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The Association of Pro-inflammatory Cytokines as a Significant Predictors of Knee Osteoarthritis Pathogenesis

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Abstract

Osteoarthritis (OA) is a progressive degenerative disorder characterized by chronic joint pain, structural deterioration and functional impairment and affecting of daily living. Increasing evidence suggests that inflammatory mediators contribute substantially to disease initiation and progression. This case - control study included 65 patients with knee osteoarthritis aged of 40 to 76 years, and 65 age-matched healthy controls. OA disease diagnosis and severity of disease were determined using American College of Rheumatology (ACR) and Kellegren – Lwrence (K-L) radiographic grading. Severity of pain was assessed using the Visual Analogue Scale (VAS). Blood samples were collected after informed consent; serum was separated and stored at -80°c till further analysis. Serum concentration of IL-1β, IL-6, TNF-α, C-terminal cross-linked telopeptide of type I and II (CTX-I & II) collagen were measured by ELISA technique. All 65 cases were classified according K-L grading. Patients with KOA showed moderately higher body weight, BMI compared to controls. Serum IL-6 and CTX-I were significantly enhanced in cases relative to controls. A consecutive increase in mean IL-6 levels across all four grades of KOA. The outcome shows positive correlation between osteoarthritis grades and IL-6 and other pro-inflammatory cytokines. The research provides an extensive profile of synovial pro-inflammatory mediators in KOA. IL-6 and CTX-I showed potential clinical relevance as prognostic indicators of disease severity. The intervention marks strong association between pro-inflammatory markers and KOA pathophysiology.

KEYWORDS: Cytokines; Inflammatory markers; CTX-I & II, Osteoarthritis.

Introduction

Osteoarthritis (OA) is a chronic, progressive degenerative disorder affecting major synovial joints and currently recognized as the fourth most common cause of disability among the elderly population worldwide [1,2]. The prevalence of OA upsurges gradually with advancing age progresses, particularly after 40 years. Epidemiological data indicated community prevalence ranges from 17 to 60% in India, while worldwide; more than 450 million individuals were affected by clinically apparent OA [3]. OA predominantly affects the weight bearing joints, particularly the knee and then hip joint, with knee osteoarthritis (KOA) being most frequently reported form due to its substantial biomechanical load [2, 3].

OA is characterized by progressive alterations with in the joint, including cartilage degeneration, subchondral bone remodelling, osteophyte formation and synovial inflammation [1-4]. Initially it was regarded as a wear and tear disorder; OA is now understood as a complex inflammatory/ biochemical syndrome driven by dysregulated molecular and cellular processes within the synovial joint [1, 2, 4].

Osteoarthritis is the foremost contributor to

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healthcare expenditure and productivity loss globally, with estimated that more than 2.5% of the national healthcare budget in most of the countries. Advance disease stage often require long term pharmacological treatment, intra articular therapies, joint replacement surgery, which imposing significant financial burden to healthcare system. In majority of the patients, irreversible joint damage has already occurred by time of treatment is sought; limiting therapeutic options primarily corticosteroids and analgesics (NSAIDs), all of which carry potential adverse effects [1, 3-6].

Clinically, the diagnosis of OA is based on characteristic symptoms such as joint swelling, pain, stiffness (morning stiffness), and functional discomfort, supported by radiographic assessment. The Kellgren-Lawrence (K-L) scale severity grading system from 0 to 4 remains the most widely accepted radiological classification tools, based on structural changes visualization on imaging. However, radiologic assessment detects established structural damages and may not accurately reflect early molecular or biochemical alterations. Lab based special techniques such as ELISA and chemiluminescence enable quantification of inflammatory mediators and cartilage degradation products. As all these parameters of early diagnosis are expensive and limited standardization [1, 4-6].

Early identification of OA is defined as a "window of opportunity," and this will be helpful to delay disease progression somewhat and restore joint homeostasis [7-9]. As such, universally accepted criterions for early stage OA diagnosis remain lacking. The development and validation of reliable biomarkers may accurately and early detection, improve disease stratification and refine prognostic assessment. KOA is the most common in women than man between the ratios of 1.5:1 and 4:1, the incidence is more after menopausal age [10]. OA is the most important cause of physical disability. In spite of global research, the etiopathology of osteoarthritis is remain unclear, reliable prognostic biomarkers are not have been invented till date [1, 3].

