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# Correlation of serum hyaluronic acid with clinical and radiological severity in primary knee osteoarthritis

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**Abstract**
**Objective:** To investigate relationship between serum hyaluronic acid (sHA) level and the presence and severity of radiographic knee osteoarthritis (OA) as well as degree of knee pain.
**Design:** A total of 150 subjects (75 controls and 75 cases) were enrolled in this study. Based on the Kellgrene Lawrence (K-L) grade, participants were radiographically classified into three groups: mild (K-L grade1 & 2), moderate (K-L grade 3) and severe (K-L grade 4). The degree of knee pain was quantified by WOMAC knee pain score. sHA levels were compared among the controls & cases and with radiological grading. In addition, the correlation between sHA level and the degree of knee pain was analyzed in each group.
**Results:** In relationship between sHA level and the severity of radiographic knee OA, sHA level of the case group was significantly higher than in the normal group ($P< 0.001$). Furthermore, sHA level correlated with the severity of radiographic knee OA (r = 0.880). sHA level had positive correlation and significant association with WOMAC knee pain in study groups. (r = 0.750, $P<0.001$)
**Conclusion:** sHA level has the potential to be useful for the diagnosis and prognosis of the severity of primary knee OA.

**Keywords:** Clinical pain score (WOMAC score), Serum hyaluronic acid (sHA) levels, Kellgrene Lawrence( K-L) grade, Osteoarthritis(OA)

## Introduction
Osteoarthritis (OA), also often called 'osteoarthrosis or degenerative joint disease', is the most common form of arthritis. It is a leading cause of chronic disability between fourth and fifth decade of life. It usually affects the large weight bearing joints, often the knee & the hip, the cervical & lumbar spine regions and small joints of the hands and feet. Knee osteoarthritis (OA) is one of the most common knee joint diseases in the elderly, and is characterized by progressive cartilage degradation and concomitant bony hypertrophy. In clinical practice, diagnosis and assessment of knee OA are conventionally based on clinical history and radiological findings [1, 2]. Patient's chief complaints are pain and stiffness of their knees, and radiological findings of knee OA include joint space narrowing, osteophyte formation, subchondral sclerosis and cysts [3]. OA should be diagnosed as soon as possible to begin treatment but the current diagnostic tools are inadequate to pick up preclinical changes. The gold standard method of diagnosis has long been plain radiography, but the sensitivity and specificity of that technique have been questioned [4]. However, when radiological diagnosis is established, significant joint damage has often already occurred. To identify patients with a high risk for destructive OA and to monitor drug efficacy, more sensitive techniques than plain X- rays are needed. Magnetic resonance imaging is currently being optimized for this purpose [5]. Magnetic resonance imaging (MRI) may allow for earlier OA diagnosis because it is capable of detecting cartilage damage, small osteophytes, subchondral bone changes, and synovitis in the presence or absence of symptoms. Nevertheless, MRI is expensive, time consuming, contraindicated in some patients and not available at all places specialy rural area.
Serum biomarkers are a potentially useful alternative tool besides conventional diagnostic imaging examination. Biomarkers allow disease activity to be objectively evaluated, are easily measured in office-based practices and can help patients understand their condition [4].

To date, various biomarkers of knee OA have been studied to potentially aid in early diagnosis and to assess minor changes in patient's bone or cartilage that are predictive factors for further development of knee OA. Amongst biomarkers, sHA is particularly promising. Several cross-sectional studies have reported that measuring sHA level may be useful for not only diagnosing knee OA but also identifying disease duration, severity, and the extent of OA-related knee pain. Therefore, sHA may have potential as a prognostic indicator of progressive knee OA, but the relationship between sHA and knee OA has only been examined in a few longitudinal studies [4, 6, 7].

