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Evaluation of Myeloperoxidase and hsCRP in Knee Osteoarthritis

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ABSTRACT

Background: Osteoarthritis (OA) is a leading cause of physical disability and impaired quality of life. There is increasing evidence of production of proinflammatory cytokines and mediators as early features of OA. Myeloperoxidase is one of the principle enzymes released from inflammatory cells, which is involved in the production of reactive oxygen species and hypochlorous acid, which have an oxidative modification effect on articular cartilage. Acute phase reactant, C-reactive Protein (CRP) is important in nonspecific host defence against inflammation. Highly Sensitive C-reactive protein (hsCRP) is probably the most widely used biochemical marker of systemic inflammation. **Aim and Objectives:** The aim of the study is to measure serum hsCRP and plasma Myeloperoxidase (MPO) levels and to correlate between MPO and hsCRP. **Material and Methods:** 60 newly diagnosed, untreated patients of knee osteoarthritis were selected for the study. 30 patients each from early and late knee OA were selected based on Kellgren-Lawrence grading score. 30 age and sex matched apparently healthy subjects were selected as controls for comparison. Levels of hsCRP and MPO were estimated by standard methods in both groups and controls. **Results:** There was highly significant rise in hsCRP and MPO value in early and late knee OA patients as compared to controls. The rise in hsCRP value was significantly high in late knee OA patients, while the rise in MPO value was significantly high in early knee OA. There is mild positive correlation between hsCRP and MPO in early knee OA while negative correlation in group B. **Conclusion:** Higher serum hsCRP concentrations in individuals with late knee OA indicate association of OA with severity and progression of disease. Rise in MPO levels in early OA may help in early detection and timely treatment of knee osteoarthritis to prevent or delay its complications.

KEYWORDS hsCRP, Inflammation, MPO and Osteoarthritis

Introduction:

Osteoarthritis (OA) is the most prevalent of the chronic rheumatic diseases and is a leading cause of pain and disability in most countries worldwide. Knee OA is more important as its prevalence rate is higher as compared with other types of OA and also its presentation is at earlier age groups particularly in younger age groups of obese women [1-3].

Osteoarthritis is characterized by changes in articular cartilage and subchondral bone within synovial joint. There is progressive destruction of aricular cartilage, thickening and fibrosis of joint capsule. These changes are thought to be due to different biochemical changes in the articular cartilage [4,5]. There is growing evidence that markers of systemic inflammation are associated with severity or clinical course of OA. Serum C-reactive Protein (CRP) is probably the most widely used biochemical marker of systemic inflammation. It has been shown that CRP in synovial fluid in patients with OA is well correlated with degree of inflammation [6]. Recently Myeloperoxidase (MPO) has been studied in synovial fluid, serum and urine of knee OA patients and has shown increase in level of MPO with early OA. They have proposed MPO as diagnostic marker for early OA detection [7, 8]. Though these two markers are well studied no one tried to find correlation between these two markers at different stages of disease. Hence the present study is an attempt to estimate and correlate highly sensitive C-reactive protein (hsCRP) and MPO in early and late knee osteoarthritis.

Material and Methods:

The present study is descriptive study with cross sectional design and has been carried out in the Department of Biochemistry in collaboration with Departments of Orthopaedics of B.J. Medical College and Sassoon General hospital, Pune from May 2012 to August 2014. The study protocol was approved by the Ethics Committee of the Institute. Informed written consent was obtained from all the study subjects enrolled in the study.

Selection of Study Subjects:

Sixty newly diagnosed and untreated patients of knee osteoarthritis from the orthopaedic OPD and ward of this institute and who were willing to participate in the study were selected. Patients were diagnosed by experienced orthopaedic surgeons based on clinical features and radiography. Based on Kellgren-Lawrence grading (K-L grade) score study subjects were divided into two groups 30 patients of early knee OA (grade 1 and 2) and 30 patients of late knee OA (grade 3&4).

30 age and sex matched apparently healthy normal subjects were selected as controls for comparison. So, three groups in this study were,

Group A = Early Osteoarthritis patients (K-L grade 1, 2)

Group B = Late Osteoarthritis patients (K-L grade 3,Group C = Controls

We excluded patients suffering from other arthropathies such as rheumatic diseases and having chronic systemic illness, other inflammatory diseases. We also excluded alcoholic and chronic smokers from the study.

