

Relation of osteopontine levels in plasma and synovial fluid of patients with knee osteoarthritis to magnetic resonance imaging findings of the knee joint

Objective: to detect the levels in plasma and synovial fluid osteopontine (OPN) in patients with primary osteoarthritis (OA) and their relation to progressive joint damage as detected by plain radiography, Magnetic Resonance Imaging (MRI) and its impact on both functional status and disease severity.

Design: 60 patients with primary knee OA and 60 healthy controls were included. Functional status was assessed using Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), pain was assessed using Visual Analogue Scale (VAS). Kellgren-Lawrence grading scale (KL) was used to assess radiological severity. MRI using magnetic resonance imaging osteoarthritis knee score (MOAKS score), levels of plasma and synovial fluid OPN were measured.

Results: Mean synovial fluid and plasma OPN of OA patients was 218.4 ± 37.7 and 136.67 ± 35.1 . Plasma OPN was significantly higher in OA than control ($p < 0.0001$). Significant positive correlation between serum and synovial OPN in OA patients ($r = 0.8, p < 0.0001$). Significant positive correlation between synovial fluid OPN and patients' age, disease duration, VAS, WOMAC, KL scale and parameters of MOAKS score. Significant positive correlation between plasma OPN and body mass index (BMI), VAS, WOMAC and KL scale parameters of MOAKS score. OPN level were significantly higher in grade IV KL than other grades. Synovial fluid OPN and bone marrow lesion (BML) score were the determining predictors for the function status ($p = 0.003$) for both and severity ($p = 0.004, p < 0.0001$). Plasma and synovial OPN show high specificity and sensitivity in relation to BML score as parameter of activity and cartilage loss score as parameter of chronicity.

Conclusion: Plasma and synovial fluid OPN correlated with the clinical features, functional status, radiological features of Knee OA and severity MOAKS score. Synovial OPN can predict both functional impact and radiological severity in patients with knee OA.

**Rasha A Abdel-Magied*¹,
Walaa F Mohammed¹
Gihan MA Omar¹,
Mustafa Abdel-Kader²,
Aliaa M Mounir³ &
Faten Ismail Mohamed¹**

¹Department of Rheumatology and Rehabilitation, Faculty of Medicine, Minia University, Egypt

²Department of Radiology, Faculty of Medicine, Minia University, Egypt

³Department of Clinical Pathology, Faculty of Medicine, Minia University, Egypt

*Author for correspondence:
rashahazem@yahoo.com

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Introduction

Osteoarthritis (OA) is a chronic painful joint disease that is characterized by structural changes to the joint including; articular cartilage loss, osteophytes development, inflammation in the synovium, changes in the subchondral bone, damage of the menisci, and laxity of the ligaments. It results from multiple factors including genetic, metabolic, biomechanical and biochemical factors [1].

Biochemical markers are used for detection of both disease and its severity. Therefore, the extracellular matrix proteins were crucial to the pathogenesis and progress of osteoarthritis. some extracellular matrix proteins such as osteopontine (OPN) was found to play important roles in promoting the inflammatory occurrence of cartilage cells in knee osteoarthritis, OPN can

mediate cellular growth, survival, adhesion and migration in osteoarthritis [2,3].

The current reference standard for grading the severity of osteoarthritis (OA) in the knee is the radiography based Kellgren and Lawrence score [4]. This technique only indirectly visualizes the cartilage and is not able to (semi) quantitatively measure cartilage quality [5]. Therefore, quality of cartilage in terms of the sulphated glycosaminoglycan (sGAG), collagen or sodium content of articular cartilage can be measured using magnetic resonance imaging (MRI) techniques [6].

In last decades, MRI had become the most important modality for assessment of pathologic changes in knee cartilage as it allows the manipulation of contrast to highlight different tissue types [7]. The etiopathogenesis of disease

as well as structure-function relationships can be detected using semi-quantitative MRI scoring. The magnetic resonance imaging osteoarthritis knee score (MOAKS) score which is a semi-quantitative scoring method for MRI assessment of knee OA which can detect scoring of Bone Marrow Lesions (BMLs) (through providing regional delineation and scoring across regions), cartilage (through sub-regional assessment), and the elements of meniscal changes (including meniscal hypertrophy, partial maceration and progressive partial maceration) and subluxation scoring [8].

