e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2024; 16(5); 981-988

Original Research Article

# Correlation of Plasma Osteopontin with Radiological Grading in Patients with Osteoarthritis in the Knee Joint

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**Received: 25-02-2024 / Revised: 23-03-2024 / Accepted: 20-04-2024**
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**Conflict of interest: Nil**

**Abstract:**

**Background:** OA (Osteoarthritis) is a common form of degenerative arthritis. Biomarkers for osteoarthritis are scarcely studied. Osteopontin and hyaluronic acid are important biomolecules in the pathogenesis of osteoarthritis. Osteopontin has varied roles, including cancer pathogenesis and vascular smooth muscle contraction. The identification and estimation of biomarkers in osteoarthritis have important implications for degenerative arthritis therapy.

**Aims:** To assess the potential of plasma osteopontin as a biomarker for tracking the severity of knee osteoarthritis, as well as its involvement in the pathogenesis of osteoarthritis through its stimulation of MMP13, which in turn raises the levels of hyaluronic acid in the serum.

**Methods:** A total of 60 cases of osteoarthritis and 30 disease-free controls were recruited and plasma levels of osteopontin, hyaluronic acid, calcium and phosphorus were estimated. Osteopontin estimation was made by quantitative ELISA and hyaluronic acid by sandwich ELISA methods.

**Results:** There was a significant difference in the levels of otseopontin ($p = 0.014$) and hyaluronic acid ($p<0.001$) among cases and controls and a significant correlation between different grades of OA in the KL classification and osteopontin levels ($p = 0.022$) and hyaluronic acid levels ($p = 0.006$). Osteopontin and hyaluronic acid could therefore be useful biomarkers for OA knee.

**Keywords:** Osteoarthritis, Biomarker, Osteopontin, Hyaluronic Acid, Radiological Grading.



## Introduction

Osteoarthritis is a “universal disorder” characterised by focal lesions of the articular cartilage with chronic, nonspecific synovial inflammation. “Osteopontin” is one of the major noncollageneous bone matrix proteins produced by various cells like activated T cells, macrophages, osteoblasts and chondrocytes. Osteopontin may be involved in the pathogenesis of osteoarthritis, at the molecular level, contributing to the progressive degeneration of articular cartilage.[1]

In early OA, there was a significant increase in the water content of articular cartilage with changes in the collagen matrix.[2]

## Biomarkers of OA Knee

The National Institute of Health funded the OA Biomarkers Network, which established "BIPED" (burden of disease, investigative, prognostic, efficacy of intervention, and diagnostic).[3]

The burden of disease and prognosis for hip and knee OA biomarkers are serum COMP, serum hyaluronic acid and urinary CTXII.[4]

The slow progression of primary OA is causing the researchers to refocus the search for OA biomarkers on secondary OA, which is more rapidly progressing after acute injury.[5]

Osteopontin is a ubiquitous glycoprotein, secreted by various cells of the body and was identified by Oldberg et al.[5] in 1986 in the osteoblast cell. Its altered expression may be associated with pathological conditions such as inflammatory disease, autoimmune disease, osteoporosis, joint diseases, and cancer metastasis.[6,7,8]

OPN influences cell mediated immunity and enhances B-cell immunoglobulin production and proliferation.[9,10]

Yumoto et al.[11] found that OPN deficiency prevents the model mice from losing proteoglycan in the articular cartilage, swelling and destruction of

joints via promotion of angiogenesis and induction of apoptosis in chondrocytes.

In their analysis of synovial fluid OPN in OA patients, Qin et al. discovered increased OPN mRNA and protein expression, which they linked to the progression of OA.[12]

Gao et al.[13] found higher OPN concentrations in articular cartilage and synovial fluid when compared to healthy subjects.

