Original Article

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Surface Plasmon Resonance Mediated Evaluation of Cartilage Oligomeric Matrix Protein in serum of Elderly Patients with Knee Osteoarthritis: An Indian Perspective

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Abstract

*The kn*ee osteoarthritis is the most common arthritis in elderly and diagnosed clinically and radiologically. New biomarkers are being tried to diagnose knee osteoarthritis. This work aims to explore the relationship between serum Cartilage Oligomeric Matrix Protein (COMP) and knee osteoarthritis in Indian patients. We recruited 72 elderly patients of knee osteoarthritis (diagnosed according to American College of Rheumatology criteria) and 23 asymptomatic healthy elderly controls from geriatric and medicine OPD. Radiographic severity was determined by K/L grade. The serum COMP level was determined by surface plasmon resonance (SPR) analysis using polyclonal rabbit antibody against COMP. Patients had significantly high level of serum COMP as compared to controls (1.75±.37ng/mL vs. 0.82±.16ng/mL; $p<0.0001$). The serum COMP significantly decreased as the duration of disease increased ($p<0.0001$). Its level was appreciably higher in patients with radiographically proven osteoarthritis ($p=0.045$). There was no significant difference in the level correlated with gender, body mass index, visual analogue scale, age and laterality of knee osteoarthritis. It was demonstrated that the serum COMP can differentiate between knee osteoarthritis elderly patients and the healthy subjects. It characterizes disease severity and early duration of osteoarthritis.

Keywords: Knee Osteoarthritis; Serum; Surface Plasmon Resonance; Cartilage Oligomeric Matrix Protein; Indian Elderly Population.

Introduction

Osteoarthritis is a painful, progressive, degenerative joint disease and characterized by loss of articular cartilage [1,2]. Knee osteoarthritis is the most common arthritis among the elderly people, and is a common cause of disability [3]. The conventional method of diagnosing knee osteoarthritis are clinical and plain radiographs of knee joint which neither capture early stages of osteoarthritis nor helps in monitoring the efficacy of treatment as well as the early progression of disease. The plain radiograph is also a subject of inter-observer variability.

There is an urgent need to identify osteoarthiritis using enhanced techniques in the early stage. Recently, several studies have been done to evaluate the use of reliable biomarkers in serum for early detection, gauge severity of knee osteoarthritis and predict the progression of disease [4]. One such potential biomarker is COMP. It is a non-collagenous, extracellular, pentameric glycoprotein of 524 kDa belonging to thrombospondin family [5,6]. It is primarily identified in cartilage, but is also found in tendon and synovium [7,8]. Its biological function is still debated but it has been suggested that COMP interacts with collagen and may be involved in regulating fibril formation and maintaining integrity of collagen network [6,9]. It has been suggested that

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COMP may increase the ability of articular cartilage to resist mechanical wear [10]. In various studies, it has been demonstrated using ELISA that the serum COMP levels increases in patients with knee osteoarthritis and predicts the progression of disease [11-15]. This study first time reports the correlation of serum COMP and knee osteoarthritis in Indian patients by label free real time surface plasmon resonance (SPR) technology.

Methods

- *Selection of Patients*

In the study, 500 elderly patients were screened on the basis of musculoskeletal symptoms (like-joint pain, joint swelling, and restriction of mobility of joints, crepitus and morning stiffness) and finally 72 knee osteoarthritis patients were selected and diagnosed according to the American College of Rheumatology Criteria (ACR) [2]. The patients were recruited from the medicine and geriatric OPD of All India Institute of Medical Sciences (AIIMS), New Delhi, India. The Ethics Committee of AIIMS approved the study protocol (A-9/25.07.2007) and informed consent was obtained from each subject. The study was performed compliant to the rules and regulations of the Ethics Committee, all subjects gave written informed consent.

The patients above 60 years of age were included in the study. The patients having any inflammatory arthritis, systematic inflammatory disease, severe/ critical illness like- chronic kidney disease, congestive heart failure, hepatic failure etc., traumatic osteoarthritis, neoplasm and those on steroid were excluded. Twenty three age, sex and ethnicity matched healthy subjects were included.