The aim of the study was to detect the levels in plasma and synovial fluid OPN of patients with primary OA and their relation to progressive joint damage as detected by plain radiography, MRI, and its impact on both functional status and disease severity.

Methods

Sixty patients with primary knee osteoarthritis with effusion candidate for aspiration were enrolled in the study; their diagnosis met the ACR Criteria for diagnosis of knee OA [9]. Sixty apparently healthy persons were also enrolled as controls. Exclusion of patients with either; diabetes mellitus, other causes of arthritis, history of receiving chondroprotective drugs or systemic corticosteroid medication in the last 6 months, recent intra-articular either corticosteroid or hyaluronic injection within the last 3 months, history of trauma, or generalized osteoarthritis.

The protocol of the study was approved by the local Ethics Committee of Faculty of Medicine and was in agreement to the World Medical Association Declaration of Helsinki and an informed consent was obtained from all patients.

Full history taking and clinical examination of patients especially for the knee joints was performed. Assessment of function status using Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC); for assessment of pain, stiffness and physical function of patients with knee OA. The scores are summed for items in each subscale as follows: pain=0-20, stiffness=0-8, physical function=0-68 and the total score of WOMAC is calculated by adding the items of the three subscales (0-96) [10]. Joint pain was evaluated using Visual Analogue Scale (VAS) [11]. Radiological severity was assessed using Kellgren-Lawrence grading scale on an antero-posterior view of the knee joint on standing position [4].

MRI was performed for the most affected knee joint using 1.5 Tesla scanners (Philips Medical Systems, Japan). Axial, sagittal and coronal fat saturated, proton density weighted dual spin echo images were from pulse sequence used in the assessment of knee OA features. Presence or absence of cartilage loss and its score, BMLs and its score, marginal osteophytes, meniscal lesion, subchondral bone cyst, subchondral bone sclerosis, ligamentous abnormalities as parameters of magnetic resonance imaging osteoarthritis knee score (MOAKS score) in painful knees were the key for evaluation of tibio-femoral joint (tibial plateau, central weight bearing and posterior portions of femoral condyles), however for statistical purpose, absence of each of these specific feature was graded zero and its presence was graded one.

Laboratory investigation included Erythrocyte Sedimentation Rate (ESR), C-Reactive protein (CRP) and plasma Osteopontin (OPN) level for both patients and controls and in synovial fluid level of OPN for patients only; these were measured by Enzyme-Linked Immuno Sorbent Assay (ELISA) (Quantkine ELISA kit D 05T00 USA). Plasma samples were collected using EDTA as anticoagulant. The results of both plasma and synovial fluid OPN levels were expressed in ng/ml relative to standards included in test kit (manufactured by Eiiab Company in China). Synovial fluid aspiration from OA patients for assessment of OPN level in synovial fluid, however synovial fluid was not aspirated from controls for ethical considerations.

Statistical analysis

The analysis of data was done using personal computer using SPSS (Statistical program for social science) version 16 (SPSS Inc., Chicago, IL, USA). The categorical and quantitative variables were respectively described in the form of numbers/percentage (%) and mean \pm SD. Comparison between variables were done using Mann Whitney or Chi-squared (χ^2) tests. Comparison of more than 2 means were done using Analysis of Variance test (ANOVA or F-test). Spearman rho correlation coefficient was used to calculate correlation between variables. Regression analysis was performed to find predictors of functional disability and disease severity of OA. Sensitivity and specificity of plasma and synovial OPN levels independent of BMLs score and cartilage loss score by MRI using Receiver Operator Characteristic (ROC) curve. p-values < 0.05 were considered significant.

Results

The OA patients' characters; demographic data, laboratory investigation, functional status, radiological grading, MRI findings and MOAKS score (Table 1), Figures 1 and 2 represent plain radiography and MRI finding. In the control the mean ESR was 7.36 ± 1.93 mm/1st hour, the mean level of serum OPN was 55.43 ± 18.56 ng/ml. Serum OPN show significantly higher level in OA patients than control group ($p < 0.0001$). Mean levels of OPN in plasma and synovial fluid in different OA grading, BML score grading and cartilage loss score grading are presented in Table 2.