Singh et al.[14] in a recent study proposed stem cells as a therapeutic choice for cartilage repair in OA patients. It has been identified that extracellular inorganic phosphate is one other modulator of OPN expression.[15]

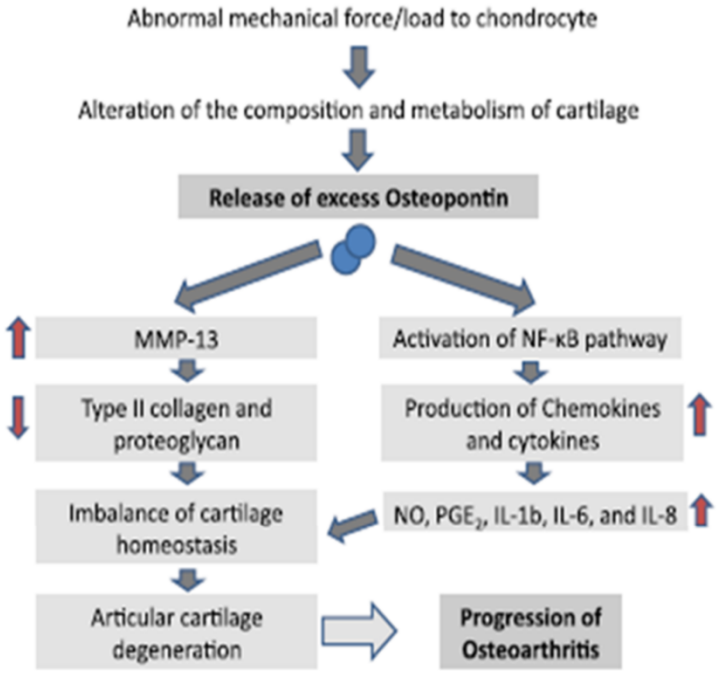



**Figure 1**

**Hyaluronic Acid:** Hyaluronic acid is a type of disaccharide polymer. It was believed that HA played a significant role in the synovial fluid.[16] The proteoglycan reflects cartilage turnover, whereas HA reflects synovial activity.[17] It is believed that the localized inflammation in the synovial lining and cartilage degradation are responsible for the increased production and release of HA.

**Aims and Objectives:** To determine whether plasma osteopontin serves as a biomarker for tracking the severity of knee osteoarthritis, and to evaluate how osteopontin contributes to the pathogenesis of osteoarthritis by triggering MMP13, which in turn raises the levels of hyaluronic acid in the serum. We also wanted to examine the relationship between plasma osteopontin level, radiological grade, and serum hyaluronic acid in individuals suffering from knee osteoarthritis.

## Materials and Methods

This case control study was carried out during the period between January 2015 and June 2015 at Madras Medical College and Rajiv Gandhi Government General Hospital. This study was conducted after obtaining Institutional Ethics Committee clearance.

### Study Population

**Cases:** The study group included 60 osteoarthritis patients, consisting of 48 adult females and 12 adult males. We selected patients who exhibited signs and symptoms, as well as radiologic evidence of OA. Patients were treated with NSAIDs and calcium supplements. The inclusion criteria for the cases included unilateral or bilateral knee osteoarthritis with a duration of knee pain of more than three months, age between 40 and 70 years, and radiological evidence of osteoarthritis.

**Control:** The control group consisted of 30 adults, age- and gender-matched individuals, with 23 females and 7 males. They had no symptoms or signs of OA, and their x-ray knee joint was normal—no evidence of OA.

**Exclusion Criteria:** Patients with secondary osteoarthritis (post-traumatic or post inflammatory), renal failure, hepatic failure and generalised osteoarthritis. The study excluded patients with both hip and knee osteoarthritis and those with malignancies.

**Osteopontin Estimation:** Plasma osteopontin level was estimated using the Sandwich Enzyme Linked Immuno Sorbent Assay (ELISA) method (Ray Biotech). The kit was stored at 2-8oC in a refrigerator.

Quantitative ELISA using the sandwich technique was performed to analyse the OPN concentration in the plasma of both cases and controls.

**Hyaluronic Acid Estimation:** The serum hyaluronic acid level was estimated using the Sandwich Enzyme Linked Immuno Sorbent Assay (ELISA) method (Cusabio). The kit was stored at 2-8 °C in a refrigerator.

A standard curve was constructed by plotting the absorbance for each standard on the y-axis against the concentration on the x-axis and a best fit curve through the points on the graph was drawn. The data may be linearized by plotting the log of the HA concentrations versus the log of the O.D., and the best fit line can be determined by regression analysis.

**Specificity:** This assay has high sensitivity and excellent specificity for the detection of human HA. No significant cross-reactivity or interference between human HA and analogues was observed.

Serum calcium was estimated in the samples using the Accucare Calcium Arsenazo III method in MERCK semi autoanalyzer.

Serum phosphorus was estimated in the samples using pathozyme phosphorus molybdate U.V. method in MERCK semi autoanalyzer.

Radiological diagnosis and staging of osteoarthritis are done by KL classification.

