**Evaluation of plasma basic fibroblast growth factor (bFGF) in primary knee osteoarthritis patients**

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**KEYWORDS**

Basic fibroblast growth factor;
Osteoarthritis;
Knee;
Ultrasonography;
Kellgren–Lawrence;
WOMAC

**Abstract**  
**Aim of the work:** The aim of this study was to investigate plasma basic fibroblast growth factor (bFGF) levels in patients with primary knee osteoarthritis (KOA) and to correlate it with physical performance, functional status and radiological severity.

**Patients and methods:** Sixty patients with primary KOA and 30 healthy individuals were recruited into this study. Measures of physical performance were assessed using Chair Stand Test, Stair Climb Test and Six-Minute Walk Test. Functional assessment was carried out using the Western Ontario and McMaster Universities (WOMAC) index. KOA severity was determined using X-ray evaluated according to the Kellgren–Lawrence (KL) grading and all underwent sonographic examination. bFGF concentrations in plasma were determined.

**Results:** The mean age of the KOA patients was 53.67 ± 7.99 years, female: male 4:1 and disease duration of 4.17 ± 1.74 years. KOA was bilateral in 71.7% and knee effusion was present in 20.39%. The WOMAC index was 53.78 ± 13.7. Plasma bFGF levels in KOA patients (43.82 ± 20.18 pg/ml) were significantly higher than in controls (12.40 ± 9.12 pg/ml) \(p < 0.001\). bFGF significantly correlated the KL radiographic grading \(r = 0.31, p < 0.027\) and negatively with cartilage thickness of medial and lateral femoral condyles \(r = -0.38, p < 0.006\).

**Conclusions:** Plasma bFGF levels were significantly increased in OA patients, and these elevated levels were significantly correlated with the degree of radiographic severity evaluated by KL grading scale and cartilage degeneration evaluated by ultrasound. These findings indicate that bFGF levels may be a monitor of disease severity and could play an essential part in the pathophysiology of degenerative process in OA.

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1. Introduction

Osteoarthritis (OA) is a common chronic disease that affects all joint tissues, causing progressive irreversible damage. Pathological changes in OA not only include joint cartilage degeneration but also subchondral bone thickening, osteophyte formation and synovial inflammation, all of which are associated with capsule laxity and decreased muscle strength [1].

Many factors have been implicated in the pathogenesis of Egyptian patients with Knee OA (KOA) as interleukin-1b, tumor necrosis factor-α, leptin, matrix metalloproteinase, nitric oxide [2] hyaluronic acid, cartilage oligomeric cartilage protein [3,4], and oxidative stress [5]. Vascular endothelial growth factor significantly correlated with clinical manifestations, functional impact, as well as radiological changes of KOA [6]. Osteopontin was found to serve as another biomarker of disease severity in KOA and could be predictive to the progression of the disease [7] and with radiological grading [8]. Osteoarthritis patients are frequently associated with subclinical atherosclerosis [9] and obese KOA patients were found to represent a high risk group for renal dysfunction [10].

Basic fibroblast growth factor (bFGF), also known as FGF-2, is a polypeptide with pleiotropic effects when applied to diverse tissues, including mitogenesis, cell migration and cell differentiation [11]. FGF-2 plays an important role in tissue repair and angiogenesis. It is responsible for wound healing and modulates many cellular functions, including proliferation, differentiation and neovascularization [12,13]. However, in systemic sclerosis, another rheumatic disease, the serum level of FGF-23 showed no significant difference between patients and normal subjects with no role in the clinical manifestations of the disease [14].

Ultrasound (US) imaging is a non-invasive technique that can be used to image soft tissues. With the advancement of technology, the newer US machine models can be equipped with high frequency probes that are very useful for musculoskeletal imaging to study the peri-articular and intra-articular structures [15,16].

The aim of this study was to investigate plasma basic fibroblast growth factor (bFGF) levels in patients with primary knee osteoarthritis and to evaluate the correlation between it and clinical and radiographic parameters including conventional X-ray and ultrasonography.