Table 1. characters of OA patients.		
Parameters mean \pm SD and/or n (%)		OA patient n.=60
Age (y)		46-72(56.3 \pm 7.95)
DD (y)		2-10 (5.8 \pm 2.33)
BMI	Over weight	4 (6.7%)
	Obese I	16 (26.7%)
	Obese II	24 (40%)
	Obese III	16 (26.7%)
VAS		5-10 (7.53 \pm 1.22)
WOMAC total score		45-96 (74.26 \pm 14.07)
KL grading	I	3 (5)
	II	21(35 %)
	III	23 (38.33 %)
	IV	13 (21.67 %)
MRI findings	BML	39 (65%)
	Bone marrow cyst	36 (60%)
	Subchondral bone sclerosis	36 (60%)
	Osteophytes	43 (71.7%)
	Ligamentous lesion	35 (58.3%)
	Meniscal lesions	47 (78.3%)
	Cartilage loss	38 (63.3%)
BMLs by MRI	0	35%
	I	38.33%
	II	20%
	III	6.67%
Cartilage loss score by MRI	0	36.67%
	I	38.33%
	II	20%
	III	5%
Plasma OPN (ng/ml)		92-222 (136.67 \pm 35.1)
Synovial fluid OPN (ng/ml)		162-298 (218.4 \pm 37.7)
ESR mm/ 1st hour		8-36 (23.97 \pm 7.4)
CRP		53 (88.3%)
OA: osteoarthritis; DD: Disease Duration; BMI: Body Mass Index; VAS: Visual Analogue Scale, WOMAC score: Western Ontario and Macmaster; K.L: Kellgran & Laurence grading scale; MRI: Magnetic Resonance Imaging; BML: Bone Marrow Lesion; BMLs: Bone Marrow Lesion score; ESR: Erythrocyte Sedimentation Rate; CRP: C-Reactive Protein; OPN: Osteopontine.		

By using post Hoc analysis for OPN level; in K-L grading, OPN shows significantly higher level in grade IV than other grades, while in comparing the different grades of BML and cartilage loss score by MRI, OPN level were significantly higher in grade III, IV than other grades.

OPN show significantly higher levels in the presence of some MRI features as cartilage loss ($t=1.94$, $p < 0.048$ for synovial fluid), subchondral cyst ($t=2.99$, $p=0.004$ for plasma, $t=3.14$, $p < 0.003$ for synovial fluid), BMLs ($t=3.74$, $p < 0.0001$ for plasma, $t=3.75$, $p < 0.0001$ for synovial fluid) and for subchondral sclerosis ($t=3.62$, $p < 0.0001$ for plasma, $t=3.66$, $p < 0.0001$ for synovial fluid), however; there was no significant difference in OPN level was found for the presence of such features as osteophytes, meniscal or ligamentous abnormalities.

Significantly positive correlation was found between OPN level in plasma with paired synovial fluid OPN level ($r=0.8$, $p < 0.0001$) (Figure 3). Plasma and synovial fluid levels of OPN correlation with the demographic, clinical, functional assessment score, VAS, laboratory data, radiological grading, and MRI findings of OA patients are presented in Table 3.

For determining the function status (using WOMAC as dependent factor), many independent factors were included; the serum and synovial fluid OPN levels, patients' age, disease duration and BML score, we found that synovial fluid OPN and BML score were the significant predictors ($p=0.003$) for both of them; while among many independent factors including the serum and synovial fluid OPN levels, patients' age, disease duration and BML score for determining severity (using Kellgren-Lawrence grading score as dependent factor); synovial fluid OPN and BML score were the significant predictors ($p=0.004$, $p < 0.0001$ respectively).

Assessment of sensitivity and specificity of plasma and synovial OPN levels independent of BML score (as parameter of activity) using ROC curve, which revealed that: at plasma OPN level (>150 ng/ml) sensitivity was 43.59% and specificity was 95.24%, accuracy is 72% and above this level BMLs appear in MRI. While at synovial fluid OPN level (>222 ng/ml), sensitivity was 53.85%, specificity was 85.71% and accuracy was 74.1%, and above this level BMLs appear in MRI (Figure 4).