The bilateral antero-posterior, weight bearing plain radiograph of knee was taken for all participants with the subject standing with toes pointed straight ahead, knees fully extended, and weight equally distributed on both feet. The X-ray beam was aimed at the lower pole of the patella and kept parallel to the joint surface. The target–film distance was 36 inches [16]. The severity of knee osteoarthritis was graded by an experienced radiologist (ASB) who was blinded of the patient's profile. Severity grading of knee radiographs was done with the Kellgren/Lawrence (K/L) grading scale [17]: grade 1 was doubtful narrowing of joint space and possible osteophytic lipping; grade 2 was definite osteophytes and possible narrowing of joint space; grade 3 was moderate multiple osteophytes, definite narrowing of joints space, some sclerosis and possible deformity of bone contour; grade 4 was large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone contour. The grade used for analysis was the higher of the two knees. We followed the good clinical practice guidelines according to Helsinki guidelines.

BMI (body mass index) was defined according to World Health organization criteria for Asian population [18]. The groups composing of disease duration were arbitrarily divided into four sets. Patients with radiographic knee OA were defined as having radiographic knee OA of K/L grade ≥2 in at least 1 knee.

- *Separation of Sera from Blood*

Five milliliter of venous blood was collected from anticubital fossa under aseptic conditions in morning hours after 30 minutes of rest. It was allowed to settle down for 1 hr at room temperature. The buffy coat was removed from the blood and centrifuged at 3000 rpm for 20 min. The serum was collected and stored at -70^{0}C in multiple aliquots.

- *Estimation of COMP Level in Serum by SPR*

The level of serum COMP was determined using Biomolecular Interaction Analysis (BIA) in BIAcore 2000 (Pharmacia, Biosensor AB, Sweden) machine that examines and characterizes bio-molecular interactions in real time and is based on SPR principle [19]. For the evaluation of serum COMP levels, the IgG-COMP of human origin (abcam®, Cambridge, USA), was immobilized on the surface of CM5 sensor chip by amine coupling. For this, equal volumes (115 µl) of N-hydroxyl succinimide (NHS) (2.3 mg in 200 µl of water) and N-ethyl-N'-(3-dimethyl-aminopropyl) carbodiimide (EDC) (15mg in 200 µl of water) obtained from Pharmacia were mixed and 75 µl of this solution was passed at the flow rate of 5 µl/min across the CM5 sensor chips to activate the carboxy methylated dextran surface. After this 0.1 µl (50 ng) of COMP antibody in 10 mM sodium acetate (209.9 µl, pH 3.9) was passed at the flow rate of 5 µl/min across the activated surface and the un-reacted groups were blocked by ethanolamine (50 µl).

To prepare the standard curve of COMP protein, seven different concentrations (0.38, 0.80, 1.60, 4.80, 8.00, 11.20 and 14.40 ng/ml) of the commercial recombinant COMP (Immunodiagnostic AG, Bensheim) were passed over the immobilized antibody and the corresponding RUs (Resonance Units) were obtained. A standard curve of RU vs.

concentration of COMP was plotted. After this 40 ìl of 1:99 dilutions (in HBS-EP buffer) of serum for each sample was passed over the immobilized COMP antibody on the sensor chip at a flow rate of 10 µl/ min. The RU for each sample was recorded and the concentration of COMP was derived from the standard curve.

The On and Off values of the COMP protein were also calculated by passing six different concentrations (0.64, 1.28, 3.84, 6.40, 8.96, and 11.52 ng/ml) of protein over the immobilized anti-COMP antibody.

- *Statistical Analysis*

The statistical analysis was done by GraphPad Prism Instat 3 statistical software package (GraphPad Software, Inc. California, USA). The continuous variables were summarized as mean ± SD, and categorical variables as proportions, n(%). Comparison between groups was done by unpaired student's t-test. The association between serum COMP and various variables of knee osteoarthritis patients was accessed by one way analysis of variance (ANOVA). The p value of <0.05 was considered statistically significant.