Collection of Blood Samples and Storage:
Random blood samples were collected from the Cubital vein. In EDTA vacutainer 2ml of blood was collected for estimation of plasma MPO level. About 3ml blood was taken in plain vacutainer for estimation of serum hsCRP. Plasma and serum were separated respectively by centrifugation at 3000 rpm for 10 minutes and stored at -20^{0}C in the deep freezer until further analysis.

Facilities and Equipment:
***Myeloperoxidase (MPO) Analysis*:**
To measure the level of MPO in the samples, we used a sandwich enzyme-linked immunosorbent assay (MPO; Molecular probes Invitrogen detection technology; Eugene, Oregon) and followed the manufacturer's instructions. In brief, MPO was captured by mouse anti-MPO antibody (primary capture antibody) and Rabbit anti–MPO antibody (secondary capture antibody) which were detected by the Amplex® UltraRed reagent, a fluorogenic substrate for horseradish peroxidase (HRP) that reacts with H_2O_2 in a 1:1 stoichiometric ratio to produce a brightly fluorescent and strongly absorbing product (excitation/emission maxima ~ 568/581nm) which is measured spectrophotometrically. Detection limit is 0.2 to 100ng/ml [3, 9].

Highly sensitive C Reactive Protein (hsCRP):
To estimate serum hsCRP levels, we used quantitative turbidimetry (Spinreact; S.A./S.A.U. Clra.Santa Coloma, Spain) and followed manufacturer's instruction. Latex particles coated with specific anti-human CRP were agglutinated when mixed with samples containing CRP. The agglutination causes an absorbance change measured at 540nm in ERBA CHEM 5 plus V2 semi autoanalyzer, depending upon the CRP contents of the patient sample that can be quantified by comparison with a calibrator of known CRP concentration. Values less than 0.05 mg/L give non-reproducible results with this method. Sample with higher concentration diluted 1/3 by NaCl 9g/l and retested. Undetectable CRP values were recorded as 0.025 mg/L. detection limit is 0.05 to 5mg/l [10, 11].

Statistical Analysis:
Data analysis was done using the SPSS (Statistical Package for the Social Science) Version 11 for window. The ANOVA test was used to find out significant difference in average values between 3 groups; 'Z' test was used to find significant difference between correlation values of 2 groups. Chi-square test was used to show association between variables in 3 groups. Scatter diagram is also used to show correlation between two variables. Bar diagram is used to show comparison between group means. A probability value of 0.05 was accepted as the level of statistical significance. P-value less than 0.05 is considered to be statistically significant (S). P-value less than 0.01 is considered to be statistically highly significant (HS). P-value more than 0.05 is considered to be statistically non-significant (NS).

Results:
The general demographic features of study groups are shown in table 1 of group A has 8 males and 22 females with mean age 48.87years. Group B has 10 males and 20 females with mean age 50.17 years and in Group C has 10 males and 20 females with mean age 48.50. Age and sex wise difference between group A, B and C is statistically not significant (P>0.05) so groups are comparable. (Table 1)

In this study, we observed a highly significant rise in hsCRP value in early and late knee OA patients as compared to controls (1.81 ± 0.93) (P value <0.01). The rise in hsCRP value is also highly significant in late knee OA patients (5.96 ± 2.84) as compared to early knee OA patients (3.59 ± 2.09) (P value <0.01). (Table2 and Fig. 1)

There is highly significant rise in MPO value in early and late knee OA patients as compared to controls (57.65 ± 17.53) (P value <0.01). The rise in MPO value is also highly significant in early knee OA (115.59 ± 33.13) as compared to late knee OA (65.82 ± 26.32) (P value <0.01). (Table 2 and Fig. 2)

Table 1 : Age and sex wise distribution of study subjects

Parameters	Group A (n=30)	Group B (n=30)	Group C (n =30)	P value
Age (years)	48.87 ± 8.35	50.17 ± 4.83	48.50 ± 5.83	>0.05
Sex (M/F)	8/22	10/20	10/20	>0.05

