Aim of work: To investigate osteopontin (OPN) levels in both plasma and synovial fluid of patients with primary knee osteoarthritis (OA) and their relationship with radiological grade.

Patient and methods: Sixty patients had knee OA and 30 control subjects were included. Anteroposterior knee radiographs were taken to determine the disease severity of the affected knee. The radiographic grading of OA in the knee was performed by using the Kellgren–Lawrence grading. Osteopontin levels in the plasma and synovial fluid were measured using enzyme-linked immunosorbent assay and compared.

Results: OA patients had higher plasma osteopontin concentrations compared to healthy controls ($p < 0.000$). Osteopontin levels in synovial fluid were significantly higher with respect to plasma sample ($r = 0.694$, $p < 0.000$). The mean plasma levels of osteopontin in KL grade 4 were greater than those in KL grade 3, and the difference was statistically significant ($p < 0.01$). The plasma osteopontin levels significantly correlated with the severity of disease ($r = 0.870$, $p < 0.000$). The synovial fluid levels of osteopontin also correlated with disease severity as regarding the radiological grade ($r = 0.817$, $p < 0.000$).

Conclusion: Osteopontin in plasma and synovial fluid is related to progressive joint damage in knee OA. Osteopontin may serve as a biochemical marker for determining disease severity as regarding radiological grade.

1. Introduction

Osteoarthritis (OA) is a chronic degenerative joint disease that is characterized by the progressive destruction of articular cartilage with varying degrees of severity within a given joint [1]. OA is a strongly age-related joint disorder that is defined by focal lesions of the articular cartilage, combined with a
hypertrophic reaction (sclerosis) in the subchondral bone and new bone formation (osteophytes) at the joint margins and mild, chronic nonspecific synovial inflammation [1].

To identify patients with a high risk for destructive OA and to monitor drug efficacy, more sensitive techniques than plain X-rays are needed. Specific and sensitive biochemical markers reflecting abnormalities of the turnover of bone, cartilage, and synovial tissues may be useful for the investigation and monitoring of OA [2].

Osteopontin is one of the major noncollagenous bone matrix proteins produced by various cell types, including activated T cells, macrophages, osteoblasts and chondrocytes [3].

Osteopontin may be involved in the molecular pathogenesis of osteoarthritis, contributing to progressive degeneration of articular cartilage [4].

Expression of osteopontin mRNA isolated from human OA cartilage was enhanced as compared with normal cartilage. Osteopontin was shown to be upregulated in human OA chondrocytes [5]. Moreover, osteopontin was found to be expressed in bone forming cells and hypertrophic chondrocytes of the embryonic epiphyseal growth plates [6]. Although circulating and/or synovial fluid levels of several cytokines have been investigated in patients with knee OA, there have been no detailed studies on plasma and synovial fluid levels of osteopontin in various clinical stages of primary knee OA [7–11].

The purposes of the present study were to investigate the concentrations of osteopontin in both plasma and synovial fluid of patients with primary knee osteoarthritis, and to evaluate the possible correlations with the radiographic grading of knee OA.

2. Patients and methods

Sixty OA patients fulfilled the American College of Rheumatology (ACR) clinical and radiographic diagnostic criteria for knee OA [12], and 30 control subjects matched for age and sex were included in the present study. The patients were recruited between December, 2010 and October, 2011 at the Rheumatology and Rehabilitation Outpatient Clinic, Minia and Beni Suef University Hospitals.

All patients gave informed consent, and this study was approved by the local ethics committee.

2.1. Inclusion criteria

 Patients with primary knee OA unilateral or bilateral with chronic knee pain more than three months and radiological evidence of early OA.

2.2. Exclusion criteria

Patients with secondary OA, e.g. post-traumatic or post-inflammatory, e.g. rheumatoid arthritis (RA), patients with systemic diseases as renal or hepatic failure or malignancy and patients with generalized OA or patients with concomitant knee and hip OA.

2.3. Clinical evaluation

All patients were subjected to: full history, clinical examination including symptoms and signs of OA, and functional evaluation, using Arabic translated and validated version of Western Ontario and McMaster University (WOMAC) index [13].