Hyaluronan, also known as Hyaluronic acid (HA) or hyaluronate is a large linear non-sulfated glycosaminoglycan (GAG) with a molecular weight between10/6 and 10/7 Da. About one-half of the body's entire Hyaluronan is found in the skin and about one fourth in the skeleton and its supporting structures like ligaments and joints. Hyaluronan is synthesized by fibroblasts and other specialized connective tissue cells. Hyaluronic acid is a common component of most connective tissues as well as being a principal component of the synovial fluid, being secreted by the fibroblastic synovial lining cells [8]. Normal concentration of hyaluronan is 0-75ng/ml in human serum.

In patients with knee OA, sHA correlates with the degree of synovial proliferation and the sizes of osteophytes. Increased sHA is observed in OA and levels are even higher in RA. Patients with higher initial values show a more rapidly progressive course of disease. sHA can correlate with the degree of joint space narrowing. RA patients with synovial inflammation show a decrease in sHA after anti-inflammatory therapy [8, 9]

The present study was undertaken to determine the relationship and correlation between sHA level with clinical findings and radiological changes in primary knee osteoarthritis.

**Methodology**

A Case control study was conducted in the Department of Orthopedics and Biochemistry in Maharaja Agrasen Medical College & Hospital, Agroha, Hisar. 75 clinically diagnosed patients with primary knee osteoarthritis of age group (40-70 years) which were recruited from OPD of Orthopedics. After history taking and thorough physical examination, cases were asked to fill the WOMAC questionnaire to access the presence and severity of the disease. The recruited cases were explained the purpose and relevance of the study. Volunteer cases were included in the study after informed and written consent. All the cases and control were subjected to bilateral knee radiograph in standing. Under all possible aseptic conditions, 5ml whole venous blood sample of the recruited cases and controls was drawn in syringe and collected in plain vial. Samples taken were kept in plain vial at room temperature before sending in to laboratory. The blood centrifuged and serum separated and stored at temperature -20 $^0$C to -80 $^o$C in small capped vials for long term use for testing. ELISA procedure was done using Sincere Biotech Human Hyaluronic Acid Elisa kit (cat no: E170986214). The kit is used for the vitro quantitative determination of human HA in serum, plasma and other biological fluids. The kit is intended for research use only, not for diagnostic or therapeutic procedure. Detection range of sHA level via this kit was 5.1ng/ml-400ng/ml. Sample is diluted with diluent to the final dilution of 5-fold. Therefore all reading with ELISA kit were multiplied by 5.

