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Raised serum IL 6 and CRP in Radiographic Knee Osteoarthritis in Eastern India

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ABSTRACT

Background: *Osteoarthritis (OA) is the most common age-related joint disorder in the world. Among the mechanisms involved in osteoarthritis, biomarkers (cytokines profile) may be related to pain and pain intensity, functional capacity, and pressure pain thresholds (PPT). Thus, the study of these relationships may offer useful information about pathophysiology and associated mechanisms involved in osteoarthritis.*

Aims & Objectives: *Therefore, the objective of this study was to investigate the concentration of pro-inflammatory cytokines (IL-6) and CRP in patients with painful knee osteoarthritis CRP in radiographic knee osteoarthritis in eastern India.*

Results: *ESR and hs-CRP were elevated in RKOA cases as compared to controls which were found statistically significant. Serum IL 6 levels were also increased in RKOA cases as compared to controls and were statistically significant (16.88 ± 7.20 versus 6.68 ± 2.89 pg/ml; P < 0.0001). However, a high positive correlation was observed between ESR with serum IL 6 level (r = 0.917; P =<0.001) among RKOA subjects while no correlation was seen in controls (r = -0.019; P = 0.251).*

Conclusion: *This study showed that serum levels of IL-6 and CRP were higher in knee OA than in healthy controls. These findings align with a physio-pathological understanding of pain and functionality that surpasses the one which currently provides basis for therapeutics in OA.*

Keywords: *Osteoarthritis, IL 6, CRP, Radiography, Knee joint, Radiological knee osteoarthritis (RKOA).*

Introduction

Osteoarthritis (OA), a common, age-related, heterogeneous group of disorders characterised pathologically by focal areas of loss of articular cartilage in synovial joints, associated with varying degrees of osteophyte formation, subchondral bone change, and synovitis [1]. The damage in the joints is caused by a mixture of systemic factors that influence the disease, and local mechanical factors that state its distribution and severity. The genetic abnormalities have been described variously, but most sporadic osteoart-hritis depends on minor contributions from several genetic loci [1,2]. Osteoarthritic joint damage may be associated with clinical disorders, but the severity of joint disease is only weakly related to that of the clinical problem. Pain is the most prominent and disabling symptom of OA which is usually nociceptive. It has been presumed that detrimental mechanical loading across the joint and inflammation, especially synovitis, may be the main factors associated with the severity of pain [1, 2].

Moreover, Inflammation is one of the key mechanisms putatively associated with the pathophysiology of knee OA. Synovitis may be considered as a secondary factor in OA which relates to the cartilage and bone changes by the release of degenerative compounds from the extracellular matrix of articular cartilage into the synovial fluid which stimulates cartilage damage [3]. Recently, the role of synovitishas been exposed to be one of the potential markers for knee pain and prognostic factors for both structural and symptomatic progression of OA[4-6]. Thus inflammation is potentially a key factor that appears to act through alteration of cytokine profiles, which occurs secondary to aging of the immune system or obesity [7–10].

Interleukin (IL)-6, a pro-inflammatory cytokine whichis produced by the synovial membrane in patients with knee OA are elevated in a variety of inflammatory conditions as well as in bone resorption [11,12]. Infusion of high doses of IL-6 does not cause symptoms of inflammation rather it suppresses the synthesis of other inflammatory cytokines and causes hepatic production of acute-phase proteins such as C-reactive protein (CRP) [13]. Increased serumhs-CRP and IL-6 have been found to be associated with reduced physical mobility and incident mobility limitation in the elderly [14]. Moreover, other cross-sectional studies also found their altered levels, especially those of CRP, are elevated in OA, although results in recent reports have been contradictory [1518]. The serum levels of IL-6 were associated with the change in knee pain over 5 years and with the future prevalence of radiological knee osteoarthritis (RKOA) [19,20]. The serum levels of IL-6 and hs-CRP were considered to be predictors of a decreased articular cartilage volume in patients with RKOA. Therefore, IL-6, which is associated with synovitis, may also be involved in the pathogenesis of pain associated with RKOA.