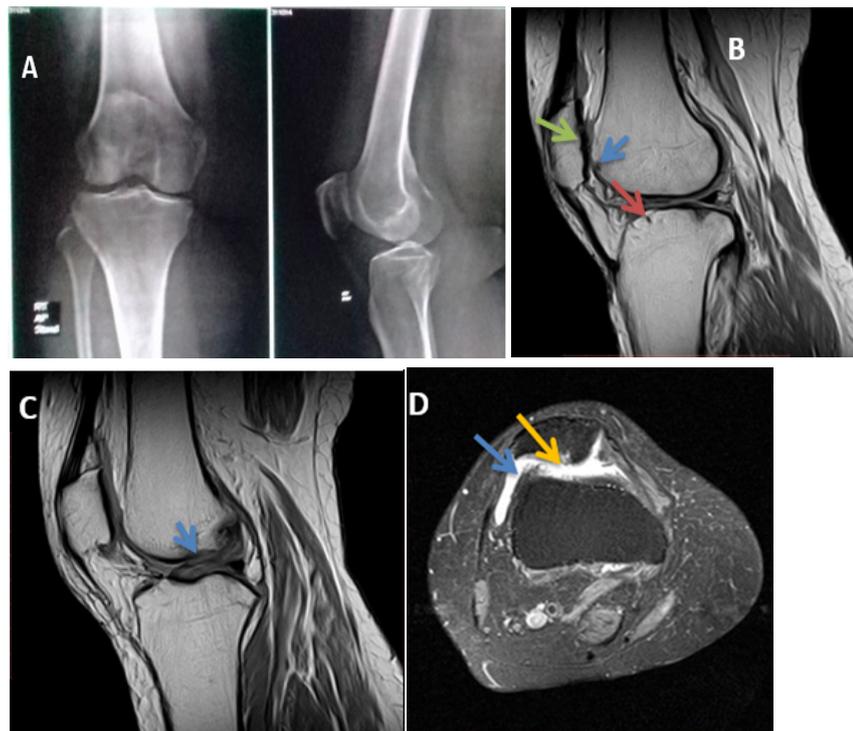


Figure 1. (A) Plain X-ray AP and lateral views of Rt. knee showed mild osteoarthritic changes in form of narrowed joint space and small marginal lipping (grade 1 OA by KL grading). MRI assessment of Rt. knee according to MOAKS score revealed. (B) Sagittal PDWI showed cartilage loss grade 1, irregularities in articular surface of tibia (red arrow) and patella (green arrow) with focal contusion in femur sub articular (blue arrow). (C) Sagittal PDWI revealed bone marrow edema grade 2 and overlying cartilage loss grade 1 (blue arrow). (D) Axial T2 Fat Sat showed subarticular marrow signal in patella (orange arrow) and mild effusion (blue arrow).