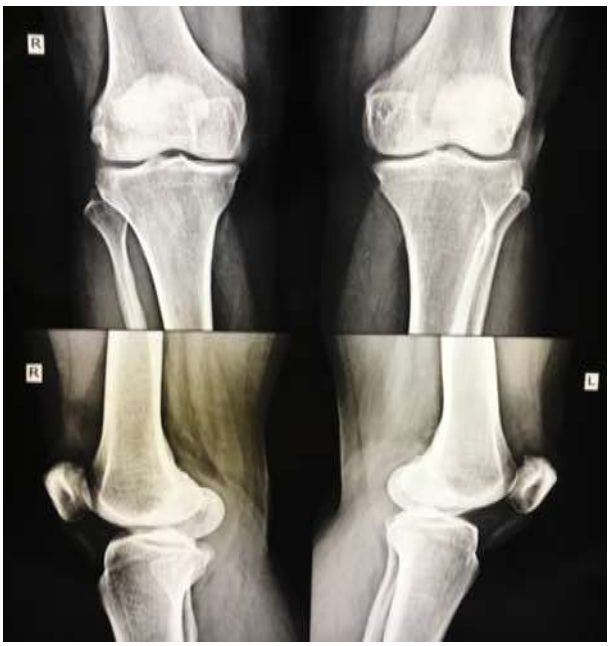
**Elisa Reader**


**Elisa Kits**

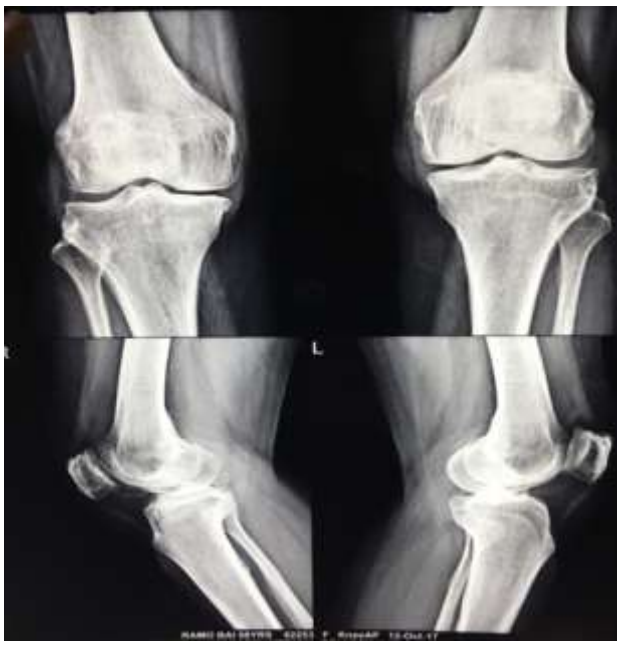
All patients (men and non-pregnant women between 40 and 70 years of age) reporting to Orthopedics OPD with signs and symptoms pertaining to primary knee osteoarthritis were screened and those who fitted into the clinical criteria of osteoarthritis American college of rheumatology were included in this study. Inclusion criteria for controls included all healthy subjects (except active sports persons & manual labourers) between 40 and 70 years of age with no signs and symptoms of Osteoarthritis.

Exclusion criteria for our study were history of rheumatoid arthritis, hepatic disease, renal disease or malignant disease, total knee arthroplasty, total hip arthroplasty and femoral head replacement. In addition, knee OA patient's under treatment and those taking oral non-steroidal anti-inflammatory drugs (NSAIDs) were also excluded.

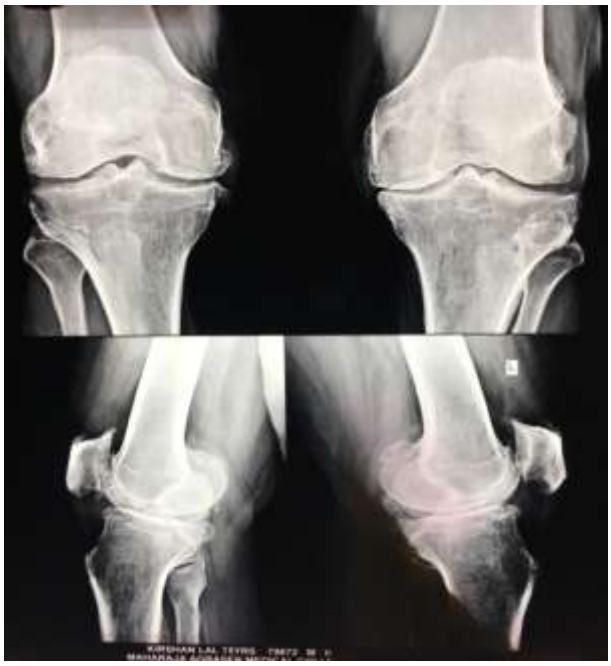
**Radiological photographs showing various K-L grades**