Thus the aim of the study was to investigate the serum IL-6 and hs-CRP levels in RKOA and assess whether the prognostic markers are associated with the pathogenesis of the disease.

Materials and Methods

The case control study consists of 42 patients presenting rheumatoid arthritis and 36 control subjects attending the outpatient department of orthopaedics in ICARE Institute of Medical Sciences and Research, Haldia. The diagnosis of RKOA was established by clinical analysis, radiographic analysis, ESR, and hs-CRP tests. Routine biochemical parameters were done for both cases as well as controls. Informed consent was taken by all individuals in both the groups. The study was approved by the Institution Ethics committee.

The radiologic and biochemical factors relevant to the present study included RKOA status obtained from radiographs of both knees, and the serum concentrations of 2 inflammatory cytokines, namely, CRP and IL-6. Radiographs were read by examiners who were blinded to the clinical information, with the readings checked against an atlas of radiographic features to determine a

global Kellgren/Lawrence (K/L) grade of RKOA (scale 0–4) and to evaluate individual features of osteophytes and joint space narrowing (scale 0–3 for both). A case of RKOA was defined as a K/L grade of ≥2 in either knee [21].

About 8 ml of venous blood was taken by arm venous puncture in sterile vials. 5 ml of blood was collected without anticoagulant and serum was separated by centrifugation at 3500 rpm for 15 - 20 mins and was used for measurement of hsCRP and IL 6. Serum hsCRP levels was determined with a high-sensitivity nephelometric method while the serum level of IL 6 was measured by immunoassay Kits (Raybiotech, USA). The rest of the blood sample was collected in sterile tube containing potassium-EDTA anticoagulant for measurement of ESR by Westergren method.

Statistical analysis of different biochemical parameters was performed by Students' *t*-test. All variables were expressed as mean ± SD (standard deviation). Means obtained from two normally distributed sample groups were compared by Student's unpaired two-tailed "*t*"-test and for nonparametric Mann-Whitney *U* "*t*" test. To find out the correlation between two variables, Pearson's product moment correlation coefficient was used. A value of $P < 0.05$ was considered as statistically significant. All statistical analyses were performed by using Graph Pad prism software (version 5, 2007, San Diego, California, USA).

Results

The demographic and biochemical profile of the RKOA subjects and healthy controls is presented in Table 1. There was no significant difference in age or sex distribution in either of the two groups between RKOA and control subjects (Table 1). The BMI were higher in RKOA patients with comparisons to control subjects and were found to be statistically significant (p<0.001). ESR and hs-CRP were elevated in RKOA cases as compared to controls which were found statistically significant (Table 1).

Serum IL 6 levels were also increased in RKOA cases as compared to controls and were statistically significant (16.88 ± 7.20 versus 6.68 ± 2.89 pg/ml; $P < 0.0001$) [Figure 1]. However, a high positive correlation was observed between ESR with serum IL 6 level ($r = 0.917$; $P = <0.001$) among RKOA subjects while no correlation was seen in controls ($r = -0.019$; $P = 0.251$) [Figure 2].

Table 1: Demographic and biochemical profile of the study participants

	Control (n = 36)	RKOA (n = 42)
Age(in years)	57.68 ± 6.1	58.09 ± 7.8
Sex (M/F)	8/28	10/32
BMI (kg/m^2)	23.72 ± 1.74	26.46 ± 1.86*
FPG (mg/dl)	88.23 ± 7.52	92.7 ± 10.47
Serum totalCHL (mg/dl)	168.3 ± 20.24	198.9 ± 62.3*
Serum HDL (mg/dl)	48.98 ± 8.12	32.24 ± 8.66*
Serum TG (mg/dl)	114.7 ± 17.34	129.6 ± 28.2
ESR (mm/h)	11.2 ± 3.24	52.24 ± 19.31*
hs-CRP (mg/L)	0.78 ± 0.12	2.56 ± 1.2*

[FPG, fasting plasma glucose; CHL, cholesterol; TG, triacylglyceride; HDL, high density lipoprotein cholesterol; ESR, Erythrocyte sedimentation rate; hs-CRP, high sensitivity- C Reactive Protein. Age, BMI, and serum levels of biochemical parameters were expressed as the means ± SD. Statistically significant, * p < 0.001 vs Control.]