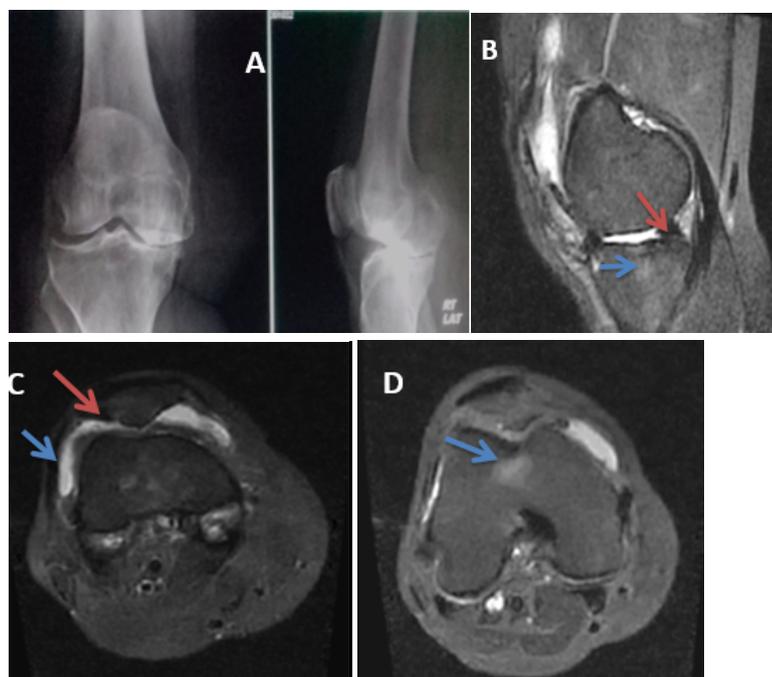


Figure 2. (A) Plain X-ray AP and Lat. views of Rt. knee showed significant osteoarthritic changes in the form of narrowed joint space, prominent tibial spine, irregular articular surface and marginal osteophytes. The patello-femoral articulation is involved with similar changes (grade 3 by KL grading). MRI assessment according to MOAKS score revealed. (B) Sagittal STIR image showed cartilage loss grade 2, irregularities in articular surface of tibia, (red arrow), bone marrow edema in tibia grade 3 (blue arrow). (C) Axial T2 Fat Sat cut of Lt knee showed bone marrow edema grade 3 in femur (blue arrow), cartilage loss grade 2 and articular irregularities in patella (red thick arrow). (D) Axial T2 Fat Sat image showed subarticular marrow signal in patella (orange arrow), mild effusion.

Table 2. Level of plasma and synovial fluid OPN in different K-L OA grading, BML score grading and cartilage loss score grading.

K-L grading	Grade I	Grade II	Grade III	Grade IV	F	P
Plasma OPN (ng/ml)	100-132 (115.3 ± 16.04)	92-164 (119.7 ± 19.7)	95-222 (139.78 ± 41.6)	115-213 (163.46 ± 28.1)	5.667	0.002
Synovial fluid OPN (ng/ml)	170-200 (183.3 ± 15.2)	162-266 (202.14 ± 29.5)	170-286 (221.47 ± 39.54)	200-298 (247.3 ± 30.54)	5.967	0.001
BMLs	Grade 0	Grade I	Grade II	Grade III	F	P
Plasma OPN (ng/ml)	92-164 (118.66 ± 19.34)	95-222 (129.8 ± 29.55)	102-210 (159 ± 35.8)	190-213 (203.2 ± 10.4)	14.348	<0.0001
Synovial fluid OPN (ng/ml)	162-266 (197.85±26.15)	170-298 (220±39.39)	(186-286) (232.16±31.7)	258-290 (275.75±14.56)	7.634	<0.0001
Cartilage loss score	Grade 0	Grade I	Grade II	Grade III	F	P
Plasma OPN (ng/ml)	97-178 (130.04 ± 23.07)	92-213 (124.26 ± 29.6)	98-222 (156.75 ± 43.4)	190-210 (200 ± 10)	7.759	<0.0001
Synovial fluid OPN (ng/ml)	162-278 (207.2 ± 28.8)	168-285 (214.65 ± 38.3)	178-298 (232 ± 41.4)	258-286 (274.66 ± 14.7)	3.99	0.01

OA: Osteoarthritis; K.L: Kellgran & Laurence grading scale; BMLs: Bone Marrow Lesion score; OPN: Osteopontine.

Table 3. Plasma and synovial fluid OPN level correlation OPN with demographic, clinical data, functional assessment score, VAS, laboratory data, radiological grading, and MRI findings of knee OA patients.

