Glory Science) were measured using ELISA. Normal serum Human MMP-13 levels ranged between 0 and 9.7 ng/mL. Normal serum IL1β: 5.4 ± 3.9 pg/ml, ranging between 0 and 13.6 pg/ml. Normal level of TNF-α was 11.2 ± 7.31 (0.0–32.5 pg/mL). Normal NO ranged from 10 to 20 μmol/L.

2.1. Statistical analysis

Data were analysed using statistical package for social science version 20( SPSS 20). Mean and standard deviation were used to describe data distribution. T test was used for comparison between 2 groups. Analysis of variance (ANOVA) was used for comparison between more than 2 groups. Pearson correlation was used to study the association between two parameters. The level of significance was 0.05.

3. Results

Eighty-four obese patients suffering from primary knee osteoarthritis were included in the study. Seventy-two (85.7%) were females and 12 (14.3%) males. The mean age was 54.6 ± 7.7 (41–72 years). The mean BMI was 35.3 ± 4.8 (30–50.2). Sixty patients (71.4%) had knee deformity.
Fifty-four patients (64.3%) had bilateral knee effusion, while 6 (7.1%) had unilateral knee effusion and the remaining 24 (28.6%) had no effusion. Table 1 shows the visual analogue scale of knee pain, Lequesne index and studied laboratory parameters in obese KOA patients.

Leptin was significantly correlated with BMI ($r = 0.394$, $p = 0.0001$), VAS ($r = 0.714$, $p = 0.0001$), and Lequesne index ($r = 0.714$, $p = .0001$). In addition, leptin significantly correlated with all tested laboratory variables as shown in Fig. 1 ($p = 0.0001$ for all). Table 2 documents that those with bilateral knee effusion had significantly higher BMI, VAS, Lequesne score and all tested laboratory variables in comparison with patients with no effusion or unilateral effusion. There was no significant difference between those with unilateral effusion and those without effusion regarding all variables. Table 3 shows that patients with knee deformity had significantly higher VAS, cytokines, NO, MMP13 and leptin than those without. Table 4 shows a comparison of the studied variables according to gender. Only the Lequesne index was significantly higher in females.

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Obese knee osteoarthritis patients ($n = 84$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>VAS knee pain</td>
<td>8.5 ± 1.5</td>
</tr>
<tr>
<td>Lequesne index</td>
<td>14.5 ± 4.5</td>
</tr>
<tr>
<td>IL1β (pg/ml)</td>
<td>587.6 ± 106.4</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>107.4 ± 27.8</td>
</tr>
<tr>
<td>NO (μmol/L)</td>
<td>64.99 ± 6.2</td>
</tr>
<tr>
<td>MMP13 (ng/ml)</td>
<td>32.9 ± 2.7</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>12.4 ± 1.9</td>
</tr>
</tbody>
</table>

VAS: visual analogue scale, IL1: interleukin-1, TNF: tumour necrosis factor, NO: nitric oxide, MMP: matrix metallo-proteinase.

### 4. Discussion

This study was conducted on 84 obese patients with KOA to assess the role of leptin and its correlation with clinical and functional derangement, cytokine production and cartilage degradation. The current study showed a significant correlation between serum leptin and BMI. Revising literature, serum leptin was elevated in obese persons and correlated with the percent of body fat [13,14,22], also leptin concentration decreased after weight loss [22]. High serum and synovial leptin in obese patients with OA were thought to control local inflammatory processes [23]. As obesity itself without leptin does not predispose to OA [24], it was suggested that elevated leptin might explain the metabolic role of obesity in knee OA [25,26].

The present study demonstrated a significant correlation between leptin and cytokines IL1β, TNFα. Gandhi et al. [27] found a significant correlation between serum and synovial level of leptin and IL1β and high serum level of TNFα and MMP13 among patients undergoing total knee replacement.

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**Figure 1** Correlation between serum leptin and MMP13 (upper left), IL1β (upper right), TNF-α (lower left) and NO (lower right) in obese knee OA patients.
In the current study, leptin was also significantly correlated with NO. Previously, this was also reported [28].

The present study documented a significant correlation between serum leptin and MMP13 level. This is in agreement with previous studies where leptin was found to increase production of metalloproteinases thus enhancing cartilage degradation [15,29–31]. Leptin modulates the degradative functions of the chondrocytes through up-regulation of matrix metalloproteases (MMP)-9 and -13 and IL-1β. Moreover, leptin was shown to enhance the synthesis of proinflammatory mediators in OA cartilage and to potentiate the IL-1