Results

- *Clinical Data of Knee OA Patients*

500 elderly patients were screened and 98 (19.6%) patients had knee osteoarthritis. But only 72 (14.4%) patients were selected for final serum COMP analysis. 26(5.2%) patients were excluded as they were having neoplasm 10(2%), severe systemic illness 6(1.2%), traumatic arthritis 4(0.8%), on steroids 3(0.6%), refused 3(0.6%).

Mean age group for patients and controls were 65.2±5.5 and 62.9±5.86 years (Mean±SD), respectively. The patients group had 26 males and 46 females whereas the control group comprised of 14 males and 9 females. The mean BMI of patients and controls were 24.5±4.8 kg/m^2 and 23.4±4.1 kg/ m^2, respectively. The patients and controls were

Table 1: Serum COMP level in different clinical parameters of knee OA patients (n=72)

Clinical Parameters	Number of Patients	Serum COMP Level (mg/ml)	p value
Subjects			
Patients	72	1.75±0.37	<0.0001
Controls	23	0.77±0.09	
Age (years)			
60-64.9	33	1.78±0.41	0.581
65-65.9	22	1.75±0.26	
≥70	17	1.66±0.43	
Sex			
Male	26	1.73±0.36	0.739
Female	46	1.76±0.39	
Duration of Disease (months)			
3-23.9	15	2.14±0.30	<0.0001
24-47.9	32	1.81±0.29	
48-71.9	15	1.45±0.20	
≥72	15	1.39±0.26	
BMI (kg/m^2)*			
<25	40	1.68±0.31	0.07
≥25	32	1.84±0.43	
Severity of OA (X-ray grading)			
<2	12	1.55±0.36	0.045
≥2	60	1.79±0.36	
Laterality of knee OA			
Unilateral	07	1.47±0.29	0.148
Bilateral	65	1.72±0.38	
VAS (Visual Analogue Scale)			
<5	50	1.72±0.41	0.37
≥5	22	1.81±0.29	