Parameters r (p)	Plasma OPN	Synovial fluid OPN
Age	0.032 (0.730)	0.800 (<0.0001)
DD	0.155 (0.236)	0.390 (0.002)
BMI	0.629 (<0.0001)	0.074 (0.574)
Patient pain VAS	0.431(0.001)	0.338 (0.008)
WOMAC total score	0.342 (<0.001)	0.358 (<0.001)
KL grading	0.358 (<0.001)	0.680 (<0.001)
ESR	0.362 (0.004)	0.087 (0.510)
CRP	0.895)) 0.017	0.689 (0.455)
Subchondral sclerosis	0.397 (0.002)	0.405 (0.001)
Cartilage loss	0.145 (0.270)	0.227 (0.08)
Cartilage loss score	0.420 (0.001)	0.381 (0.003)
BML	0.379 (0.003)	0.403 (0.001)
BML score	0.625 (<0.0001)	0.521(<0.0001)
Bone marrow cyst	0.339 (0.008)	0.360 (0.005)
Osteophytes	0.039 (0.481)	0.083 (0.529)

OA: osteoarthritis; DD: Disease Duration; BMI: Body Mass Index; VAS: Visual Analogue Scale; WOMAC: Western Ontario and Macmaster; K.L: Kellgran & Laurence grading scale; MRI: Magnetic Resonance Imaging; BML: Bone Marrow Lesion; BMLs: Bone Marrow Lesion score; ESR: Erythrocyte Sedimentation Rate; CRP: C-Reactive Protein; OPN: Osteopontine.

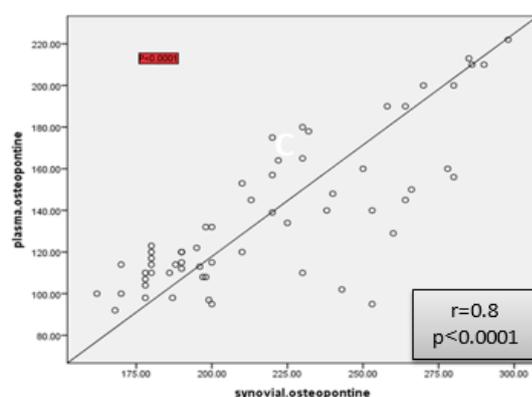


Figure 3. Correlation between plasma and synovial fluid OPN levels in patients with primary knee OA.

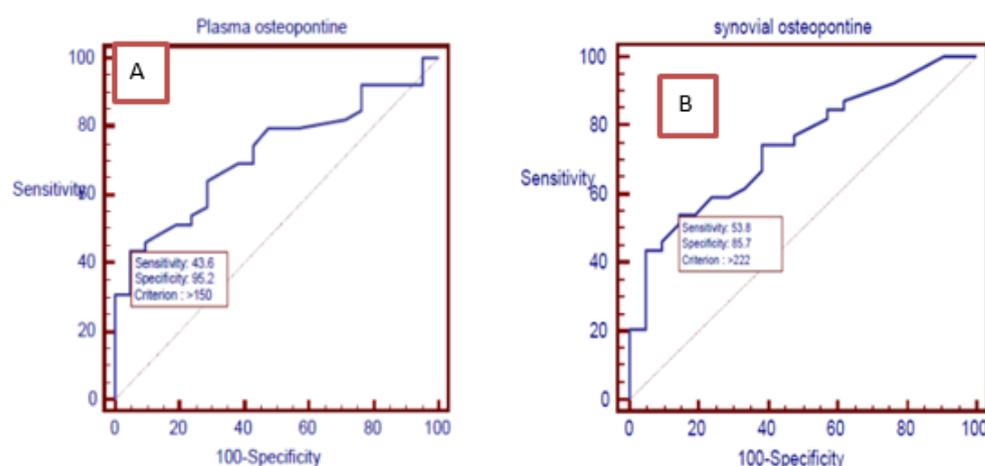


Figure 4. (A) ROC curve for plasma osteopontine (OPN) and BML score, (B) ROC curve for synovial fluid osteopontine (OPN) and BML score.