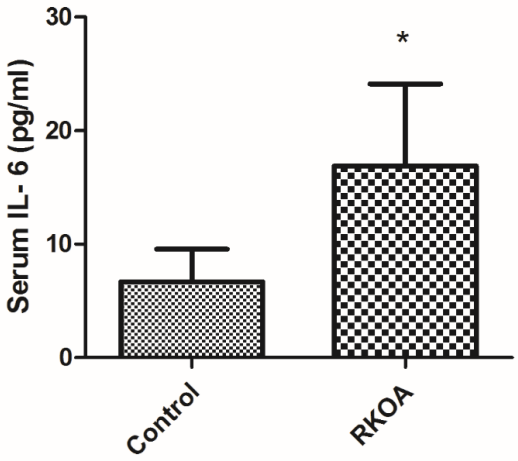

Figure 1: Serum IL 6 levels in RKOA cases as compared to controls

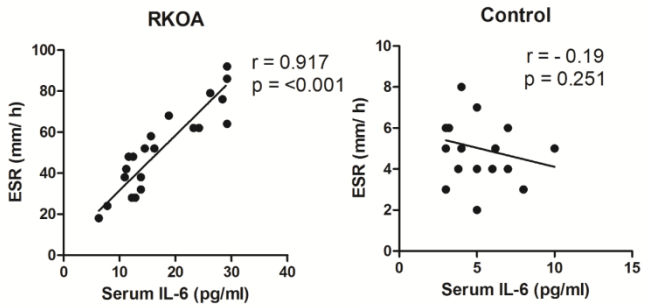

Figure 2: Correlation between ESR with serum IL 6 level among RKOA subjects and in controls.

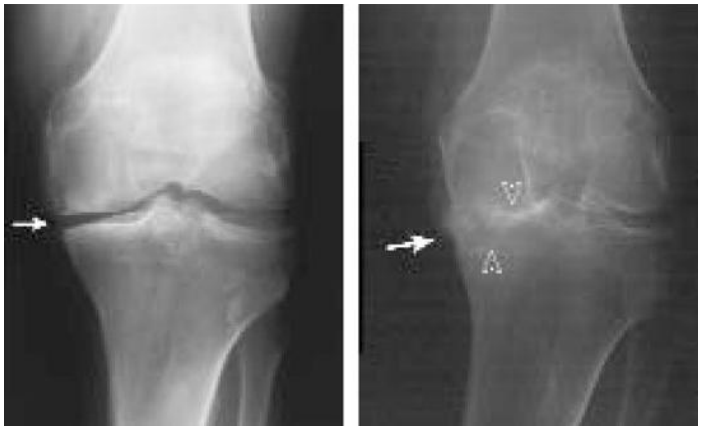

Figure 3: Radiological knee osteoarthritis (RKOA) in study participant

Discussion

A number of facts suggest that development of OA is usually accompanied by inflammation [22]. Many findings from epidemiologic studies found the severity and progression of tibiofemoral cartilage damage to be more common and severe in patients having reactive and inflamed synovial fluid [23]. Further, higher levels of cytokines such as IL-1β and TNFα, both of which are regulators of inflammation, are found in the disease progression[16,24]. Several studies have observed increased levels of CRP have been associated with the prevalence and progression of knee and hip OA, although other studies have found non significant association after adjustment for BMI [15, 17, 19, 25].

Synovitis is seen from the early stage of knee OA [26,27]. The gradual changes in the cartilage matrix turnover might be detected by several potential molecular biomarkers which accounts for synovitis and knee pain in patients with an early-stage knee OA [27]. Severe chronic synovitis was visualised in the advanced stage of knee OA which was correlated with the pain severity and the level of disability than that in the early stage [28,29]. However, an increased degree of monon-uclear cell infiltration and an overproduction of proinflammatory cytokines were more frequently observed in the early-stage of knee OA [26].