**Kellgren and Lawrence Classification**

**Grade 1:** Doubtful narrowing of joint space and possible osteophytic lipping

**Grade 2:** Definite osteophytes and possible narrowing of joint space

**Grade 3:** Definite narrowing of joint space, moderate multiple osteophytes, some sclerosis and possible deformity of the bone contour

**Grade 4:** Marked narrowing of joint space, large osteophytes, severe sclerosis and definite deformity of bone contour-joint displacement.[18]

**Statistical Analysis:** Statistical analysis was performed for the present study using the SPSS software (Statistical Package for Social Sciences). Student's t-test was performed for comparison between groups. Pearson's correlation coefficient was employed to determine the correlation between the concentrations of plasma osteopontin and serum hyaluronic acid and the Kellgren-Lawrence (K/L) grades. P-values <0.05 were considered statistically significant.

**Results**

In the present study, 60 patients with varying grades of OA, confirmed by x-rays of the knee joint and 30 controls were enrolled. The mean and standard deviation of OA patients and controls were 56.60+/-7.907 and 56.67+/-8.683 respectively. There was no statistical difference in the age between OA patients and controls (p = 0.971).

In both cases and controls, the gender distribution among patients with OA knees shows that females constitute a higher percentage than males. There is no significant difference in gender between cases and controls (p = 0.715).

There is a highly statistically significant difference with respect to weight and BMI (p=<0.001**) among cases and controls, while there is no significant difference among cases and controls with respect to height, systolic and diastolic BP.

**Table 1: Sociodemographic Details Including Age, Sex, Height, Weight and BM**

| | Group | N | Mean | Std. Deviation | | P-Value |
|---|---|---|---|---|---|---|
| Age distribution | Control | 30 | 56.67 | 8.683 | | 0.971 |
| | Cases | 60 | 56.60 | 7.907 | | |
| Sex distribution | | Controls | | Cases | | P-Value |
| | Males | 7 (36.8%) | | 12 (63.2%) | | 0.715 |
| | Females | 23(32.4%) | | 48(67.6%) | | |
| Anthropometry | | **Group** | **N** | **Mean** | **Std. Deviation** | **P-Value** |
| | Height | Control | 30 | 161.73 | 4.571 | 0.123 |
| | | Cases | 60 | 155.28 | 6.325 | |
| | Weight | Control | 30 | 59.43 | 4.305 | <0.001** |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | Cases | 60 | 62.87 | 9.675 | |
| | BMI | Control | 30 | 22.7543 | 1.51611 | <0.001** |
| | | Cases | 60 | 26.0972 | 3.77687 | |
| | ** Highly Significant | | | | | |
| Distribution of patients according to blood pressure | | **Group** | **N** | **Mean** | **Std. Deviation** | **P-Value** |
| | SBP | Control | 30 | 118.00 | 11.861 | 0.234 |
| | | Cases | 60 | 121.17 | 11.802 | |
| | DBP | Control | 30 | 73.93 | 4.346 | 0.458 |
| | | Cases | 60 | 74.80 | 5.575 | |
| | ***** Highly significant, weight and BMI among cases and controls.** | | | | | |

**Table 2: Comparison of Concentration of Calcium, Phosphorus, OPN and HA in Patients with OA Knee Cases and controls**

| | **Group** | **N** | **Mean** | **Std. Deviation** | **P-Value** |
|---|---|---|---|---|---|
| Calcium | Control | 30 | 9.540 | 0.2328 | 0.105 |
| | Cases | 60 | 9.460 | 0.2109 | |
| Phosphorus | Control | 30 | 3.967 | 0.2881 | 0.365 |
| | Cases | 60 | 4.035 | 0.3569 | |
| OPN | Control | 30 | 611.050 | 207.5941 | 0.014* |
| | Cases | 60 | 984.912 | 804.0174 | |
| HA | Control | 30 | 1.87573 | 0.480887 | <0.001** |
| | Cases | 60 | 2.58292 | 0.908696 | |

Table 2 shows the mean, standard deviation and p-value of the analytes- calcium and phosphorus. The mean and standard deviation of calcium and phosphorus in patients with OA knee and controls are 9.46+/-.21, 9.54+/-.23 and 4.03+/-.35, 3.97+/-.28 respectively. There is no statistically significant difference between cases and controls with respect to calcium and phosphorus (p-values of 0.105 and 0.365 respectively).