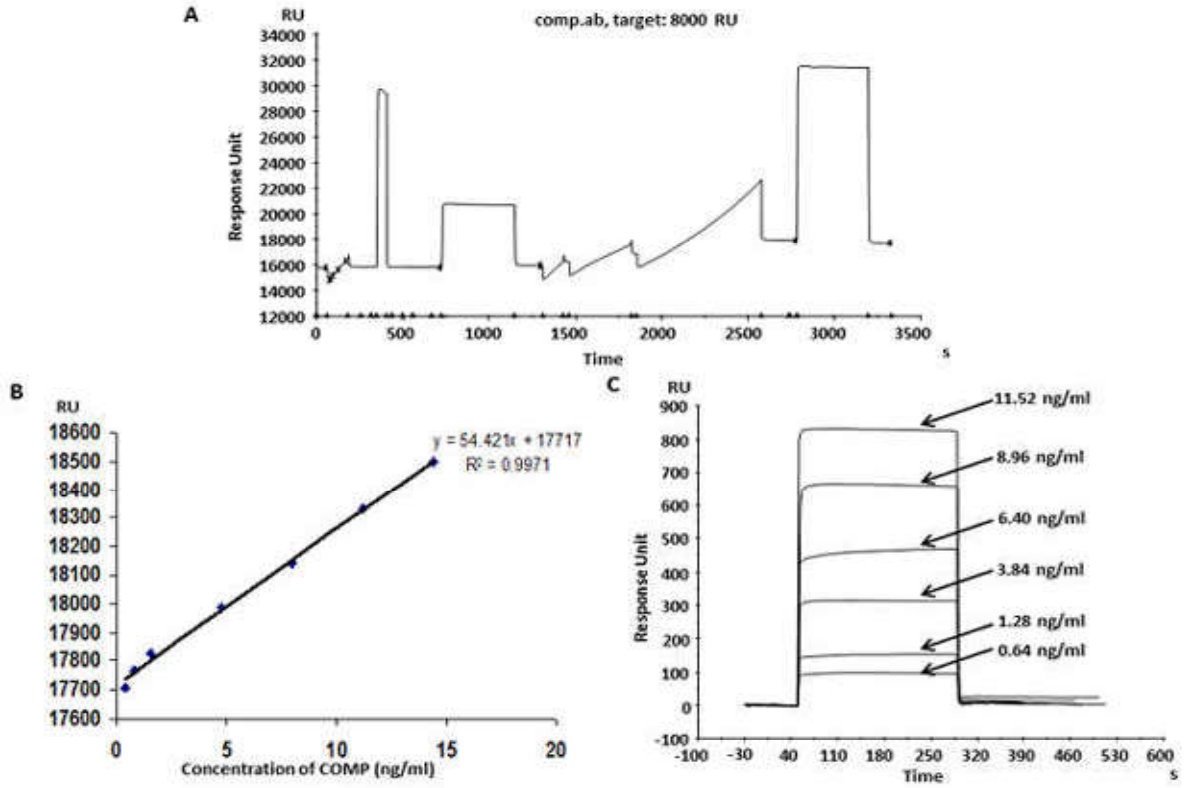

Fig. 1: Assessment of COMP using SPR. (A) Immobilization profile of anti-COMP antibody on the CM5 chip, (B) A standard graph was plotted between different concentrations of COMP and their respective RU, (C) Six different concentrations of COMP passed over the immobilized antibody to calculate the on and off values.

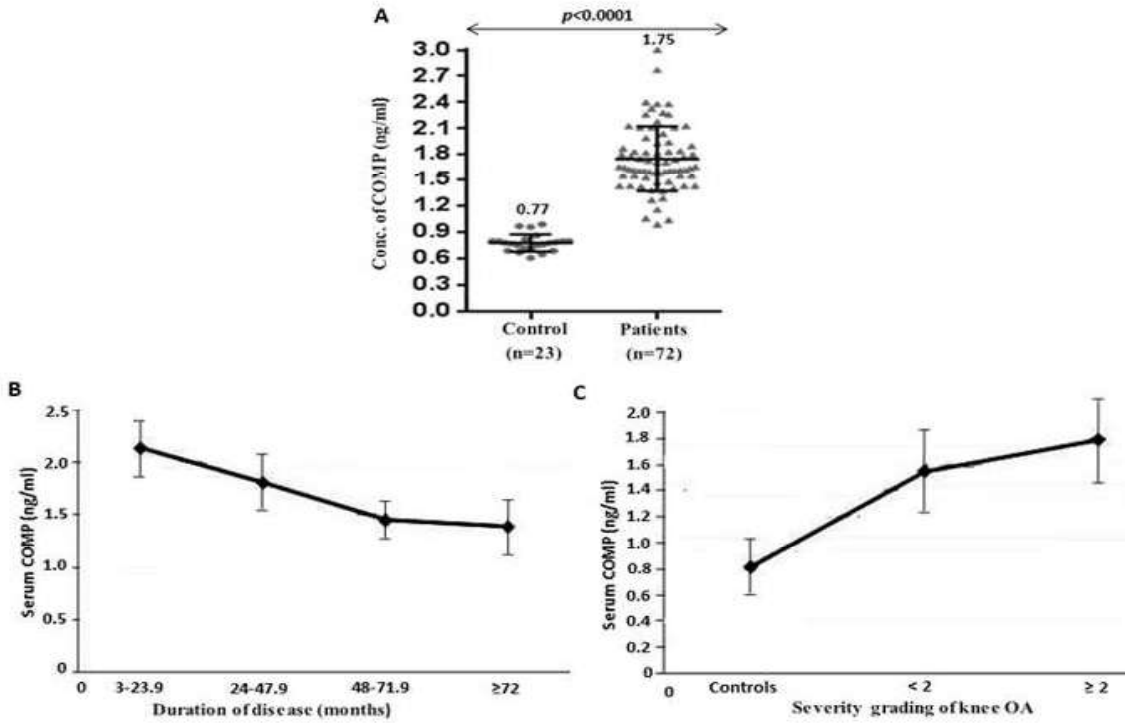

Fig. 2: Evaluation of COMP. (A) Scatter diagram exhibiting the comparison of mean COMP values in control and patients, (B) A comparison of mean COMP levels in patients according to the duration of the disease, (C) A comparison of mean COMP concentrations according to the grading of the knee in OA patients. Error bar shows the standard deviation

matched for age and BMI.