2.4. Radiological evaluation

The severity of the disease was determined using weight bearing anteroposterior radiographs of the affected knee. Knee radiographs were evaluated according to the Kellgren and Lawrence classification [14]: grade 1, doubtful narrowing of joint space and possible osteophytic lipping; grade 2, definite osteophytes and possible narrowing of joint space; grade 3, moderate multiple osteophytes, definite narrowing of joint space, some sclerosis and possible deformity of bone contour; grade 4, large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone contour. The grading scale used for analysis was the higher of the two knees. Radiographic OA was considered present if K–L grade was more than 2 [15].

2.4.1. Laboratory investigations

Routine and special laboratory tests were done for all patients and controls. Synovial fluid was aspirated (only from patient) from the affected knee centrifuged to remove cells and joint debris and stored immediately at −80 °C until the day of measurement, also plasma was stored at −80 °C for quantitative detection of osteopontin by commercial enzyme-linked immunosorbent assay (ELISA). Kit was supplied by R&D Systems (Berlin Germany).

2.4.2. Principle of osteopontin assay

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for OPN has been pre-coated onto a microplate. Standards and samples were pipetted into the wells and any OPN present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for OPN was added to the wells, a substrate solution was added to the wells and color developed in proportion to the amount of OPN bound in the initial step. The color development was stopped and the intensity of the color was measured.

Statistical analysis: Statistical analyses were performed using SPSS version 17.0 (SPSs, Chicago, IL). Continuous data were expressed as mean ± standard deviation (SD). The differences among OA patients and control were determined by independent sample t-test. The correlations between OPN and disease severity parameters were done by Spearman correlation test. Statistical significance was set at p < 0.05.

3. Results

3.1. Demographic, clinical and laboratory findings of OA patients and control group

The study population included 60 patients with knee OA, their mean age was (54.33 ± 6.94), ranging from 45 to 70 years old. The mean duration of disease was 6.81 ± 3.19 ranging from 1 to 15 years and 30 healthy volunteers matched for age and sex served as controls Table 1.

Regarding the clinical findings detected in OA patients NO (%) Knee swelling was detected in 43 (71%), Tenderness in 47
(78%), Crepitus in 23 (38%), Effusion in 38 (65%), Muscle waisting in 45 (75%), Bony swelling in 12 (20%), Limited motion in 14 (23%), Baker cyst in 5 (8%), Deformity in 7 (11%), Instability in 3 (5%) of patients while the mean ± SD of: Inactivity stiffness was (8.67 ± 3.31) minutes, WOMAC mean (52.69 ± 22.78), K/L (2.65 ± 1.01), Synovial osteopontin (366.83 ± 148.42) knee swelling was the most common symptoms while knee tenderness was the most common sign.

OA patients had higher plasma osteopontin concentrations compared to healthy controls ($p < 0.000$) Table 1.

Osteopontin levels in synovial fluid were significantly higher with respect to plasma samples ($r = 0.694$, $p < 0.000$) Fig. 1.

According to the Kellgren and Lawrence (KL) grading scale, 7 (12%) patients were KL grade 1, 23 (38%) patients were KL grade 2, whereas 14 (23%) patients were KL grade 3 and 16 (27%) patients were KL grade 4 osteoarthritis.

3.2. Comparison of the plasma and synovial fluid levels of osteopontin in relation to radiological KL grading of OA: Table 2 and Figs. 2 and 3

The plasma osteopontin level was higher in KL grade 4 (252.18 ± 57.93).

These results showed that plasma osteopontin levels in KL grades 3 and 4 were significantly higher than those of KL grade 2 independently ($p < 0.001$). The mean plasma levels of osteopontin in KL grade 4 were greater than those in KL grade 3, the difference was statistically significant ($p < 0.01$) Fig. 2.

In addition, the synovial fluid levels of osteopontin from KL 4 were 192.85 ± 10.74. The data revealed that synovial fluid osteopontin levels in KL grades 3 and 4 were significantly elevated compared with those of KL grade 2 independently ($p < 0.001$).

3.3. The correlation between the plasma and synovial fluid levels of osteopontin with other disease parameters Table 3

The correlation was positive only with age, disease duration, WOMAC index and KL grades (total).