The mean and standard deviation of the OPN values in patients with OA knees and controls are tab

ulated. The mean OPN concentration in patients with OA knee was 984.91+/−804pg/mL while in controls it was 611.05+/−207.59 pg/mL. The difference in OPN values between OA knee patients and controls was statistically significant (p = 0.014).

The mean and standard deviation for the values of HA among the cases and controls. The mean HA in OA knee patients was 2.58+/−0.90ng/mL while in controls it was 1.87+/−0.48 ng/mL. There was a highly significant difference in the value of HA between cases and controls, with a p-value of <0.001**.

**Table 3:**

| **K/L-Grade** | **N** | **Osteopontin (pg/mL)** | | **P-Value** | **95% Confidence Interval for Mean** | |
|---|---|---|---|---|---|---|
| | | **Mean** | **Std. Deviation** | | **Lower Bound** | **Upper Bound** |
| 2 | 9 | 558.489 | 107.1066 | **0.022*** | 476.159 | 640.818 |
| 3 | 37 | 915.238 | 693.5673 | | 683.991 | 1146.485 |
| 4 | 14 | 1443.179 | 1108.5290 | | 803.133 | 2083.224 |
| ***Comparison of OPN Levels with Various K/L Grades of OA among Cases*** | | | | | | |
| *Significant<br>K/L Grade- 2, 3, 4 & OPN level | | | | | | |
| **K/L-Grade** | **N** | **Hyaluronic Acid (ng/mL) Mean** | **Std. Deviation** | **P-Value** | **95% Confidence Interval for Mean** | |
| | | | | | **Lower Bound** | **Upper Bound** |
| 2 | 9 | 1.74178 | 0.530247 | **0.006**** | 1.33419 | 2.14936 |
| 3 | 37 | 2.67124 | 0.998217 | | 2.33842 | 3.00407 |
| 4 | 14 | 2.89021 | 0.448811 | | 2.63108 | 3.14935 |
| ***Comparison of HA Levels with Various K/L Grades of OA among Cases*** | | | | | | |
| K/L Grade- 2,3,4 & HA level,<br>** Highly significant | | | | | | |

Table 3 shows the comparison of plasma osteopontin levels in relation to radiological K/L grading of OA in the present study and it was found that the mean plasma OPN levels in K/L grades 2, 3 and 4 were 558.48+/−107.10pg/mL, 915.238+/−693.56pg/mL and 1443.179+/−1108.52pg/mL. This implies that the plasma OPN levels were significantly higher in K/L grades 2, 3 and 4 (p = 0.022).

Table 3 shows the comparison of serum HA levels in relation to radiological K/L grading of OA in the present study and it was found that the mean serum HA levels in K/L grades 2, 3 and 4 were 1.74+/−0.53 ng/mL, 2.67+/−0.99ng/mL and 2.89+/−0.44ng/mL .This implies that the serum HA levels were significantly higher in K/L grades 2, 3 and 4 (p = 0.006).

**Table 4: Pearson Correlation Coefficient between OPN, HA and K/L Grade in the Study Population**

| | | K/L Grade |
|---|---|---|
| **OPN** | **Pearson Correlation** | 0.349**(**)** |
| | Sig. (2-tailed) | **0.006**** |
| | N | 60 |
| **HA** | **Pearson Correlation** | 0.358**(**)** |
| | Sig. (2-tailed) | **0.005**** |
| | N | 60 |
| ** Correlation is significant at the 0.01 level (2-tailed) | | |

The Pearson correlation coefficient on the variables OPN and K/L grade is shown in Table 5. In order to measure the strength of the linear relationship between OPN and K/L grade, Pearson correlation was done and it was observed that there was a positive linear relationship between OPN and K/L grade with r = 0.349, p-value = 0.006.

The Pearson correlation coefficient was calculated for variables HA and K/L grade in order to measure the strength of linear relationship between the two variables as tabulated. It was observed that as K/L grade increases the concentration of HA also increases, as indicated by a positive linear relationship with r = 0.358, and a p-value of 0.005.

We determined the Pearson coefficient between the variables OPN and HA. We conducted this study to determine the existence of a linear relationship between these variables. In the present study, it was found that there was no statistical significance (p-value = 0.256).