- *COMP Assessment by SPR*

The SPR signal for immobilization of antibody of COMP was found to be 17671.6 RU (Fig. 1A). Five different concentrations of recombinant COMP in HBS-EP buffer were passed over the immobilized COMP antibody and the RUs obtained were (17709.6, 17768.6, 17825.9, 17987.2 18140.9, 18330.2 and18500.0). The standard curve was plotted between RU obtained from the sensorgram and respective concentrations of COMP protein as mentioned above (Figure 1B). The binding of the COMP as ligand was linear and was shown in the Figure 1B The RU was increasing linearly as the concentration of COMP increases. The On (Ka) and Off (Kd) values calculated for the COMP were 1.74 x 10^{12} M and 5.73x10^{-13} M, respectively (Figure 1C).

- *Correlation of COMP with Clinical Parameters*

The correlation of expression levels of COMP with clinical parameters in knee OA patients are shown in Table 1. As shown in the Table 1, significant correlations were found between patients (1.75±0.37ng/ml) and control group (0.77±0.09ng/ml; p<0.0001), severity of the diseases on K/L scale (p=0.045) and duration of disease (p<0.0001). However, there was no significant association with other clinical parameters like age, gender, BMI, laterality of knee OA and VAS. The levels of serum COMP of patients and controls ranged from 0.86 to 3.00 ng/ml, and 0.61 to 1.40 ng/ml, respectively. The mean serum COMP level decreased progressively as the duration of disease increased (p<0.0001). The mean serum COMP level was significantly elevated in patients with knee OA of K/L grade ≥2 (p=0.045) (Figure 2). There was a decreasing trend in the mean serum COMP level with the advancement of age, but it did not reach significance level (p=0.581). The level did not vary differently in both the sexes, although mean serum COMP was slightly higher in females (p=0.739). The patients having higher BMI had higher mean serum COMP level but was not statistically significant (p=0.07). Same trends were seen in unilateral vs. bilateral knee OA (p=0.148) and low and high VAS scale (p=0.37).

Discussion

Our study is different in many aspects from earlier studies. The relationship between serum COMP and knee OA has been investigated previously in several studies in different population, however, to the best of our knowledge, this is the first study in which serum COMP level has been measured using BIA core which utilizes the natural phenomenon of SPR to deliver high quality data in labels free real time. SPR biosensor offers better sensitivity than traditional antibody based methods such as ELISA and Western blotting.

The serum COMP level was significantly higher in patients (1.75±0.37 ng/ml) as compared to asymptomatic healthy controls (0.77±0.09 ng/ml; p<0.0001) in Indian populations. In this study, the mean serum COMP level significantly decreased as the duration of knee OA advanced (p<0.0001). It has been demonstrated that there is loss of cartilage volume, as disease progressed [20,21]. It has been postulated that level of serum COMP may be related to the volume of cartilage [22]. So, it could be possible that longer duration of disease is associated with less volume of cartilage and decreases in level of serum COMP.

The mean serum COMP was higher in radiographically defined knee OA (K/L grade ≥2) than less severe grade (K/L grade <2) {1.79±0.36 vs. 1.55±0.36; p=0.045}. It has been suggested that COMP up-regulates and is responsible for degradation of cartilage in osteoarthritis patients [23].