The plasma osteopontin levels significantly correlated with disease severity as regarding the radiological grade ($r = 0.870$, $p < 0.000$), and also the synovial fluid levels of osteopontin significantly correlated with disease severity ($r = 0.817$, $p < 0.000$).

There was significant correlation between plasma and synovial fluid levels of osteopontin in OA patients ($r = 0.694$, $p < 0.000$) Fig. 4.

4. Discussion

Expression of OPN was shown to be upregulated in OA cartilage and synovium. OPN could be involved in synovial cell attachment to chondrocytes or cartilage matrix, in addition to its involvement in destruction of cartilage matrix by the production of collagenases in articular chondrocytes [4,5,16,17].

Our study was designed to investigate osteopontin (OPN) levels in both plasma and synovial fluid of patients with primary knee osteoarthritis (OA) and their relationship with severity of the disease as regarding the radiological grade.

Our findings suggest enhanced local and systemic production of Osteopontin in primary knee osteoarthritis.

This study showed a marked increase of Osteopontin level in plasma of patients with primary knee osteoarthritis compared to the control plasma levels (165.33 ± 67.22 vs 56.06 ± 5.77 ng/mL, $p < 0.000$). This is consistent with the results of Honsawek et al. [18] who found that, OA patients had higher plasma osteopontin concentrations compared to healthy controls (168.8 ± 15.6 vs 67.2 ± 7.7 ng/mL, $p < 0.0001$).

In this study osteopontin level in synovial fluid was significantly higher with respect to plasma samples (366.83 ± 148.42 vs 165.33 ± 67.22 ng/mL, $r = 0.694$, $p < 0.000$). This is in agreement with the result of Honsawek et al., [18] who found that, OPN levels in synovial fluid were significantly higher with respect to paired plasma samples (272.1 ± 15.0 vs 168.8 ± 15.6 ng/mL, $p < 0.001$). Also this is in agreement with the result of Gao et al. [17] who found that, OA patients had higher synovial fluid OPN concentrations compared to healthy controls (4519.60 ± 303.39, 95%CI 3999.42 and 5039.79 vs 1179.70 ± 303.39, 95%CI 1035.53 and 1438.74 pg/ml, $p < 0.001$). But our finding is inconsistent with the results of...
Hasegawa et al. [19] who found that, the levels of non-thrombin-cleaved osteopontin (OPN full-length) in OA knees were not statistically different from those in controls ($p = 0.134$). In contrast, the levels of N-terminal half of thrombin-cleaved osteopontin (OPN N-half) were significantly higher in OA knees than those in controls ($p = 0.042$). The % OPN N-half increased significantly in OA knees compared to controls ($p = 0.003$).

Elevated levels of OPN in the SF were possibly caused by either the release of OPN residing in the local tissues, including the synovium, articular cartilage, and bone, or the increase in its production, or both [17,18]. It is suggested that cell adhesion, migration, or inflammation could be involved in the release of OPN [20].

Previous studies have demonstrated the immunohistochemical expression of OPN in the synovial lining cells [10], fibroblasts in the synovial tissues [21] and articular chondrocytes [5,21].

In our study, there was a statistically significant difference in plasma osteopontin levels among patients with OA with different age ($p < 0.014$), but there was no statistically significant difference in synovial fluid osteopontin levels among patients with OA ($p < 0.396$). This is consistent with the result of Honsawek et al. [18] who found that there was no statistically significant difference in synovial fluid osteopontin levels among patients with OA with different age ($p = 0.5$).

In our study, comparing the plasma level of osteopontin in relation to radiological KL grading of OA, we found that plasma osteopontin levels in KL grades 3 and 4 were significantly higher than those of KL grade 2 ($p < 0.001$). The mean plasma levels of osteopontin in KL grade 4 were greater than those in KL grade 3, the difference was statistically significant ($p < 0.01$). So the plasma osteopontin levels significantly correlated with the severity of disease ($p < 0.000$). This is consistent with the result of Honsawek et al. [18] who found that, plasma osteopontin levels in KL grades 3 and 4 were significantly higher than those of KL grade 2 ($p < 0.001$). Although the mean plasma levels of osteopontin in KL grade 4 were greater than those in KL grade 3, the difference was not statistically significant ($p = 0.835$). So the plasma osteopontin levels significantly correlated with the severity of disease ($p < 0.001$).