According to the data of 60 patients with varying grades of OA knee and 30 normal subjects as controls, the ROC curve was plotted. When 617.95 pg/mL was set as the cut-off level of plasma OPN, the sensitivity to predict the susceptibility of patients to developing OA knees was 67%, and the specificity was 63%. For OPN, the area under the curve was 0.683. Therefore, we can use OPN as a biomarker to evaluate the severity of OA knee.

When 2.06 ng/mL was set as the cut-off level of serum HA, the sensitivity to predict patients with OA knees was 75% and the specificity was 70%. The area under the curve (AUC) was 0.761 for HA. This implies that HA can be used as a biomarker to assess the cartilage damage in OA knees.

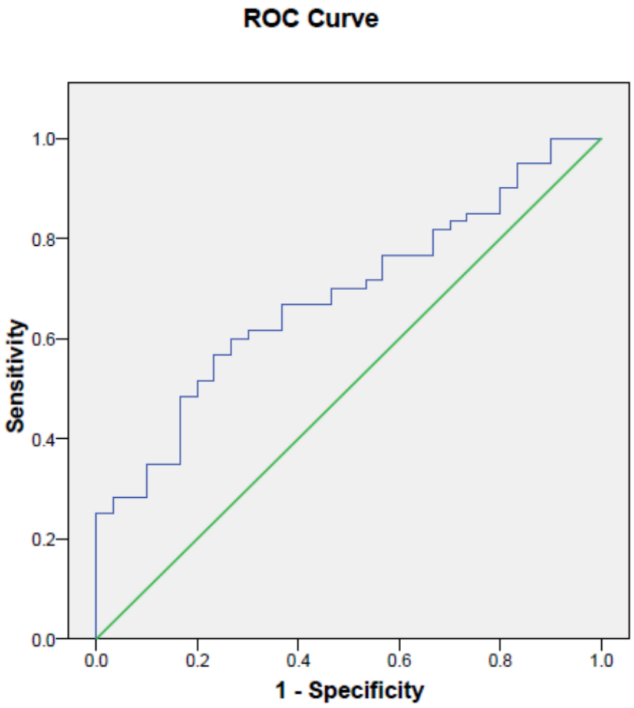
## Discussion

OA knee is a chronic degenerative disease with multiple pathogenic pathways of chondrocyte triggering and downstream transcription factors, which leads to the final common pathway of activation of MMP-13 mediated type-II collagen breakdown, which ultimately causes cartilage erosion. OPN also has a crucial role in MMP-13 activation, leading to cartilage destruction and the release of HA from the cartilage. Finally, impairment of the regenerative capacity of the cartilage occurs due to cell death caused by deranged autophagy.[19] To date, only plain x-rays are available to assess the severity of OA in the knee joint. Recently, newer biomarkers on detecting cartilage matrix synthesis and degradation are gaining clinical importance in the diagnosis of the severity of OA knee and recent advances in the proteomics and microRNA have enabled the detection of newer OA biomarkers.

In the present study, the plasma concentration of OPN was evaluated to assess its utility as a biological marker in patients with OA knee, to correlate with radiological grade and to assess if osteopontin contributes to the pathogenesis of the degenerative process of osteoarthritis by stimulating MMP13 and thereby increasing hyaluronic acid levels in the serum in patients with OA knee.

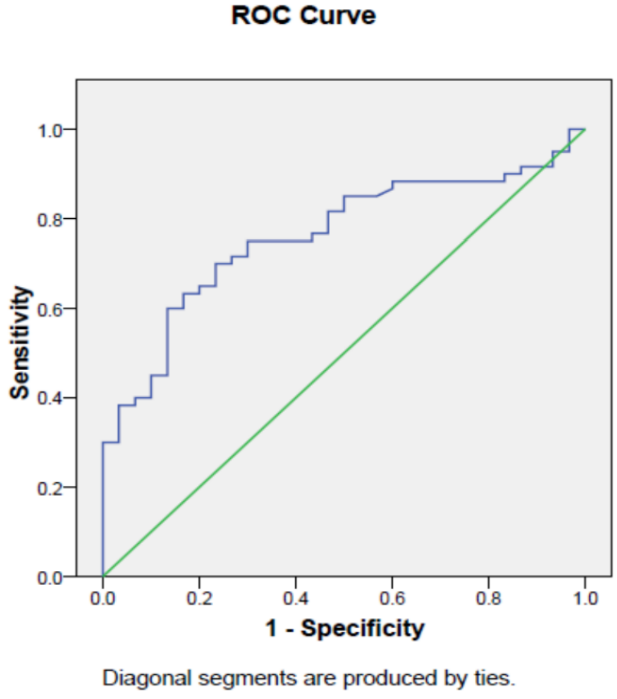
Insignificant p-values were obtained for variables viz., age, gender, height, systolic and diastolic BP while comparing the cases and controls. This implies that the cases and controls were perfectly matched with respect to the confounding variables. There was a statistically significant difference between cases and controls with respect to weight and BMI. This indicates that obesity has a significant role in pathogenesis of OA knee.