M – male, F – female

Table 2: Comparison of Serum hsCRP and plasma MPO Values in Study Groups

Parameters	Group A Mean ± SD (n=30)	Group B Mean ± SD (n=30)	Group C Mean ± SD (n=30)	F test	P value
hsCRP (mg/L)	3.59 ± 2.09	5.96 ± 2.84	1.81 ± 0.93	29.34	P<0.01
MPO ng/ml	115.59 ± 33.13	65.82 ± 26.32	57.65 ± 17.53	42.16	P<0.01

Gr. A Vs Gr. B: P<0.01 Gr. A Vs Gr. C: P<0.01 Gr. B Vs Gr. C: P<0.01

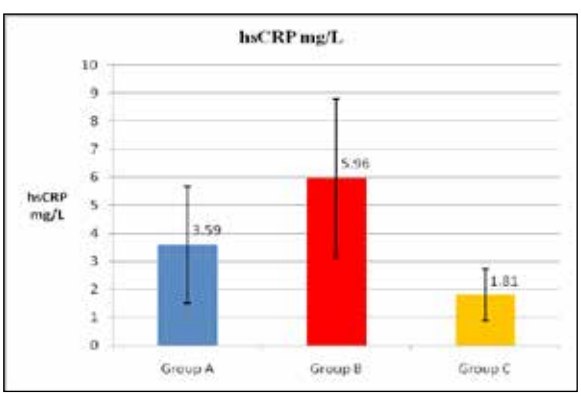

Fig. 1: Bar diagram showing Comparison of Serum hsCRP in Study Groups

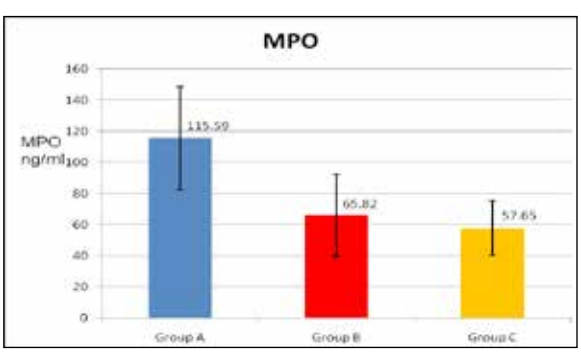

Fig. 2: Bar Diagram showing Comparison of Serum MPO in Study Groups

In our study, statistically there is significant difference observed

in correlation value of hsCRP and MPO in early and late osteoarthritis patients. (Table 3, Fig. 3 and Fig. 4)

Table 3: Comparison of Correlation Values between HSCRP Values and MPO Values in Early and Late Osteoarthritis Respondents

Correlation coefficient	Group A	Group B	Z test	P value
HSCRP vs MPO	0.29	-0.32	2.31	P=0.02

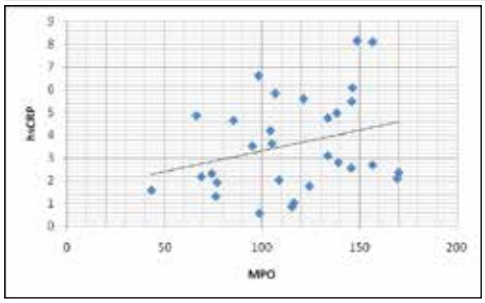

Fig. 3: Scattered Diagram showing Correlation between Serum hsCRP and Plasma MPO in Study Subjects of Group A

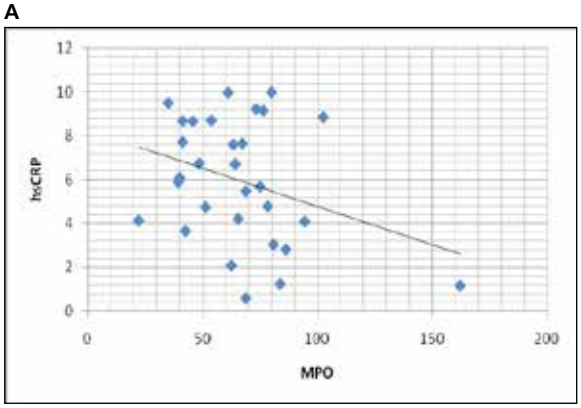

Fig. 4: Scattered Diagram showing Correlation between Serum hsCRP and Plasma MPO in Study Subjects of Group B

Discussion:

In this study, we have observed highly significant rise in hsCRP value in early and late osteoarthritis as compared to controls (1.81 ± 0.93)(P value <0.01). The rise in hsCRP value is also highly significant in late osteoarthritis (5.96 ± 2.84) as compared to early osteoarthritis (3.59 ± 2.09) (P value <0.01). (Table 2 and Fig. 1)

These findings are in accordance with the findings of that of Sharif ***et al*** (2000) which show raised serum CRP reflecting events that precede a period of later radiographic progression in knee OA [12]. Spector ***et al*** (1997) have shown that in response to inflammation tissue necrosis factor (TNF) and interleukin1 (IL1) are released; they stimulates interleukin6 (IL6) which stimulates synthesis of CRP from liver [13, 6].

Earlier studies have suggested a low-grade inflammatory response in OA, although OA inherently lacks significant systemic inflammatory response [14]. Serum hsCRP concentrations are higher in individuals with OA and are associated with OA severity as well as progression of disease. Thus, indicating role of inflammation in pathogenesis of OA.

There is highly significant rise in MPO value in early and late osteoarthritis as compared to controls (57.65 ± 17.53) (P value <0.01). The rise in MPO value is also highly significant in early osteoarthritis (115.59 ± 33.13) as compared to late osteoarthritis (65.82 ± 26.32) (P value <0.01). (Table 2 and Fig. 2)

Our results are consistent with Sunita ***et al*** (2012) who have demonstrated the presence of elevation of plasma and urine MPO levels in early OA patients as compared to late OA and control group, which is indicative of presence of inflammatory infiltrates [8]. Steinbeck ***et al*** (2007) also have shown that synovial fluid samples of patients with early OA have elevated levels of MPO and chlorinated peptides [7]. Punzi ***et al*** (2012) have demonstrated that serum levels of MPO and the ratio Coll2-1NO2/Coll2-1 are higher in erosive hand osteoarthritis (EHOA) than in non-EHOA [16].

Myeloperoxidase (MPO) is released from azurophilic granules of neutrophils and monocytes-macrophages present in the joint in inflammation. Due to inflammation, permeability of cells increases which leads to leakage of MPO from synovial fluid to blood. Presence of MPO indicates an inflammatory involvement in the development of OA, mononuclear cell infiltration within the synovial membrane of patients with OA. H_2O_2, MPO and halide catalyzes reaction to producing hypochlorous acid (HOCl) and Cl_2. HOCl and Cl_2 react with components of articular cartilage such as tyrosine, tryptophan, lysine, and pyridinoline crosslinks and cause subsequent proteolytic cleavage, leading to cartilage destruction [7, 8, 17].

In our study, we have observed mild positive correlation between hsCRP and MPO in group A; while surprisingly we have found negative correlation in group B. (Table 3, Fig. 3 and Fig. 4) In early OA, active inflammation and availability of articular cartilage for destruction are responsible for significantly high levels of MPO in blood as compared to late OA and controls. But in Late OA, even though there is active inflammation as indicated by raised hsCRP, very little cartilage is remaining for destruction. As disease progresses capsule and synovium are often thickened and cellular activity decreases. In late stages; there is marked fibrosis of the capsular tissues decreasing escape of MPO molecules into the blood. Combine effect of these factors causes only slight raise in MPO levels in blood [7, 8]. Though previous studies have proved presence of hsCRP and MPO in synovial fluid as well as in the serum, there is paucity of study of these markers in the stages of disease along with correlation between them.

Limitations :

Study is done in small number of subjects which may not reflect entire population. Despite some limitations, the associations we found were highly significant and consistent with other studies addressing the above mentioned associations. Further Longitudinal studies with repeated assessments of these biochemical markers along with radiological progression within the course of OA are needed to assess the possible value of increase of hsCRP and MPO for early detection, monitoring or predicting the clinical course of OA.

Conclusion :

We can conclude that hsCRP can be marker for progression of knee OA as results showing increase in hsCRP levels goes on increasing as Kellgren-Lawrence grading increases, that is highly significant rise in hsCRP levels in late knee OA as compared to early knee OA. There is highly significant rise in levels of MPO in early knee OA as compared to late knee OA, which is suggestive of MPO can detect disease in early stages. Combination of these parameters can be used for early detectio n and to assess progression of knee OA. This can help in timely treatment and to delay in disease progression.

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