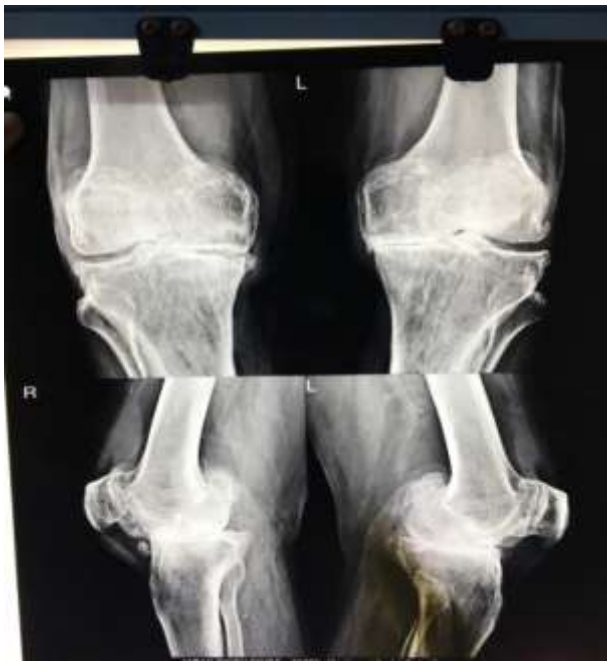
**K-L grade 1**



**K-L grade 2**



**K-L grade 3**



**K-L grade 4**

## Results

The age of subjects in the present study were in the range of 40-70 years with a mean age in control group was 48±7.14years and in case group was 53.97±8.47. There were 82 females and 68 males in study groups. There was significant female preponderance in the present study (56%).

Mean WOMAC score of control group in present study was 11.37±9.143 and mean WOMAC score of case group in present study was 40.43±13.828 (Table 1 &Fig 1). A statistically significant difference in WOMAC score of controls and cases was found (P <0.001).

**Table 1:** Mean WOMAC score in controls and cases

| | Controls | Cases |
|---|---|---|
| Mean womac score | 11.37±9.143 | 40.43±13.828 |

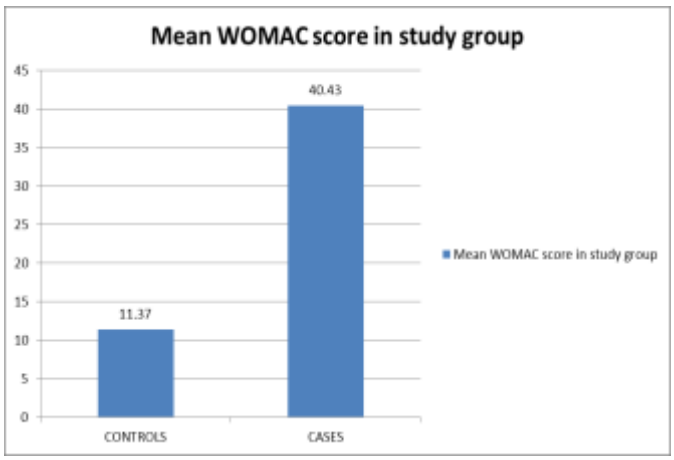



**Fig 1:** Showing mean WOMAC score in controls and cases

There was significant correlation of WOMAC score with various K-L grades of OA patients. It was observed that WOMAC score persistantely increased as the severity of knee OA increased (K-L grading). (Table 2 & Fig 2)

**Table 2:** Showing correlation of WOMAC score in respective of K-L Grades

| | K-L Grade 1 | K-L Grade 2 | K-L Grade3 | K-L Grade 4 |
|---|---|---|---|---|
| WOMAC score | 29.94 | 35.24 | 49.93 | 62.33 |