There was no significant elevation of serum COMP level in various age groups and different gender. Clark et al. showed the increasing level of serum COMP with the advancement of age [12], which was not observed in the present study and in Brazilian population [24]. Serum COMP level did not vary significantly with gender in some earlier studies [12,24] and in the present study; but another study by Jorden et al had shown that serum COMP varies with sex in Caucasian population [25]. It may be due to the ethnic variability, shown in other studies [25,26]. Study done by Valdes et al. had reported that there is a role of genetic polymorphism in various ethnic groups and both the genders, which may be one of the reasons for variable serum COMP levels in different ethnic groups and genders. In this study, serum COMP level was higher in patients having higher BMI, but did not reach the significant level as shown by other studies [12,24]. The different cutoff values for classifying BMI were applied in Asian population.

There was increase in serum COMP in bilateral knee OA, but did not reach significant level as shown by Clark et al. The involvement of OA joints lead to increase in serum COMP level [12]. In the present study, the number of patients having bilateral OA knee was higher as compared to the unilateral knee OA, hence, was not statistically significant (p=0.148).

The association of serum COMP level with different VAS scale in patients was not observed, which was reported in Brazilian and Egyptian population [24,27]. It was probably due to the fact that symptoms were not well correlated with severity of disease; and the perception of pain was subjective one.

Conclusion

It can be concluded that the serum COMP is higher in knee OA patients compared with healthy controls and can be used as a biomarker for symptomatic radiographic knee OA in Indian patients. It is related with severity of knee OA and early duration of disease. We need to follow up the study for further characterizing the role of COMP in knee OA with treatment.

Key Messages

- The level of serum COMP was found to be significantly higher in osteoarthritis patients as compared to controls (1.75±.37ng/mL vs. 0.82±.16ng/mL; $p<0.0001$).
- The serum COMP significantly decreased as the duration of disease increased ($p<0.0001$).
- It was demonstrated that the serum COMP can differentiate between knee osteoarthritis elderly patients and the healthy subjects.

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Conflict of Interest

There was no conflict of interest.

References

1. Lane NE. Osteoarthritis of the hip. N Engl J Med 2007;357:1413-1421.
2. Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, et al. Development of criteria for the classification and reporting of osteoarthritis. Arthritis Rheum. 1986;29:1039-1049.
3. Katz WA. Rheumatology and the rheumatology work- up. In: Katz WA, ed. Diagnosis and management of rheumatic diseases, 2nd edn. Philadelphia: JB Lippincott Co.; 1987.p.3–7.
4. Davis CR, Karl J, Granell R, Renner JB, Dragomir AD, Vilim V, et al. Can biochemical markers serve as surrogates for imaging in knee osteoarthritis? Arthritis Rheum. 2007;56:4038-4047.
5. Oldenberg A, Antonsson P, Lindblom K, Heinegard D. COMP (cartilage oligomeric matrix protein) is structurally related to the thrombospondins. J Biol Chem. 1992;267:22346223-22346250.
6. Posey KL, Hecht JT. The role of cartilage oligomeric matrix protein (COMP) in skeletal disease. Curr Drug Targets. 2008;9:869-877.
7. Di Cesare PE, Carlson CS, Stollerman, ES, Chen PS, Leslie M, Perris R. Expression of cartilage oligomeric matrix protein by human synovium. FEBS Lett. 1997;412:249–252.
8. Di Cesare PE, Hauser N, Lehman D, Pasumarti S, Paulsson M. Cartilage oligomeric matrix protein (COMP) is an abundant component of tendon. FEBS Lett. 1997;354:237–240.
9. Rosenberg K, Olson H, Morgelin M, Heinegard D. Cartilage oligomeric matrix protein shows high affinity zinc-dependent interaction with triple helical collagen. J Biol Chem. 1998;273:20397-20403.
10. Posey KL, Hankenson K, Veerisetty AC, Bornstein P, Lawler J, Hecht JT. Skeletal abnormalities in mice lacking extracellular matrix proteins, thrombospondin-1, thrombospondin-3, thrombospondin-5, and type IX collagen. Am J Pathol. *2008;*172:1664-1674.
11. Hoch JM, Mattacola CG, Medina McKeon JM, Howard JS, Lattermann C. Serum cartilage oligomeric matrix protein (sCOMP) is elevated in patients with knee osteoarthritis: a systematic review and meta-analysis. *Osteoarthritis Cartilage.* 2011;19:1396-1404.
12. Clark AG, Jordan JM, Vilim V, Renner JB, Dragomir AD, Luta G, et al. Serum cartilage oligomeric matrix protein reflects osteoarthritis presence and severity: the Johnston County Osteoarthritis Project. Arthritis Rheum. 1999;42:2356-2364.
13. Sharif M, Saxne T, Shepstone L, Kirwan JR, Elson CJ, Heinegård D, et al. Relationship between serum cartilage oligomeric matrix protein levels and disease progression in osteoarthritis of the knee joint. Br J Rheumatol. 1995;34:306–310.
14. Petersson IF, Boegard T, Svensson B, Heinegard D, Saxne T. Changes in cartilage and bone metabolism identified by serum markers in early osteoarthritis of the knee joint. Br J Rheumatol. 1998;37:46–50.
15. Sharif, M., Kirwan, J. R., Elson, C. J., Granell, R., & Clarke S. Suggestion of nonlinear or phasic progression of knee osteoarthritis based on measurements of serum cartilage oligomeric matrix protein levels over five years. Arthritis Rheum. 2004;50:2479-2488.
16. Leach RE, Gregg T, Siber FJ. Weight-bearing