2. Patients and methods

Sixty patients with primary KOA selected from the outpatient clinic of Physical Medicine, Rheumatology and Rehabilitation Department, Tanta University Hospitals. Patients fulfilled the criteria of the American College of Rheumatology (ACR) [17] for diagnosis of osteoarthritis knee. Thirty healthy volunteers matched in age, sex and body mass index (BMI) were selected as controls. The study was approved by the local research ethics committee of Faculty of Medicine, Tanta University and the patients gave an informed consent before being enrolled. Patients with other forms of arthritis, chronic inflammatory diseases, diabetes, cancer and patients with past history of knee trauma or corticosteroid injection within the past three months were excluded from the study.

The following were clinically assessed for the patients: BMI, pain using Visual Analogue Scale (VAS) [18], and duration of morning stiffness and knee effusion. Measures of physical performance were assessed using Chair Stand Test (CST), Stair Climbing Test (CCT) and Six-Minute Walk Test (6MWT) [19]. Functional assessment was carried out using the WOMAC (Western Ontario and McMaster Universities) index [20].

Venous blood samples were collected from all participants with sterile disposable syringes in sterile EDTA vacuum tube, and samples were centrifuged at 1000g for 15 min for plasma separation by means of dry clean Pasteur pipette. The plasma samples were frozen at –70°C until used for assay of plasma basic fibroblast growth factor (bFGF) level by ELISA technique [21], (Quantikine ELISA Human FGF basic Immunoassay kit Catalog Number DFB50 supplied by Clini Lab Scientific Service Company).

Osteoarthritis severity was determined using weight-bearing anteroposterior and lateral radiographs of the affected knee and was evaluated according to the Kellgren Lawrence (KL) grading system [22]. All the patients underwent sonographic examination at ultrasound unit of Physical Medicine, Rheumatology and Rehabilitation Department of Tanta University Educational Hospital using SAMSUNG MEDISON (UGEO H60) by an expert radiologist blinded to the clinical and radiological data, using linear array transducers (with frequencies ranging between 7.5 and 12 MHz). Power Colour Doppler was used to check for blood flow in the synovium. The following were considered during the ultrasound assessment: supra-patella recess effusion (mm), synovitis, synovial thickness (mm), cartilage thickness at medial and lateral femoral condyles (maximal and minimal thickness in mm), medical and lateral menisci (positive or negative degeneration) and presence of osteophytes [23].

2.1. Statistical analysis

Statistical analysis was carried out using the SPSS software, version 16.0. Demographic data between patients and controls were compared by chi-square tests and unpaired Student’s t tests where appropriate. Pearson’s correlation coefficient (r) was employed to determine correlation between concentrations of bFGF in plasma and clinical and radiographic parameters. Data are expressed as mean ± standard deviation (SD). p values < 0.05 were considered statistically significant for differences and correlation.

3. Results

Sixty knee OA patients and thirty healthy controls were enrolled in this study. There was no significant difference in demographic data between OA patients and controls. However, there was a significant difference between OA patients and controls regarding physical performance assessments and plasma bFGF levels as demonstrated in Table 1.

Regarding KL criteria; 35.9% and 32% of KOA patients revealed KL grades 1 and 3 respectively, while 25.3% and 6.8% revealed grades 2 and 4 respectively. Ultrasound findings in OA patients were demonstrated in Table 2. The decreased cartilage thickness of medial and lateral femoral condyle in knee osteoarthritis patient is shown in Fig. 1.
Evaluation of plasma basic fibroblast growth factor (bFGF) in primary knee osteoarthritis patients.