One of the vital sources of these pro-inflammatory cytokines is adipose tissue, which accompanies obesity and is known to be associated with high levels of biomarkers of inflammation, including IL-6 [30,31]. One of the studies suggests that synthesis of TNFα in adipose tissue could induce the production of IL-6 and the acute-phase reactant CRP [31]. The development of OA may be due to increased levels of these cytokines. Our study also has shown an increase in IL6 levels and hs CRP levels which support this observation. In our study, we observed significantly higher BMI values in individuals affected with RKOA. It may be due to fat tissue causes systemic release of proinflammatory cytokines. Circulating IL-6 also known to stimulate CRP production by the liver. Moreover, we found a consistent positive correlation between the ESR levels and IL-6 levels, and significantly higher CRP levels in RKOA-affected individuals.

The current study was associated with some limitations. First, the sample size is less in our present study. Secondly, the patients were not divided into subgroups with advanced-stage OA or early-stage OA [Figure 3]. Thirdly, the levels of serum IL-6 have been reported to show a circadian rhythm which we have not studied. Finally, OA patients may be associated with some other diseases such as diabetes mellitus, hypertension, cardiovascular disease which we have not excluded in our present study which might act as a confounding factor. Despite these limitations, there shows a clear rise in serum IL6 and hsCRP levels and also an association between IL6 and ESR levels which helps in identification and progression of the disease. However, a large study needs to be done to conclude the fact.

Conflict of Interest: None declared.

References

1. Felson DT. The sources of pain in knee osteoarthritis. CurrOpinRheumatol. 2005; 17: 624– 628
2. Dieppe PA, Lohmander LS. Pathogenesis and management of pain in osteoarthritis. Lancet. 2005; 365: 965–973.
3. Ayral X, Pickering EH, Woodworth TG, Mackillop N, Dougados M. Synovitis: a potential predictive factor of structural progression of medial tibiofemoral knee osteoarthritis – results of a 1 year longitudinal arthroscopic study in 422 patients. Osteoarthritis Cartilage. 2005; 13: 361–367.
4. noue R, Ishibashi Y, Tsuda E, Yamamoto Y, Matsuzaka M, Takahashi I., et al. Knee osteoarthritis, knee joint pain and aging in relation to increasing serum hyaluronan level in the Japanese population. Osteoarthritis Cartilage. 2011; 19: 51–57.
5. Scanzello CR, Goldring SR. The role of synovitis in osteoarthritis pathogenesis. Bone. 2012; 51: 249–257.
6. Sellam J, Berenbaum F. The role of synovitis in pathophysiology and clinical symptoms of osteoarthritis. Nat Rev Rheumatol. 2010; 6: 625–635.
7. Penninx BW, Kritchevsky SB, Newman AB, Nicklas BJ, Simonsick EM, Rubin S, et al. Inflammatory markers and incident mobility limitation in the elderly. J Am Geriatr Soc. 2004;52:1105–1113.
8. Park HS, Park JY, Yu R. Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNF-α and IL-6. Diabetes Res ClinPract.2005;69:29–35.
9. Miller GD, Nicklas BJ, Loeser RF. Inflammatory biomarkers and physical function in older, obese adults with knee pain and self-reported osteoarthritis after intense weight-loss therapy. J Am Geriatr Soc. 2008;56:644–51.
10. Bullo M, Garcia-Lorda P, Megias I, Salas-Salvado J. Systemic inflammation, adipose tissue tumor necrosis factor, and leptin expression. Obes Res. 2003;11:525–531.
11. Tsujinaka T, Fujita J, Ebisui C, Yano M, Kominami E, Suzuki K, et al. Interleukin 6 receptor antibody inhibits muscle atrophy and modulates proteolytic systems in interleukin 6 transgenic mice. J Clin Invest. 1996;97:244–249.
12. Kwan Tat S, Padrines M, Theoleyre S, Heymann D, Fortun Y. IL-6, RANKL, TNF-α/IL-1: interrelations in bone resorption pathophysiology. Cytokine Growth Factor Rev. 2004;15:49–60.
13. Aderka D, Le JM, Vilcek J. IL-6 inhibits lipopolysaccharide-induced tumor necrosis factor production in cultured human monocytes, U937 cells, and in mice. J Immunol. 1989;143:3517–3523.
14. Cesari M, Penninx BW, Pahor M, Lauretani F, Corsi AM, Rhys Williams G, et al., Inflammatory markers and physical performance in older persons: the InCHIANTI study. J Gerontol A BiolSci Med Sci. 2004;59:242–248.
15. Spector TD, Hart DJ, Nandra D, Doyle DV, Mackillop N, Gallimore JR, et al. Low-level increases in serum C-reactive protein are present in early osteoarthritis of the knee and predict progressive disease. Arthritis Rheum. 1997;40:723–727.
16. Goldring MB, Goldring SR. Osteoarthritis. J Cell Physiol. 2007; 213:626–634.
17. Pearle AD, Scanzello CR, George S, Mandl LA, DiCarlo EF, Peterson M, et al. Elevated high-sensitivity C-reactive protein levels are associated with local inflammatory findings in patients with osteoarthritis. Osteoarthritis Cartilage. 2007;15:516–523.
18. Engstrom G, Gerhardsson de Verdier M, Rollof J, Nilsson PM, Lohmander LS. C-reactive protein, metabolic syndrome and incidence of severe hip and knee