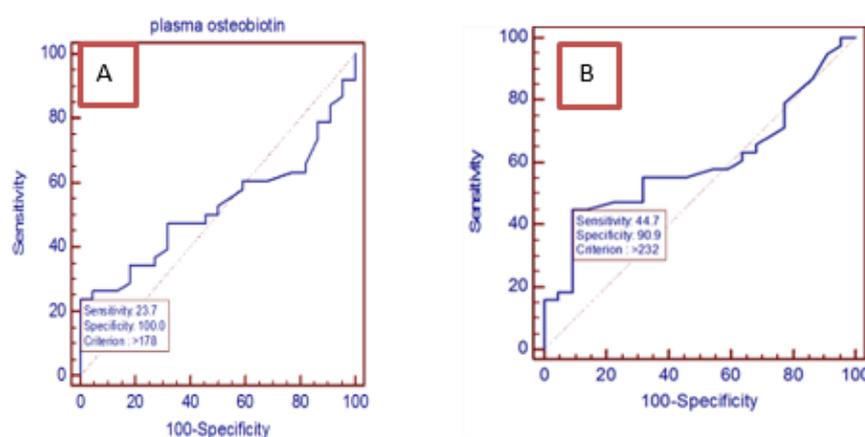


Figure 5. (A) ROC curve for plasma osteopontine (OPN) and cartilage loss score, (B) ROC curve for synovial fluid osteopontine (B) and cartilage loss score.

Also by assessment of sensitivity and specificity of plasma and synovial OPN levels independent of cartilage loss score (as parameter of chronicity) using ROC curve, which revealed that: at plasma OPN level (>178 ng/ml) sensitivity was 23.68% and specificity was 100%, accuracy was 52.2% and above this level cartilage loss appear in MRI. While at synovial fluid OPN level (>232 ng/ml), sensitivity was 44.74%, specificity was 90.9% and accuracy was 59.7% and above this level cartilage loss appear in MRI (Figure 5).

Discussion

Osteoarthritis (OA) is a low-grade inflammatory disease involving synovial joints and is considered the most common form of arthritis that cause chronic pain and physical disability [12]. The changes that are observed in both articular cartilage and subchondral bone in OA include sclerotic changes, articular cartilage thinning, subchondral plate thickening and thickness

subchondral cortex, formation of osteophyte, vascular invasion of the calcified cartilage leading to both bone marrow lesions and bone cysts in the subchondral compartment development [13,14].

Bone marrow lesions (BMLs) which are focal changes in the subchondral bone are features that can be detected by magnetic resonance imaging (MRI) and associated with the severity features of OA including symptoms such as pain and degeneration of osteo-chondral unit which occur in both early asymptomatic OA and in severe late-stage OA [15-18].

Osteopontine is a multifunctional phosphoprotein which is considered as a critical intrinsic regulator which plays an important role in the progression of OA [19,20]. The increased expression of OPN has been observed to be related to the severity of joint involvement and the inflammatory status in OA [21,22].

This aim of the study was to detect OPN levels in both plasma and synovial fluid in patients with primary knee OA, and to determine the relation between these levels and both disease severity as detected MRI of osteoarthritic knee using MOAKS score (for cartilage loss and BML) and functional status. 60 patients with knee OA were included and 60 apparently healthy individuals were matched as regard age and sex and served as a control group.

In this study WOMAC score and K-L grading show positive correlation with both plasma and synovial OPN levels. These results were in agreement with many previous studies as; Mohammed et al. [23] Lee et al. [24] and Kim et al. [25] who found positive correlation between plasma OPN level with WOMAC index and K-L grades, while synovial fluid OPN level positively correlated with K-L scale.

In our study plasma osteopontine show statistically significant higher level in OA patients than control group and OPN levels in synovial fluid of OA patients were significantly higher with respect to paired plasma level. Plasma OPN level show significant positive correlation with paired synovial fluid OPN level and both of these levels were correlated with VAS of pain. These results were previously proven in many studies as detected by Honsawek, et al. [21], Mohammed et al. [23], Qin et al. [3] and Haider et al. [26].

Although these findings were inconsistent with Hasegawa et al. [2] as they proved that no statistically significant difference between the levels of non-thrombin-cleaved osteopontine (OPN full-length) in OA knees and controls. In this study statistically significant positive correlation between plasma OPN and body mass index, also a statistically significant positive correlation was found between synovial fluid OPN and age and disease duration. Mohammed et al. [23] found positive correlation between plasma OPN with age and disease duration. While a positive correlation between synovial fluid OPN and disease duration was detected in their study. Elsebaie et al. [27], Haider et al. [26] and Honsawek et al. [21] disagree with our result; they did not found such significant correlations.