Table 1 Demographic, clinical and laboratory data in knee osteoarthritis patients and controls.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>KOA patients</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>n = 60</td>
<td>n = 30</td>
<td></td>
</tr>
<tr>
<td>53.67 ± 7.99</td>
<td>51.53 ± 4.46</td>
<td></td>
<td>0.11</td>
</tr>
<tr>
<td>Sex (female/male)</td>
<td>48/12</td>
<td>20/10</td>
<td>0.17</td>
</tr>
<tr>
<td>BMI</td>
<td>33.94 ± 7.07</td>
<td>32.67 ± 2.48</td>
<td>0.22</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>4.17 ± 1.74</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Bilateral</td>
<td>43 (71.7)</td>
<td>17 (28.3)</td>
<td>–</td>
</tr>
<tr>
<td>Unilateral</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>VAS</td>
<td>6.53 ± 1.56</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Morning stiffness (min.)</td>
<td>7.42 ± 5.78</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Knee effusion</td>
<td>21 (20.39)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>WOMAC index</td>
<td>53.78 ± 13.7</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CST (times/30 s)</td>
<td>5.65 ± 1.72</td>
<td>11.07 ± 0.78</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>SCT (s)</td>
<td>12.98 ± 6.37</td>
<td>6.47 ± 1.01</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>6MWT (/m)</td>
<td>93.5 ± 22.16</td>
<td>187.33 ± 37.5</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Plasma bFGF level (pg/ml)</td>
<td>43.82 ± 20.18</td>
<td>12.40 ± 9.12</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

KOA: knee osteoarthritis; BMI: body mass index; VAS: Visual Analog Scale; WOMAC: Western Ontario and McMaster Universities; CST: Chair Stand Test; 6MWT: Six-Minute Walk Test; SCT: Stair Climb Test; bFGF: basic fibroblast growth factor.

* Significantly different at p < 0.05.

Table 2 Ultrasound findings in the knees of knee osteoarthritis patients.

<table>
<thead>
<tr>
<th>Ultrasound findings</th>
<th>KOA patients (n = 60)</th>
<th>Findings in 103 knees</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effusion (ml)</td>
<td>25 (24.3)</td>
<td>0.61 ± 0.85</td>
</tr>
<tr>
<td>Synovial thickening (mm)</td>
<td>24 (23.3)</td>
<td>3.92 ± 2.48</td>
</tr>
<tr>
<td>Synovial activity</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Osteophytes</td>
<td>66 (64.08)</td>
<td>–</td>
</tr>
<tr>
<td>Meniscal degeneration</td>
<td>4 (3.88)</td>
<td>–</td>
</tr>
<tr>
<td>Cartilage thickness (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial femoral condyle</td>
<td>1.16 ± 0.59</td>
<td>Maximum: 1.72 ± 0.77</td>
</tr>
<tr>
<td>Lateral femoral condyle</td>
<td>1.41 ± 0.58</td>
<td>Maximum: 2.15 ± 0.52</td>
</tr>
</tbody>
</table>

KOA: knee osteoarthritis.

Plasma bFGF level significantly correlated with the KL radiographic grading of KOA (r = 0.31, p = 0.027) (Fig. 2). In addition, plasma bFGF levels negatively correlated with cartilage thickness of medial and lateral femoral condyles measured by ultrasonography. (r = −0.38, p = 0.006) (Table 3). However, there was no significant correlation between plasma bFGF levels and other sonographic findings. Moreover, no significant correlation was found between plasma bFGF levels and clinical data and physical performance.

4. Discussion

Knee osteoarthritis was more frequent in females in the present study. In another study on Egyptian patients with primary KOA, females were significantly more involved [2].

Fibroblast growth factors (FGFs) represent a large cytokine family of structurally related multifunctional polypeptide mitogens of widespread tissue distribution [24]. Basic FGF is one of the most well-characterized members of the family and one of the most powerful angiogenic polypeptides [25].