In this study, we found positive correlation between plasma and synovial fluid levels of osteopontin ($p = 0.000$), this is in agreement with the result of Honsawek et al. [18], who found positive correlation between plasma and synovial fluid levels of osteopontin ($p = 0.035$).

In our study, analyzing and comparing the synovial fluid level of osteopontin in relation to radiological KL grading of OA, we found that synovial fluid osteopontin levels in KL grades 3 and 4 were significantly elevated compared with those of KL grade 2 ($p < 0.001$). So the synovial fluid levels of osteopontin correlated with disease severity ($p < 0.000$). This is consistent with the results of Honsawek et al. [18], and Gao et al. [17], but inconsistent with the results of Hasegawa et al. [19]. Where, Honsawek et al. [18] revealed that synovial fluid osteopontin levels in KL grades 3 and 4 were significantly elevated compared with those of KL grade 2 ($p < 0.01$), so the synovial fluid levels of osteopontin correlated with disease severity ($p < 0.01$).

### Table 2
Plasma and synovial fluid osteopontin levels at KL grading.

<table>
<thead>
<tr>
<th>OA grades</th>
<th>KL I ($n = 7$)</th>
<th>KL II ($n = 23$)</th>
<th>KL III ($n = 14$)</th>
<th>KL IV ($n = 16$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma osteopontin</td>
<td>92.85 ± 8.09</td>
<td>124.73 ± 13.35</td>
<td>181.78 ± 14.4</td>
<td>252.18 ± 57.93</td>
</tr>
<tr>
<td>Synovial osteopontin</td>
<td>192.85 ± 10.74</td>
<td>274.56 ± 36.17</td>
<td>417.14 ± 110.69</td>
<td>531.56 ± 124</td>
</tr>
</tbody>
</table>

KL = Kellgren and Lawrence grades.
And Gao et al. [17] revealed that synovial fluid OPN levels in KL grade 4 were significantly elevated compared with those of KL grade 2 and 3 ($p < 0.01$), so the synovial fluid level of OPN correlated with disease severity (Spearman’s $r^{1/4} 0.581$, 95%CI 0.335, 0.726, $p < 0.001$).

But Hasegawa et al. [19] found that, in rank correlation tests, the SF levels of OPN full-length showed no difference ($p = 0.191$); however, the levels of OPN N-half and percentage of N-half correlated with disease severity ($p < 0.001$). Apart from one clear outlier in K/L grade 4, showing an extremely high concentration of OPN N-half (7.8 nM), OPN N-half levels correlated with disease severity ($p < 0.001$). In addition, statistically significant correlation was found between OPN N-half and disease severity by K/L grades 1, 2, 3, and 4 ($p < 0.001$). After adjusting the levels of OPN N-half as well as % N-half for sex and age, the correlation between % N-half and the radiographic grading levels remained statistically significant ($p = 0.015$); however, the levels of OPN N-half showed no difference ($p = 0.089$).

Recent studies have showed that osteopontin protein deposition and mRNA expression increased with morphological signs and the severity of matrix degradation in human osteoarthritic cartilage [11]. These findings indicate that osteopontin expression in osteoarthritic cartilage strongly correlates with the severity of OA disease. Furthermore, Yagi et al. [22] showed that the advanced OA cartilage had significantly higher osteopontin mRNA expression than the minimal OA cartilage.

Limitation of this study is that, the sample size was not large enough to make strong conclusions and the effect of joint sites other than the knee needs to be taken into account. Further investigations with standardized assay system in a large population are warranted to gain insight into the potential utility of OPN in OA patients, especially in synovial fluid.

6. Conclusion

Patients with primary knee OA had higher levels of plasma osteopontin compared with healthy controls. Osteopontin concentrations in plasma and synovial fluid significantly correlated with the severity of radiological grade. Further investigations are needed to elucidate the contribution of osteopontin to the pathogenesis of the degenerative process of osteoarthritis.

Conflict of Interest statement

There is no conflict of interest of the authors.

References