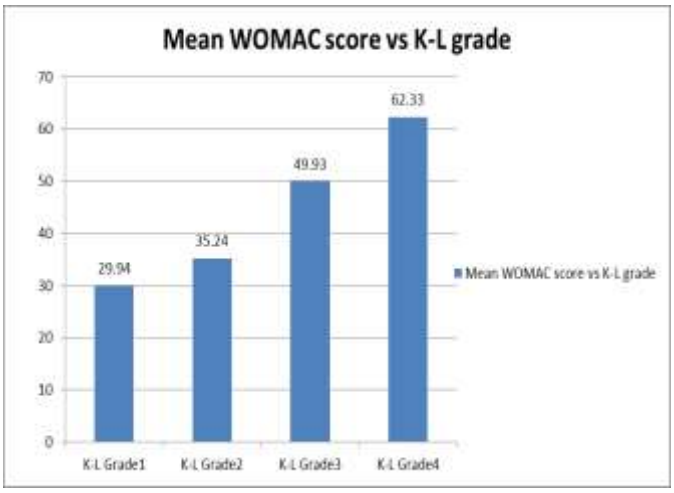



**Fig 2:** Showing correlation of mean WOMAC score with K-L Grades

Mean sHA level in controls was 64.8±13.51ng/ml and in cases was 309.55±173.32ng/ml. (Table 3 and Fig 3). Significantly higher mean sHA levels were found among the cases as compared to controls ($P$<0.001).

**Table 3:** Mean sHA concentration in both controls and cases

| | Controls | Cases |
|---|---|---|
| Mean sHA level | 64.8±13.51 | 309.55±173.32 |

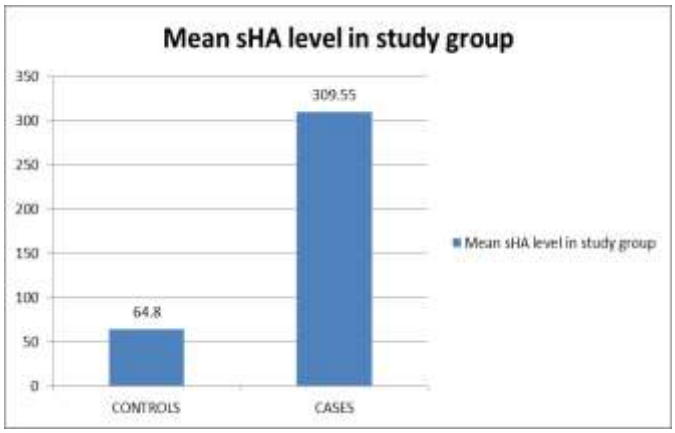



**Fig 3:** Showing mean sHA levels in both controls and cases group

Within case group and control group, there was no significant difference in sHA levels between two genders (P >0.05). Mean sHA levels of males in case group (mean322.23ng/ml) was higher than mean sHA levels of females in the same group (mean: 302.7ng/ml). On the other hand mean sHA levels of males in control group (63.40ng/ml) was lower than in mean sHA levels of females in the same group(66.20ng/ml).

There were significant correlation of sHA levels in various K-L grades of OA patients. It was observed that sHA levels persistantely increased as the severity of knee OA increased (K-L grading). (Table 4 & Fig 4)

**Table 4:** Showing correlation of sHA level in respective of K-L Grade

| | K-L Grade 1 | K-L Grade 2 | K-L Grade3 | K-L Grade 4 |
|---|---|---|---|---|
| sHA | 93.1 | 207.7 | 363.78 | 585.29 |