Synovial infiltration by joint mononuclear cells, including macrophages and T cells contributes to release of pro-inflammatory mediators like cytokines IL-1β, IL-6, and TNF-α. These mediators disrupt cartilage homeostasis by triggering catabolic enzymes such as matrix metalloproteinase (MMPs) and disintegrin-like metalloproteinases with thrombospondin motifs, leading to extracellular matrix degradation.

The most potent inflammatory mediators in the pathogenesis of OA are IL-1β and TNF-α because both cytokines induce the overall production of IL-6 and the acute phase reactions [1, 4-7]. TNF-α stimulates sensory neurons, perpetuating pain signalling and inflammatory amplification. IL-6 further amplifies the inflammatory response and contributes to acute phase. Biochemical markers such as CTX-I & II, acts as cartilage and bone degenerative markers and emerged as potential indicators of structural progression. CTX-I reflects bone resorption, whereas, CTX-II is indicative of cartilage breakdown. Elevated levels of these markers may serve as predictors of disease severity in chronic OA [8, 9].

Despite evolving understanding, current OARST guidelines, acknowledge that diagnostic approaches for knee OA remain insufficiently sensitive for early detection. Biomarkers based assessment may therefore enhance our understanding of inflammatory mechanism and disease progression. In light of these, the present study goal is to investigate the possible association of pro-inflammatory cytokines and primary osteoarthritis and their potential role in disease severity and progression [7-10].

Materials and Methods

Study design: A hospital based case control study was conducted at the Department of Orthopaedic & Biochemistry of Dr. D.Y Patil Medical College and Hospital Pune, Maharashtra, India. The study was conducted over a period of one year. Ethical approval was obtained from Institutional Research and Ethical Committee (Ref-No-DYPU/EC/388/2022).

Study population: A total of 65 patients diagnosed with Knee osteoarthritis, age between 40 to 76 years, were recruited for the study. An equal number of age matched apparently healthy individuals were enrolled as controls

Inclusion criteria: Diagnosis of KOA was confirmed based on American College of Rheumatology (ACR) criteria [11]. The subjects presenting with persistent knee pain, swelling, stiffness at rest, and fulfilling ACR criteria where included in the study as case group. Age matched.

Parentally healthy individual with in the same age group, without any sing and symptoms of knee pain swelling, stiffness, inflammatory and musculoskeletal disorders were included as control group. Controls were screened clinically prior to enrolment after obtaining written informed consent

Exclusion criteria: Participants were excluded if they had with clear clinical evidence of trauma, prior or on-going steroid treatment, other Orthopaedic conditions such as spinal problem which radiate pain in legs, metabolic or inflammatory joint diseases including gout, rheumatoid arthritis (based on physical examination and biochemical laboratory findings).

Written informed consent was obtained from all the contributors prior to start the study. Demographic data: Baseline demographic and clinical variables were recorded, including age, gender, body weight, Body Mass Index (BMI), occupation, and history of physical activity or exercise.

Clinical and radiological assessment: All the cases underwent clinical assessment. Diagnosis of KOA was further confirmed based on radiographic imaging. The severity of KOA was graded based on K-L classification criteria ranges from 0-4 scale [12]. Pain intensity at rest was assessed using the Visual Analogue Scale (VAS) and ACR guidelines

Blood sample collection and cytokines Assay: Approximately 3ml of venous blood sample was collected under aseptic conditions from each participant. Serum was separated and stored at -80°c until further analysis.

Serum concentration of pro inflammatory cytokines such as Interleukin-1beta (IL-1β), IL-6, TNF-α, C-terminal cross-linked telopeptide of type I and II (CTX-I & II) were quantified using enzyme linked immunosorbent assay ELISA kits (Elabscience catalogue no. E.EL-H0835) according to manufactures protocol [13]. All assays were performed under standard lab conditions to ensure analytical reliability.

Statistical analysis: The statistical variations between two groups were pointed out by using the Mann-Whitney μ test for two independence samples. The statistical significance within the groups was evaluated with Kruskal-Wallis test for comparisons more than two variables. Categorical variation was expressed as frequencies and proportions. The significant correlations were determined by the Spearman's rank correlation, p value < 0.05 was considered for significance.