In this study plasma and synovial fluid OPN levels show significant positive correlation with K-L grading. Plasma & synovial fluid OPN levels were significantly higher in grade IV K-L than other grades. These results were previously proven in the studies of Honsawek et al. [21],

Gao et al. [28], Hasegawa et al. [2], Honsawek et al. [29], Elsebaie et al. [27], Mohammed et al. [23] and, Haider et al. [26].

However, our results disagree with Matsui et al. [22] who noticed that severity OA changes in mice was the result of OPN deficiency and not increases. In this study a statistically significant correlation was found between age and BML, bone marrow cyst, cartilage loss, subchondral sclerosis, and meniscal lesion. These findings not studied before. Our results revealed that total WOMAC score and K-L scale was positively correlated with subchondral bone sclerosis, BML, BML score, meniscal lesion.

In this study we found near similar percentage of different grades of K-L score for knee OA to the results of the studies of Quatman et al. [30] and Haider et al. [26]. However as regarding MRI done for the same patients, Quatman et al. [30] found lower percentage than ours regarding articular cartilaginous defects (37.1%), meniscal lesions (32.3%), and ACL lesions (38.7%), BME-like lesions (45.2%), osteophytes (45.2%) and joint effusions (67.7%) of knees, however they found synovitis in 100% of knees. Haider et al. [26] found higher percentage than our results regarding the cartilage loss in medial tibiofemoral compartment (80%) and Marginal osteophytes (74%), the other MRI finding were at lower percentage than ours; subchondral cyst (52%), Bone marrow edema (44%), subchondral sclerosis (32%), meniscal abnormalities (12%), ligamentous abnormalities (4%) and synovial effusion (24%).

In this study; the plasma and synovial fluid OPN show significantly higher level in OA patients with grades III, IV according to BML classification in MOAKS score by MRI when compared to patients with grades I and II. Also they show significantly higher level in grade III in comparison with other grades according to cartilage loss classification in MOAKS score by MRI. The presence of some MRI features as cartilage loss, subchondral cyst, BMLs and subchondral sclerosis were associated with significantly higher OPN levels. However, the presence of osteophytes or meniscal or ligamentous abnormalities was not associated statistical significant difference in OPN level.

There was significant positive correlation between plasma and synovial fluid OPN and BML, BML score, subchondral sclerosis, cartilage loss score and bone marrow cysts, however no such significant correlation were found between

plasma, synovial fluid OPN levels and meniscal lesion, ligamentous lesions, cartilage loss and osteophyte. This was in agreement with Haider et al. [26] apart from significant results regarding marginal osteophytes and synovial effusion and non-statistical significant difference regarding subchondral sclerosis.

Our study revealed that K-L scale show significant positive correlation with subchondral bone sclerosis, BML, BML score, meniscal lesion. This finding was previously proven in the studies of Joshi et al. [31] and Schiphof et al. [32].

In our study MRI lesions was found in older age group, and both cartilage loss and marginal osteophytes were the commonest MRI findings in OA patients and subchondral bone cyst and bone marrow edema were found in about half of patients. There was a significant positive correlation between BML and VAS. These were in agreement with the studies of Guermazi et al. [5] and Kim et al. [33].

Assessment of sensitivity and specificity of plasma and synovial OPN levels independent of BML score by using ROC curve, the cut-off value for plasma OPN level (>150 ng/ml) and for synovial fluid OPN level was (>222 ng/ml). For cartilage loss score the cut-off value for plasma OPN level (>178 ng/ml), for synovial fluid OPN level (>232 ng/ml).

Honsawek et al. [21] found that the determination of an accurate cut-off value for circulating osteopontine level is one critical issue in its clinical application. The documented normal circulating osteopontine levels in previous reports are highly variable, with a range from 31 ng/mL to 200 ng/mL. The exact reason is unclear but it could be attributed to the different assay systems and conditions of sample collection utilized in those studies.

Conclusion

In conclusion Plasma and synovial fluid OPN correlated with the clinical features, functional status, radiological features of Knee OA and severity MOAKS score. Synovial OPN can predict both functional impact and radiological severity in patients with knee OA.

Conflict of interest

The author(s) declared no potential conflicts of interest.

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