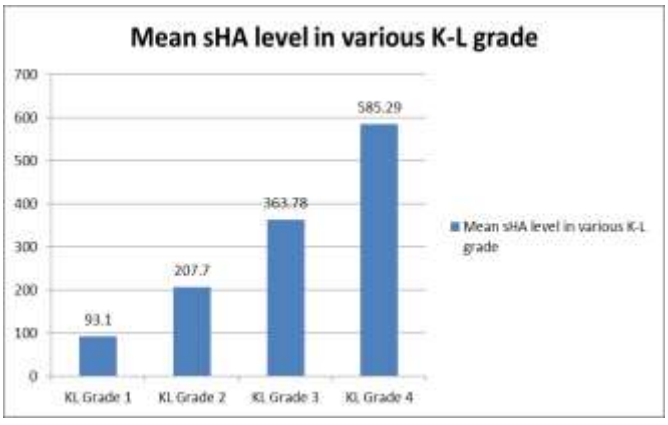



**Fig 4:** Showing correlation of mean sHA level with K-L Grades

## Discussion

Knee Osteoarthritis (OA) is one of the most prevalent condition resulting to disability particularly in elderly population. OA is the most common articular disease of the developed world and a leading cause of chronic disability[10]. OA is characterized by progressive cartilage degradation and concomitant bony hypertrophy. In clinical practice, diagnosis and assessment of knee OA are conventionally based on clinical history and radiological findings [11]. Patient's chief complaints are pain and stiffness of their knees and radiological findings of knee OA include joint space narrowing, osteophyte formation, subchondral sclerosis and cysts [12]. However, radiological findings don't always reflect patient's knee symptoms. Recently, several alternative techniques have been used to assess knee OA, especially in its early stages. Magnetic resonance imaging and ultrasonography are useful techniques for assessing cartilage lesions of knee OA [4, 5]. To date, various biomarkers of knee OA have been studied to potentially aid in early diagnosis and to assess minor changes in patient's bone or cartilage that are predictive factors for further development of knee OA [4-6].

Cibere *et al.* [16] suggested that specific biomarker ratios combining cartilage degradation markers and synthesis markers were better able to differentiate OA stages compared with individual marker levels. Early diagnosis and prediction of progression are of particular importance from the standpoint of prevention and therapeutic strategy. However, although several biomarkers for knee OA have been investigated, there is no established marker for pre-radiographic knee OA. The present study was undertaken to determine the correlation of sHA(biomarker) concentration with radiographic changes and clinical finding in primary

knee osteoarthritis.

The age of subjects in the present study was in the range of 40-70 years with a mean value of 50.98 years. Darwish *et al.* [17] reported mean age 58.9 years and Sasaki *et al.* [4] reported mean age of 55.4years in their respective studies..There was significant female preponderance in the present study (56%). Sasaki *et al.* [4], Garnero *et al.* [5] and Inoue *et al.* [18] reported that OA prevalence more in female than male.

Significant difference of WOMAC score between control and case groups found as $P<0.001$. Inoue *et al.* [18], Darwish *et al.* [17] and Ishijima *et al.* [19] also found significant difference of WOMAC score between both case and control groups.

In present study WOMAC score had a direct correlation and significant difference in various K-L grades ($r=0.872$, $P<0.001$). Inoue *et al.* [18] and Salaffi *et al.* [20] also reported the positive correlation between WOMAC score and K-L grading.

In present study Significantly higher mean sHA levels were found among the cases as compared to controls ($P <0.001$). Sasaki *et al.* [4], Turan *et al.* [7] and Inoue *et al.* [18] also reported that there is significant difference of sHA levels in both case and control groups. In our study there was no significant difference between two genders of study groups as also reported by Sharif *et al.* [21] and Inoue *et al.* [18].

sHA level had a direct positive correlation and significant difference with radiological severity (K-L grading). Elliott *et al.* [6], Turan *et al.* [7] and Inoue R *et al.* [18] reported that sHA level was positively associated with the severity of radiographic knee OA in their study.

In relationship between sHA level and the WOMAC knee pain score in each group, sHA level was positively correlated with WOMAC knee pain score in control and case groups. Because the degree of knee pain seems to reflect synovial inflammation [22] and cartilage degeneration at the time [23], these positive correlations suggest that measurement of sHA level is useful as a biomarker in primary knee OA. High level of sHA may reflect not only a high degree of knee pain but also the severity of radiographic knee OA. George *et al.* [24] and Pavelka *et al.* [25] reported that sHA level had a predictive value for further development of knee OA.

## Conclusion

sHA estimation as a biochemical marker in primary knee OA of knee is still under extensive Research throughout the world for diagnostic, prognostic and therapeutic role. In present study, sHA level had strong significant correlation with severity of radiological primary knee OA (K-L grading). sHA concentration also had direct strong correlation and significant association with WOMAC score (degree of knee pain). Further Research is required to make sHA estimation as a potent reliable tool in prediction or early diagnosis, assessment of severity, progression and therapeutic evaluation in primary OA of the knee.

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