osteoarthritis: a population-based cohort study. Osteoarthritis
Cartilage. 2009;17:168-173.
19. Stannus OP, Jones G, Blizzard L, Cicuttini FM, Ding C. Associations between serum levels of inflammatory markers and change in knee pain over 5 years in older adults: a prospective cohort study. Ann Rheum Dis. 2013; 72: 535–540.
20. Livshits G, Zhai G, Hart DJ, Kato BS, Wang H, Williams FM, et al., Interleukin-6 is a significant predictor of radiographic knee osteoarthritis: the Chingford Study. Arthritis Rheum. 2009; 60: 2037–2045.
21. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. Ann Rheum Dis. 1957;16:494–502.
22. Felson DT. Clinical practice: osteoarthritis of the knee. N Engl J Med. 2006;354:841–848.
23. Ayral X, Pickering EH, Woodworth TG, Mackillop N, Dougados M. Synovitis: a potential predictive factor of structural progression of medial tibiofemoral knee osteoarthritis: results of a 1 year longitudinal arthroscopic study in 422 patients. Osteoarthritis
Cartilage. 2005;13:361–367.
24. Goldring MB, Otero M, Tsuchimochi K, Ijiri K, Li Y. Defining the roles of inflammatory and anabolic cytokines in cartilage metabolism. Ann Rheum Dis. 2008;67(Suppl 3):iii75–82.
25. Sturmer T, Brenner H, Koenig W, Gunther KP. Severity and extent of osteoarthritis and low grade systemic inflammation as assessed by high sensitivity C reactive protein. Ann Rheum Dis. 2004;63:200–205.
26. 26.Benito MJ, Veale DJ, FitzGerald O, van den Berg WB, Bresnihan B. Synovial tissue inflammation in early and late osteoarthritis. Ann Rheum Dis. 2005; 64: 1263–1267.
27. Ishijima M, Watari T, Naito K, Kaneko H, Futami I, Yoshimura-Ishida K, et al., Relationships between biomarkers of cartilage, bone, synovial metabolism and knee pain provide insights into the origins of pain in early knee osteoarthritis. Arthritis Res Ther. 2011;13:R22.
28. Haywood L, McWilliams DF, Pearson CI, Gill SE, Ganesan, A, Wilson D. et al., Inflammation and angiogenesis in osteoarthritis. Arthritis Rheum. 2003; 48: 2173–2177.
29. Krasnokutsky S, Belitskaya-Levy I, Bencardino J, Samuels J, Attur M, Regatte R, et al., Quantitative magnetic resonance imaging evidence of synovial proliferation is associated with radiographic severity of knee osteoarthritis. Arthritis Rheum. 2011; 63: 2983–2991.
30. Das UN. Is obesity an inflammatory condition? Nutrition. 2001;17:953–956.
31. Carman WJ, Sowers M, Hawthorne VM, Weissfeld LA. Obesity as a risk factor for osteoarthritis of the hand and wrist: a prospective study. Am J Epidemiol. 1994; 39:119–129.