**ROC Curve of OPN for Analysis of the Diagnostic Values of Plasma OPN in OA Patients**




| Area | Std. Error(a) | Asymptotic Sig.(b) | Asymptotic 95% Confidence Interval | |
|---|---|---|---|---|
| 0.683 | 0.056 | 0.005 | 0.572 | 0.793 |

*ROC Curve and Area under the Curve–OPN*




| Area | Std. Error(a) | Asymptotic Sig.(b) | Asymptotic 95% Confidence Interval | |
|---|---|---|---|---|
| 0.761 | 0.051 | 0.000 | 0.662 | 0.860 |

*ROC Curve and Area under the Curve for HA*

The common secondary causes of OA knee-post traumatic were ruled out by history and the other most common cause was CPPD (Calcium Pyrophosphate Deposition) disease in the elderly by estimation of calcium and phosphorus. In this study, the levels of serum calcium and phosphorus were normal in both cases and controls, implying that the secondary causes of OA in the study groups have been excluded.

In this study, plasma OPN concentrations were significantly higher in patients with OA knees when compared to controls. Significant differences in OPN concentrations were observed among the cases when they were classified into three groups (K/L grade 2, 3 and 4) based on their radiological findings. The patients with OA knees showed an increase in plasma OPN concentration as their radiological grade advanced. In this study, the OPN levels did not differ significantly by gender in both study groups. This study also observed a positive correlation between OPN and K/L grade, suggesting that an advancement in K/L grade also leads to an increase in OPN concentration. Hence, plasma OPN levels significantly correlate with the severity of the disease. Honsawek et al. obtained similar results.[20] An increase in OPN level among cases when compared to controls and an increase in osteopontin levels with radiological grades 3 and 4 when compared to K/L grade 2 were also observed by Honsawek et al.[20]

In the present study, serum HA concentrations were significantly higher in patients with OA knees than controls. Significant differences in HA concentrations were observed among the cases when they were classified into three groups (K/L grade 2, 3 and 4) based on their radiological findings. The patients with OA knee showed an increase in serum HA concentration as their radiological grade advanced. This increase in HA level with radiological grade was statistically significant.

In this study, the HA levels did not differ significantly by gender in both study groups. Also, a positive correlation was observed between HA and K/L grade in this study, indicating that as K/L grade advances, the HA concentration also increases. Hence, HA levels significantly correlate with the severity of the disease and the amount of cartilage damage. Sharif et al.,[21] observed increased serum HA levels in patients with late stages of OA than in patients with early stages of OA. A positive correlation was demonstrated between serum HA level and the amount of involved cartilage by another study.[22]

## Conclusion

In the present study, a statistically significant increase in concentration of both OPN and HA with respect to radiological grade and a positive correlation between OPN and K/L grade and HA and K/L grade implies that OPN has a significant role in activating MMP-13, causing degradation of articular cartilage and release of HA into the circulation in osteoarthritis.

The correlation between OPN and HA did not show any statistical significance, implying that the HA level increase is contributed by many factors other than OPN.

The diagnostic value of plasma OPN and serum HA was determined by plotting the ROC (Receiver Operator Characteristic) curve. Hence, both the increase in plasma OPN (cut-off value of 617.95pg/mL) and the increase in serum HA (cut-off value of 2.06ng/mL) can be used as biomarkers to assess the severity of the disease and cartilage damage in patients with OA knee.

