

**Role of Serum Hyaluronic Acid as diagnostic indicator of knee Osteoarthritis**

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**Abstract**

**Introduction:** Osteoarthritis (OA) is a chronic degenerative joint disease, with knee joint most commonly involved. In clinical practice, diagnosis and assessment of knee OA are conventionally based on clinical history and radiological findings. But radiographs provide positive results only after significant joint damage has already occurred. The present study was undertaken to determine the relationship and correlation between serum hyaluronic acid (sHA) level with clinical findings and radiological changes in primary knee osteoarthritis to help in early diagnosis osteoarthritis.

**Materials and Methods:** A case control study was conducted including patients of age group (40-80 years), 100 patients with non-traumatic knee pain who fit into the clinical criteria of American college of rheumatology meeting inclusion criteria were included in case group and all healthy subjects of same age group with no signs and symptoms of knee osteoarthritis, preferably first-

degree relatives of the cases were enrolled as controls. All subjects were asked to fill the WOMAC questionnaire to assess the presence and severity of the disease and were subjected to bilateral knee radiography to ascertain Kellgren-Lawrence grade (K-L Grading). Venous blood samples of all patients were tested for levels of Hyaluronic acid (HA) by enzyme linked immuno-sorbent assay (ELISA).

**Results:** The age of subjects in the present study were in the range of 40-80 years with a mean age in case group was 54.9±6.5 years and in control group was 53.2±7.1years (p>0.05). There were 32 males (32%) and 68 females (68%) in Case Group, while Control Group consisted of 38 males (38%) and 62 females (62%). The difference in K-L grades of Cases and Controls was statistically significant (p<0.001). Mean WOMAC score of cases group in present study was 51.37±12.7 and mean WOMAC score of control group in present study was 12.4±9.38 (p <0.001). Mean sHA levels of Cases Group

was  $308.9 \pm 132.46$  ng/dl and in Control Group was  $65.95 \pm 13.89$  ng/dl and this difference was significant ( $p < 0.001$ ).

**Conclusion:** Serum hyaluronic acid levels are significantly associated with knee osteoarthritis and are able to predict the disease severity in consonance with WOMAC scoring. Hence, they can play a crucial role in identification, gradation and management of patients with knee osteoarthritis.

**Keywords:** Hyaluronic acid, WOMAC score, Osteoarthritis, K-L grade

### Introduction

Osteoarthritis (OA) is a chronic degenerative joint disease characterized by progressive damage of articular cartilage and underlying bone. It is a leading cause of chronic disability between fourth and fifth decade of life. Knee joint is involved most commonly followed by hand and hip. In clinical practice, diagnosis and assessment of knee OA are conventionally based on clinical history and radiological findings<sup>1</sup>. 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<sup>2</sup>. OA should be diagnosed as soon as possible to begin treatment but radiographs provide positive results only after significant joint damage has already occurred<sup>3</sup>. Magnetic resonance imaging is a non-invasive technique and may allow for earlier OA diagnosis but cost and availability prevents routine use. Serum biomarkers are a potentially useful alternative tool besides conventional diagnostic imaging. Biomarkers allow disease activity to be objectively evaluated, are easily measured in office-based practices and can help patients understand their condition<sup>4</sup>. 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, serum hyaluronic acid (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<sup>4,5,6</sup>. 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<sup>7</sup>. 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<sup>7,8</sup>. The present study was undertaken to determine the relationship and correlation between sHA level with clinical findings and radiological changes in primary knee osteoarthritis.

### Materials and Methods

A case control study was conducted including patients of age group (40-80 years) reporting to out patients department with complaints of non- traumatic knee pain and those who fit into the clinical criteria of American college of rheumatology were included in this study. Patients were excluded if they had a) secondary osteoarthritis, b) any other pathology affecting knee joint,

c) renal, hepatic or malignant disease, d) were on treatment of osteoarthritis, e) alcohol or drug abuse, f) have hypersensitivity to non-steroidal anti-inflammatory drugs, g) pregnant and lactating mothers or h) active sportsperson. All healthy subjects between 40 and 80 years of age with no signs and symptoms of knee osteoarthritis, preferably first-degree relatives of the cases were enrolled as controls. The recruited subjects (cases and controls) were explained the purpose and relevance of the study and those who volunteered were included in the study after informed and written consent. After screening we finally selected 100 osteoarthritis cases and 100 normal controls to meet our objective.