Table 2  Relation of the studied variables according to knee effusion in obese KOA patients (n = 84).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Knee effusion</th>
<th>Unilateral</th>
<th>Bilateral</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAS</td>
<td>6.6 ± 0.7</td>
<td>7.5 ± 0.5</td>
<td>9.4 ± 0.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lequesne index</td>
<td>12.2 ± 5.8</td>
<td>9.3 ± 0.8</td>
<td>16.1 ± 2.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI</td>
<td>34.4 ± 3.4</td>
<td>35.2 ± 4.1</td>
<td>40.1 ± 11.1</td>
<td>0.032</td>
</tr>
<tr>
<td>IL1β (pg/ml)</td>
<td>478.7 ± 96.2</td>
<td>534.7 ± 44.9</td>
<td>641.9 ± 69.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>86.1 ± 5.2</td>
<td>89 ± 6.6</td>
<td>118.9 ± 28.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NO (μmol/L)</td>
<td>57.9 ± 3.4</td>
<td>61.3 ± 4.1</td>
<td>68.6 ± 3.95</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MMP13 (ng/ml)</td>
<td>29.8 ± 1.6</td>
<td>30.8 ± 2.02</td>
<td>34.5 ± 1.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>10.3 ± 0.5</td>
<td>11.1 ± 1.3</td>
<td>13.5 ± 1.4</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

KOA: knee osteoarthritis, VAS: visual analogue scale, BMI: body mass index, IL1: interleukin-1, TNF: tumour necrosis factor, NO: nitric oxide, MMP: matrix metallo-proteinase. Bold values = significant at p < 0.05.

Table 3  Comparison between different studied variables according to the presence and absence of knee deformity in obese patients with KOA (n = 84).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Knee deformity</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAS</td>
<td>Yes (n = 60)</td>
<td>No (n = 24)</td>
</tr>
<tr>
<td>Lequesne index</td>
<td>9.1 ± 1.3</td>
<td>6.96 ± 0.5</td>
</tr>
<tr>
<td>BMI</td>
<td>15.03 ± 3.9</td>
<td>13.2 ± 5.6</td>
</tr>
<tr>
<td>IL1β (pg/ml)</td>
<td>35.6 ± 5.1</td>
<td>34.5 ± 3.95</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>62.11 ± 98.8</td>
<td>503.9 ± 74.6</td>
</tr>
<tr>
<td>NO (μmol/L)</td>
<td>115.4 ± 29.1</td>
<td>87.4 ± 4.4</td>
</tr>
<tr>
<td>MMP13 (ng/ml)</td>
<td>67.3 ± 5.7</td>
<td>59.2 ± 2.2</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>33.98 ± 2.2</td>
<td>30.1 ± 1.7</td>
</tr>
</tbody>
</table>

KOA: knee osteoarthritis, VAS: visual analogue scale, BMI: body mass index, IL1: interleukin-1, TNF: tumour necrosis factor, NO: nitric oxide, MMP: matrix metallo-proteinase. Bold values = significant at p < 0.05.

This study showed a significant correlation between knee pains documented by VAS and serum leptin. Bas et al. [34] and Perrucio et al. [35] found an association between pain severity and serum adipokine including leptin in knee and hip OA. Lübbeke et al. concluded that high synovial leptin is associated with severe preoperative pain in obese women with hip and knee OA [36].

The present study showed a significant correlation between serum leptin and Lequesne index which denotes worse functional status of the patients. No previous literature discussed the relation of serum leptin and functional status of obese OA patients as worse function denotes usually a more advanced and severe disease. The positive relation between the severity of knee OA and serum leptin was documented before [37].

This study showed that patients with bilateral knee effusion had significantly higher BMI, worse clinical manifestation measured by knee pain and Lequesne index as well as higher cytokines, MMP13 and leptin as compared with patients without or with only unilateral effusion. Knee effusion is a manifestation of ongoing synovitis and inflammation [38]. So this result is an additional proof that knee inflammation is proportionate to the degree of obesity, hence leptin and all the following cascade of inflammation.
The current study proved that patients with knee deformity had significantly higher knee pain, cytokines, NO, MMP13 and leptin than those without. Knee deformity usually occurs in advanced knee OA [39]. This also denotes that those with knee deformity i.e. advanced OA had higher leptin and cytokines and cartilage degradation. In a previous study, serum leptin significantly correlated with the severity of knee OA [37].

In the present study, we did not find significant difference between male and female regarding all tested variables except for significantly higher Lequesne in females. Less number of male patients may be the cause, but the patients were recruited randomly and sequentially.

A previous study detected higher serum leptin in women compared to men [40]. One study suggested that the higher serum leptin in women might partially account for gender disparity of KOA [26]. Since the aim of the study was originally mere correlation, control group was not included, but as the clinical and laboratory results prove a strong relation between leptin and severity of KOA in obese patients, additional longitudinal studies on a larger population and taking into consideration a control group of non obese KOA patients are encouraged to verify our results and performing a regression analysis could further confirm the role of leptin in this group of patients.

In conclusion, leptin is proportionate with the degree of obesity and is extremely related to the degree of inflammation and cartilage degradation in obese patients with knee osteoarthritis, hence the severity of KOA.

Conflict of interest
None.

References