## References


1. Attur MG, Dave MN, Stuchin S, Kawaiski AJ, Steiner G, Abramson SB, et al. Osteopontin: an intrinsic inhibitor of inflammation in cartilage. Arthritis Rheum. 2001; 44:578–84.
2. Maroudas AI. Balance between swelling pressure and collagen tension in normal and degenerate cartilage. Nature 1976; 260(5554) :808–9.
3. Firestein GS, Budd RC, Gabriel SE, McInnes IB, O'Dell JR. Kelley's Textbook of Rheumatology. 9th edn. Pathogenesis of OA. Elsevier 2012; 1632.
4. Catterall JB, Stabler TV, Flannery CR, Kraus VB. Changes in serum and synovial fluid biomarkers after acute injury (NCT00332254). Arthritis Res Ther 2010;12(6):R229.
5. Oldberg A, Franzen A, Heinegard D. Cloning and sequence analysis of rat bone Sialoprotein (osteopontin) cDNA reveals an Arg-Gly-Asp cell binding sequence. Proc Natl Acad Sci USA. 1986; 83:8819-23.
6. Murry CE, Giachelli CM, Schwartz SM, Vracko R. Macrophages express osteopontin during repair of myocardial necrosis. Am J Pathol 1994;145(6):1450-62.
7. Wang KX, Denhardt DT. Osteopontin: role in immune regulation and stress responses. Cytokine Growth Factor Rev 2008;19(5-6):333-45.
8. Shiraga H, Min W, Van Dusen WJ, Clayman MD, Miner D, Terrell CH, et al. Inhibiion of calcium oxalate crystals growth invitro by uropontin: Another member of the aspartic acid - rich protein superfamily. Proc Natl Acad Sci USA. 1992; 89:426-30.
9. Wang KX, Denhardt DT. Osteopontin: role in immune regulation and stress responses. Cytokine Growth Factor Rev 2008;19(5-6):333-45.
10. Yumoto K, Ishijima M, Rittling SR, Tsuji K, Tsuchiya Y, Kon S, Nifuji A, et al. Osteopontin deficiency protects joints against destruction in anti-type II collagen antibody-induced

arthritis in mice. Proc Natl Acad Sci USA 2002;99(7):4556-61.
11. Qin LF, Wang WC, Fang H, Mao XZ, Huang GL, Chen Y, et al. Expression of NF-κB and osteopontin of synovial fluid of patients with knee osteoarthritis. Asian Pac J Trop Med 2013;6(5):379-82.
12. Gao SG, Li KH, Zeng KB, Tu M, Xu M, Lei GH. Elevated osteopontin level of synovial fluid and articular cartilage is associated with disease severity in knee osteoarthritis patients. Osteoarthritis Cartilage 2010;18(1):82-7.
13. Fatherazis S, Matsa-Dunn D, Foster BL, Rutherford RB, Somerman MJ, Presland RB. Phosphate regulates osteopontin gene transcription. J Dent Res 2009;88(1):39-44.
14. Singh A, Goel SC, Gupta KK, Kumar M, Arun GR, Patil H, Kumaraswamy V, Jha S. The role of stem cells in osteoarthritis: an experimental study in rabbits. Bone Joint Res 2014;3(2):32-7.
15. Meyer K, Hobby GL, Chaffee E, Dawson MH. The hydrolysis of hyaluronic acid by bacterial enzymes. J Exp Med 1940;71(2):13746.
16. Firestein GS, Budd RC, Gabriel SE, McInnes IB, O'Dell JR. Kelley,s Textbook of rheumatology. 9th edn. Pathogenesis of OA. Elsevier 2012;1633.
17. Hedin PJ, Weitoft T, Hedin H, Engstrom-Laurent A, Saxne T. Serum concentrations of hyaluronan and proteoglycan in joint disease: lack of association. J Rheumatol 1991; 18(10):1601-5.
18. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. Ann Rheum Dis 1957;16(4):494-502.
19. van den Berg WB. Osteoarthritis year 2010 in review: pathomechanisms. Osteoarthritis Cartilage 2011;19(4):338-41.
20. Honsawek S, Tanavalee A, Sakdinakiattikoon M, Chayanupatkul M, Yuktanandana P. Correlation of plasma and synovial fluid osteopontin with disease severity in knee osteoarthritis. Clin Biochem 2009;42(9):808-12.
21. Sharif M, George E, Shepstone L, Knudson W, Thonar EJ, Cushnaghan J, et al. Serum hyaluronic acid level as a predictor of disease progression in osteoarthritis of the knee. Arthritis Rheum 1995;38(6):760-7.
22. Goldberg RL, Huff JP, Lenz ME, Glickman P, Katz R, Thonar EJ. Elevated plasma levels of hyaluronate in patients with osteoarthritis and rheumatoid arthritis. Arthritis Rheum 1991; 34(7):799-80.