radiography in osteoarthritis of the knee. Radiology. 1970;97:265-268.

17. Kellgren JH, Lawrence JS. Radiological assessment of osteoarthrosis. Ann Rheum Dis. 1957;16:494-502.
18. Barba C, Cavalli-Sforza T, Cutter J, Darnton-Hill I, Deurenberg P, Gill T, et al. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet. 2004;363:157-163.
19. Jason-Moller L, Murphy M, Bruno JA. Overview of Biacore Systems and Their Applications. Curr Protoc Protein Sci. 2006;45:19.13.1-19.13.14.
20. Raynauld JP, Martel-Pelletier J, Berthiaume MJ, Beaudoin G, Choquette D, Haraoui B, et al. Long term evaluation of disease progression through the quantitative magnetic resonance imaging of symptomatic knee osteoarthritis patients: correlation with clinical symptoms and radiographic changes. Arthritis Res Ther. 2006;8:R21.
21. Cicuttini FM, Wluka AE, Wang Y, Stuckey SL. Longitudinal study of changes in tibial and femoral cartillage in knee osteoarthritis. Arthritis Rheum. 2004;50:94–97.
22. Sharif M, Granell R, Johansen J, Clarke S, Elson C, Kirwan JR. Serum cartilage oligomeric matrix protein and other biomarker profiles in tibiofemoral and patellofemoral osteoarthritis of the knee. Rheumatology. 2006;45:522–526.
23. Helminen HJ, Saamanen AM, Salminen H, Hyttinen MM. Transgeneic mouse model for studing the role of cartilage macromolecules in osteoarthritis. Rheumatology. 2002;41:848-856.
24. Fernandes FA, Pucinelli ML, da Silva, NP, Feldman D. Serum cartilage oligomeric matrix protein (COMP) levels in knee osteoarthritis in a Brazilian population: clinical and radiological correlation. Scand J Rheumatol. 2007;36:211-5.
25. Jordan JM, Luta G, Stabler T, Renner, JB, Dragomir AD, Vilim V, et al. Ethnic and sex differences in serum levels of cartilage oligomeric matrix protein: the Johnston County Osteoarthritis Project. Arthritis Rheum. 2003;48:675-681.
26. Valdes AM, Loughlin J, Oene MV, Chapman K, Surdulescu GL, Doherty M, et al. Sex and ethnic differences in the association of ASPN, CALM1, COL2A1, COMP, and FRZB with genetic susceptibility to osteoarthritis of the knee. Arthritis Rheum. 2007;56:137-146.
27. Awadallah AM, Sabry GH, Khater TM. Serum Level of Cartilage Oligomeric Matrix Protein as a Screening Modality for Osteoarthritis among Knee Joint Pain Patients. J Am Sci. 2010;6:1059-1066.