All subjects were asked to fill the WOMAC questionnaire to assess the presence and severity of the disease and were subjected to bilateral knee radiography to ascertain Kellgren-Lawrence grade (K-L Grading). Five ml whole venous blood sample of the recruited cases and controls was drawn in syringe and collected in plain vial taking all aseptic precautions and the samples were tested for levels of Hyaluronic acid (HA) by enzyme linked immuno-sorbent assay (ELISA). The samples taken were kept in plain vial at room temperature before sending in to laboratory. The blood samples were centrifuged and serum was separated and stored in small capped vials for long term use at -200C until tested. In Case Group the assessment of severity was done on the basis K-L grades and the group was subdivided as mild grade (K-L Grade II), moderate grade (K-L grade III) and severe grade (K-L grade IV) of the disease. K-L grading of the Control group was also done

**Results**

The age of subjects in the present study were in the range of 40-80 years with a mean age in case group was 54.9±6.5 years and in control group was 53.2±7.1years

(p>0.05). There were 32 males (32%) and 68 females (68%) in Case Group, while Control Group consisted of 38 males (38%) and 62 females (62%). Number of subjects in different K-L grades in Cases Group and Control Group are given in Table 1. The difference in K-L grades of Cases and Controls was statistically significant (p<0.001). Mean WOMAC score of cases group in present study was 51.37±12.7 and mean WOMAC score of control group in present study was 12.4±9.38. A statistically significant difference in WOMAC score of controls and cases was found (p<0.001) (Table 1). Mean sHA levels of cases Group was 308.9±132.46 ng/dl and in Control Group was 65.95±13.89 ng/dl and this difference was significant (p<0.001) (Table 1).

Table 1: Comparison of K-L GRADE, WOMAC SCORE and sHA Level

		Cases	Control	Statistical significance
K-L GRADE	0	0	24	p<0.001
	I	6	76	
	II	38	0	
	III	42	0	
	IV	14	0	
WOMA C SCORE	mean±S D	53.37±12.7	12.4±9.38	p<0.001
sHA LEVEL	mean±sd	308.9±132.46	65.95±13.89	p<0.001

Observed that WOMAC score and sHA level persistently increased as the severity of knee OA increased (K-L grading) (Table 2).

Table 2: Correlation of WOMAC score and sHA level in respective of K-L Grade

K-L GRADE (Increasing)	N	WOMAC score (mean±sd)	sHA level (mean±sd)	Statistical significance

severity)				
I	6	17.9	92.9	p<0.001
II	38	41.6	206.9	
III	42	59.4	364.2	
IV	14	70.5	584.7	

### Discussion

Knee Osteoarthritis (OA) is one of the most prevalent conditions resulting to disability particularly in elderly population. A diagnosis of knee OA is traditionally based upon clinical (WOMAC score) and radiological (K-L Grade) criteria. Usually, joint tissue degeneration is already advanced by the time the diagnosis is made, hence the research focus has now shifted to a find method to diagnose this condition at the early stage of the disease and for this very purpose biomarkers came in to vogue. Although several biomarkers for knee OA have been investigated, there is no established marker for preradiographic 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 40-80 years with a mean value of 54 years, comparable to study conducted by Darwish et al.<sup>9</sup> (58.9 years) and Sasaki et al.<sup>4</sup> (55.4 years) in their respective studies. There was significant female preponderance in the present study (65%), Sasaki et al.<sup>4</sup>, Garnero et al.<sup>10</sup> and Inoue et al.<sup>11</sup> 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.<sup>11</sup>, Darwish et al.<sup>9</sup> and Ishijima et al.<sup>12</sup> 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 (p<0.001). Inoue et al.<sup>11</sup> and Salaffi et al.<sup>13</sup> 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.<sup>4</sup>, Turan et al.<sup>6</sup> and Inoue et al.<sup>11</sup> also reported that there is significant difference of sHA levels in both case and control groups. sHA level had a direct positive correlation and significant difference with radiological severity (K-L grading). Elliott et al.<sup>5</sup>, Turan et al.<sup>6</sup> and Inoue R et al.<sup>11</sup> 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<sup>14</sup> and cartilage degeneration at the time<sup>15</sup>, 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.<sup>16</sup> and Pavelka et al.<sup>17</sup> reported that sHA level had a predictive value for further development of knee OA.

### Conclusion

On the basis of our results, it can be concluded that hyaluronic acid levels are significantly associated with knee osteoarthritis and are able to predict the disease severity in consonance with WOMAC scoring. Hence, they can play a crucial role in identification, gradation and management of patients with knee osteoarthritis.

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