Our study demonstrated that the plasma basic fibroblast growth factor (bFGF) levels in OA patients were significantly higher than the control group. These results were in agreement with, Honsawek et al. [26] who measured plasma and synovial bFGF levels in thirty-five patients with primary KOA and found that their levels were significantly elevated compared with that of healthy controls. Jingushi et al. [27] showed that when radiolabeled FGF-2 was injected into knee joints of C57Bl/10 mice, a transient binding was observed in the superficial and intermediate zones of the articular cartilage as well as in the synovium and perichondrium. FGF-2 injection (5 μg) caused synovial hyperplasia adjacent to the articular cartilage on day 7, cartilage formation adjacent to the articular cartilage on day 14, and osteophyte on day 21. These changes were dose-dependent. No destructive changes in the joints were observed. Im et al. [28] documented that FGF-2 induces matrix metalloproteinase-13 (MMP-13), the most potent collagen-type II degrading enzyme in human articular cartilage, resulting in collagen breakdown. They used chondrocytes isolated from human adult articular cartilage 24 h after death. Treatment of cells in monolayer with bFGF significantly stimulated MMP-13 production, and the induction occurred in both mRNA and protein levels in a dose-dependent manner.

bFGF stimulates MMP-13 via molecular cross-talk between mitogen-activated protein kinases and protein kinase C delta pathways in articular chondrocytes. Li et al. [29] provide a biochemical explanation for the conflicting effects induced by FGF-2 on articular cartilage human and mice, using human articular cartilage (ex vivo) and a medial meniscal destabilization animal model (in vivo). The differences in the expression patterns of FGFR receptors (FGFRs) explain these conflicting effects. In normal human cartilage, FGFR1 and FGFR3 are predominantly expressed, with negligible levels of FGFR2 and FGFR4. In OA cartilage, FGFR3 expression level is markedly reduced, and this is closely linked with enhanced catabolic signaling in the presence of FGF-2. FGF-2 highly up-regulates FGFR3 in murine chondrocytes, whereas FGF-2 significantly down regulates FGFR3 in human articular chondrocytes. Thus, in mice, FGFR3 may also play an anabolic role in articular cartilage after injection with FGF-2.

The WOMAC index was used for functional assessment of the present KOA patients and a non-significant correlation was found with the plasma bFGF levels. The severity of KOA in our patients was detected by KL grading and ultrasonography of knee joint to assess cartilage thickness, effusion, synovial thickening and meniscal degeneration. KL grades 1, 2, 3 and 4 were found in 35.9%, 25.3%, 32% and 6.8%. The US revealed...
effusion in 24.3%, synovial thickening in 23.3%, osteophytes in 64.08% and meniscal degeneration in 3.88%. The cartilage thickness was more reduced over the medial condyle.

Saarakkala, et al. [30] investigated the diagnostic performance of non-invasive knee US to detect degenerative changes of articular cartilage compared to arthroscopic grading as the gold standard. They concluded that the sum of cartilage grades significantly correlated between both modalities and that knee US is a promising technique for screening but a negative finding does not rule out degenerative changes of articular cartilage.

Our study found that plasma bFGF levels significantly correlated with KL grades of OA knees and negatively with cartilage thickness of both medial and lateral femoral condyles in OA knees detected by US. This was in agreement with Honsawek et al. [26] who found that plasma and synovial bFGF levels were significantly increased in OA patients and correlated with radiographic severity using KL grading. They observed that plasma bFGF levels were more pronounced in end-stage compared with early OA patients. The correlation of plasma bFGF levels with OA severity was explained by Li et al. [29] who documented that bFGF plays a catabolic and anti-anabolic role in cartilage homeostasis, driving homeostasis toward degeneration.

In conclusion, the study revealed that plasma bFGF in patients with primary knee OA was significantly elevated compared with that of healthy controls. Plasma bFGF levels significantly correlated with the degree of radiographic severity evaluated by KL grading scale and negatively with cartilage thickness of both medial and lateral femoral condyles. These findings suggest that bFGF could be a useful biochemical parameter to reflect disease severity in KOA. Further studies are needed to explore the role of bFGF in the pathogenesis and progression of primary KOA and the possibility of being a novel therapeutic target.

Conflict of interest
None.

References