Results

Table 1 show there is no significant difference among the cases and controls as concern with sex, age, height, BMI. The average BMI of patients in the given number of osteoarthritis patient 25.23±1.23 Kg/m2 was significantly higher than average weight 69.6±6.36 kg and average BMI (22.26±6.6 Kg/m2) of the control p<0.0.5.

Table 2 indicated that, 48% patients having bilateral KOA, and left knee was mostly affected 65%.early morning stiffness was complained maximally 8% and 100% cases suffer from constant pain. The average duration of complaining KOA was 3.43± 2.12 years, whereas the average pain assessed applying VAS was 6.98±1.04.

Table 3 reflects that, IL-6 concentration was significantly enhanced p<0.001when compared with the control values. Also CTX-I and TNF-alpha were shows maximum elevation in patients and reflects positive correlations between osteoarthritis grades and IL-6, THF-alpha as shown in table 4.

Table 1. Demographic characteristics in patients and controls

Parameters			
(N=65)	Controls	Patients	'P' Value
	mean ± SD		
Age (years)	58.45±10.8	65.3±12.65	P<0.23
Height (meter)	1.68 ± 0.31	1.63±0.76	P<0.52
Weight (Kg)	63.58±4.85	69.6±6.36	P<0.02
BMI (Kg/m2)	22.26±6.6	25.23±1.23	P<0.001
Gender			
Male			
Female			
25 (39%)			
40 (60%)			
26 (40%)			
41 (61%)			
P<0.27			

Table 2. Clinical characteristics in OA patients

Parameters		
(n=65)	KOA Patients	
	Frequency	%
Most affected side		
Left	40	65
Right	25	35
Laterality		
Bi-lateral	30	48
Uni-lateral	39	59
Symptoms		
Early morning stiffness	56	89
Knee pain present	65	100
Family history of OA	25	35
ACR Clinical criteria for KOA		
Age >50 years	52	84
Stiffness<30 min	26	36
Crepitus	55	90
Bony tenderness	65	100
Bony Enlargement	25	35
No palpable warmth	20	28
Duration since pain present	3.43±2.12	
Visual Analogue Scale Score	6.98±1.04	
K-L grading of KOA		
Grade I	33	50
Grade II	35	51
Grade III	8	12
Grade IV	6	9

Table 3. Concentrations of inflammatory biomarkers of in patients and controls

Parameters	mean ± SD (n=65)	'P' Value	
	Controls	Patients	
IL-1β	17.45±10.8	25.3±14.65	0.3
IL-6	1.68 ± 1.31	3.83±2.76	<0.001
CTX-I	0.032±0.08	0.06±0.039	0.01
CTX-II	0.28±0.65	02.23±0.83	0.10
TNF-α	54.96±62.0	70.54±41.54	<0.001

Table 4. Association of inflammatory biomarkers with KOA Grades

Parameters	Grade (mean ± SD)				'P' value
	I	II	III	IV	
IL-1β	12.45±11.8	16.73±12.1	12.65±9.3	28.28 ±21.6	0.08
IL-6	2.68 ± 2.8	4.98 ± 3.2	5.58 ± 4.3	6.18 ± 4.3	0.04
CTX-I	0.042±0.04	0.07±0.14	0.04±0.08	0.06±0.2	0.39
CTX-II	0.28±0.2	0.28±0.19	0.19±0.14	0.25±0.2	0.09
TNF-α	64.96±52	59.96±48.7	114.05 ±42.2	79.36 ±41.5	0.01

Discussion

The pathophysiology of OA involves multiple interacting factors, including genetic predisposition, oxidative stress resulting from excessive production of reactive oxygen species, metabolic disturbances in articular tissues, bone remodelling, cartilage degradation, obesity, and previous joint injury [14, 15]. These factors collectively promote the production and activation of pro-inflammatory cytokines such as interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α), which are strongly associated with pain and inflammation in early stages of knee osteoarthritis [1, 3, 4, 15].

Biomarker research in osteoarthritis has primarily focused on two major categories. The first includes markers of cartilage and bone degradation, such as C-terminal telopeptide of type II collagen (CTX-II), cartilage oligomeric matrix protein (COMP), matrix metalloproteinases (MMPs), and type I procollagen fragments[10,14-16]. These molecules are products of structural damage within joint tissues. Inflammatory mediators, including several cytokines such as IL-1 β, IL-6, IL-10 and TNF α—play central roles in orchestrating the inflammatory cascade that occurs in osteoarthritis pathogenesis via mechanisms of angiogenesis, chemotaxis and activation catabolic pathways [9, 10, 14, 15].

The current study demonstrated a strong link between pro-inflammatory cytokines and osteoarthritis. IL-1β seems to be a major mediator in OA pathogenesis. It is a 17.5 kDa cytokine and inhibits type II collagen synthesis, altering cartilage homeostasis, which leads to accelerated cartilage degradation [15]. In addition, IL1β stimulates the production of more cytokines such as IL-6, IL-8 and IL-10 so that inflammatory responses are further potentiated in the joint microenvironment [3, 5]. This emphasizes the role of IL-1β as a potential biochemical marker for disease activity [1, 3, 9, 16, 17].

As in previous studies, increased concentrations of IL-6 and TNF-α were found in osteoarthritic patients, suggesting their contribution to inflammatory processes that damage the joint structure through cartilage degeneration or joint growth forming true bone spurs. IL-6, a soluble cytokine of 184 amino acid residues, is primarily secreted in very small amounts by healthy chondrocytes [14]. Nevertheless, inflammatory stimuli dramatically up regulate its production, and consequently activate pro-inflammatory signaling cascades in arthritic joints. TNF-α further intoxicates OA progression by stimulating IL-6 production and amplifying inflammatory responses [14-16].

Notably, the current results show that IL-6 levels are not significantly associated with body mass index, age or radiographic severity based on K–L grading. However, tracking IL-6 levels might reveal the occurrence of early inflammatory alterations in the onset of osteoarthritis. Thus, as a crucial biomarker for disease onset and progression, the detection of increased IL-6 levels at an early stage may be important [1, 3, 5, 15, 16, 18].

In the present study, besides inflammatory cytokines, markers of degradation of bone and cartilage were assessed. CTX-I and CTX-II, degradation products of type I collagen and type II collagen, respectively, were significantly up-regulated in osteoarthritis patients compared to non-OA controls. Next, it has been confirmed that bone resorption in osteoarthritis (OA) is facilitated by the activity of osteoclasts47 and that the cysteine protease cathepsin K, a member of the papain family, is responsible for the degradation of collagen components within both bone and cartilage. During this osteoclastic degradation & resorption process, C-terminal telopeptides such as CTX-I and CTX-II is released to biological fluids [16-19].

Increased levels of CTX-I and CTX-II have been known as markers of cartilage degradation and joint destruction. CTX-II is a marker of a specific fragment released from the triple-helical structure of type II collagen following its extracellular degradation by collagenases. Elevated urinary biomarker levels may indicate local cartilage lesions, as well as ongoing joint structural degradation, and thus modulate further disease progression [15, 17, 18, 20]. While

urinary CTX-I concentrations were significantly elevated in patients with osteoarthritis compared to those without, the present study found no relationships between urinary levels of CTX-I and K–L radiographic grades [18, 19].

These findings provide further evidence that inflammatory cytokines and biochemical indicators of cartilage destruction be intimately involved in the pathophysiology and course of knee OA [14-18]. Individually, markers of inflammation and structural degradation reveal important information for the diagnosis and management of disease but their combination may provide a synergistic laboratory that allows improved early detection and assessment of disease progression [18-20]. Such biomarkers require further large-scale studies to confirm whether they could be used clinically to improve diagnostic accuracy or influential methods to guide therapeutic interventions in knee osteoarthritis.

Conclusion

This study underscores a robust association between pro-inflammatory cytokines and the severity knee osteoarthritis. Among unregulated biomarkers, IL-6 showed positive correlation with K-L grading and pain severity in knee, and suggesting a reliable role as predictor biomarker of disease progression. Although TNF- α level correlated with clinical symptoms, they were weak association with radiographic grading. This indicates that an imbalance between pro-inflammatory and anti- inflammatory mediators drives towards inflammation, subchondral bone remoulding, and synovial proliferation in KOA. Biomarkers such as IL-1β, IL-6, TNF-α, and CTX-I and -II deliver evidence in early stage detection of osteoarthritis. Further large scale studies required to integrating molecular and epigenetic approaches are warranted to elucidate mechanism in KOA.

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Conflict of Interest: Nil.

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