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Analysis of pathogenesis of osteoarthritis: Role of cytokines and review of literature

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Abstract

Background: Osteoarthritis (OA) is the most common diseases of joints that affect almost all people after 5th -6th decade. Traditionally it has been classified as a degenerative disease. Recently there has been a great interest in its pathogenesis. This study was conducted to analyze its pathogenesis especially the role of cyto-chemokines.
Methods: This study was conducted at Aware Global Hospital, Hyderabad, India. 100 patients of different grade of OA (Histology and arthroscopically proven) were selected as cases and 100 patients who never had joint pain, swelling and restricted range of motion were selected as control group. Synovial fluid analysis was done using ultrasensitive MSD multiplex ELISA system. Data was subjected to a one-way analysis of variance using a post hoc Student's Newman-Keuls test.
Results: Analysis of cell free synovial fluid (Table 2) by ultrasensitive MSD multiplex ELISA showed consistent increase in pro-inflammatory cyto-chemokines (RANTES, IL- 1 beta, IFN-beta, IL-6, TNF-alfa, IL-8,) and pro-inflammatory MMPs (MMP-1,MMP-3 and MMP-9) with increase in severity of OA. Anti-inflammatory cyto-chemokines showed significant decrease with progression of OA (IL-10, IL-12p70 and IFN-gama).
Conclusion: Our study suggests that OA is linked with increased levels of proinflammatory cyto-chemokines, particularly those cytokines associated with TLR3 activation. Inflamamatory cyto-chemokines are detected at early stages of OA, grade I-II.
Clinical relevance: Inflammatory cyto-chemokines can be target of therapeutics in early OA to prevent its progression.

Key words – Osteoarthritis, Synovial fluid, Pathogenesis, Cyto-chemokines.

INTRODUCTION

Osteoarthritis (OA) is characterized by progressive damage to articular cartilage resulting from complex interaction of mechanical, genetic and metabolic factors with mediating role of inflammatory molecules. There has been a great interest in understanding of pathogenesis of osteoarthritis in recent years and now it is no longer considered a non inflammatory disease. Age is the most important independent factor, other being the trauma, malalignment and genetics. Water content and other constituents like chondritin sulfate to keratin sulfate ratio and activity of degradative enzymes are different in osteoarthritic cartilage from aging cartilage. With aging cartilage senescence occurs, but osteoarthritis is more than mere aging of cartilage. In the last decade there has been a flood of studies signifying the roll of inflammation in osteoarthritis.

Normal synovial joint can withstand stresses of normal routine activities for life time without developing osteoarthritis.[1,2] Why some patients do develop OA early in their life and why do some patients progress to advanced OA faster than other? Thus age and mechanical factors might play a role but certainly not solely responsible. So osteoarthritis can be considered as failure of tightly regulated remodeling by chondrocytes between synthesis and degradation of cartilage matrix. We think osteoarthritis as end result of many different pathways initiated by factors like age, trauma etc, mediated by inflammatory molecules and landing up in joint destruction. This study deals with the role of different inflammatory molecules and innate immunity in pathogenesis of osteoarthritis.

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METHODS AND MATERIALS

This study included 100 patients who presented to our outpatient department at Aware Global Hospital (AGH) with knee pain in June 2012. Inclusion criteria were strictly followed and study included biopsy proven patients of osteoarthritis. Definition of primary osteoarthritis was observed according to American College of Rheumatology classification criteria for OA [3]. Mean age was 63.4 years (47 to 87 years). Average BMI of patients was 27.89 (21 to 37.33). Exclusion criteria were patient with post traumatic, post infective and inflammatory arthritis, crystal deposition and other metabolic diseases. No antibiotics and anti-inflammatory drugs were given to patients 5 days prior to synovial fluid sample collection. Antero-posterior, lateral and skyline views of the knee were taken in all cases. Kellgren- Lawrence system [33] was used for radiological grading of osteoarthritis. This is a simple system based on joint space narrowing, osteophytes, bone ends sclerosis and deformity. Thirty % patients (n=30) were of grade 4, 18% (n=18) were of grade 3, 27% (n=27) were of grade 2 and 25% (n=25) patients were graded as grade 1. Hundred patients without any evidence of osteoarthritis (who never had joint pain, swelling, restricted range of motion and radiological evidence of arthritis on x ray) were taken as control. Both cases and control were selected from the same population (Andhrapradesh, India). Mean age of control group was 62 years (50-80 years). Average BMI of control group was 27.2 (24 – 52). Synovial fluid was collected from knee joint in all patients during

Table 1: No. of patients in different grade of osteoarthritis.

Grade	Score	No. of patients
1	1-3	25
2	4-7	27
3	8-10	18
4	11-15	30

Table 2: Analysis of different cyto-chemokines in synovial fluid

	RANTES	IFN- beta	IL-6	TNF- alfa	IL-8	IL-1 beta	IFN- alfa	IL-10	IL-12	MMP 12	MMP 3	MMP1		
Mean Value in synovial fluid Pg/ml (Control)		390	100	100	1	10	.35	4.65		6.2	4	1500	2,10000	3,90000
Mean Value in synovial fluid Pg/ml (Grade 4 osteoarthritis)	1400	4500	2600		7.5	180		3.4	.65	2.8	.1	4500	7,40000	6,80000
P value		<.001	<.001	<.01	<.01	<.01	<.01	<.001	<.05	<.01	<.01	<.01	<.01	

arthroscopy. Radiological findings were confirmed arthroscopically and synovial biopsy was sent for Histopathological examination to rule out other diagnoses. All Histopathological slides were formalin fixed, paraffin embedded and H & E stained. Synovial fluid was examined for cytology and biochemical analysis. Analysis of pro-inflammatory cyto-chemokines in grade-specific OA synovial fluid using ultrasensitive MSD multiplex ELISA system was done. Data was subjected to a one-way analysis of variance using a post hoc Student's Newman-Keuls test. Ethical committee approval was taken for the project at AGH.

RESULTS

Cytological and biochemical examination was not contributory except mild to moderate leukocytosis. Arthroscopy confirmed the radiological grading in all patients. Histopathological examination of synovium included lining layer hyperplasia, degree of vascularity, lymphoid aggregates, presence of plasma cells and polymorphonuclear leukocytes. Scoring system described below was used to grade the inflammation. (Table 1)

Mean synovial lining layer thickness/hyperplasia (score: 1 =mean of 1–2 cell layers, 2 = mean of 3–5 cell layers, 3 = mean of >5 cell layers).

Vascularity of the sub lining layer: (two blind scores, (score 0 is lowest expression) and (score 3 is highest expression).

Global cellular infiltration of the sub lining layer: (two blind scores, (score 0 is lowest expression) and (score 3 is highest expression).

Presence/absence of lymphoid aggregates: (absent – score 0 and present –score1).

Plasma cells: (two blind scores, (score 0 is lowest expression) and (score 3 is highest expression).

Polymorphonuclear cells (PMCs): (two blind scores, (score 0 is lowest expression) and (score 3 is highest expression).

Analysis of cell free synovial fluid (Table 2) by ultrasensitive MSD multiplex ELISA showed consistent increase in pro-inflammatory cyto-chemokines, RANTES, IL-1beta, IL-8, IL-6, TNF-alfa, IFN-beta and pro-inflammatory MMPs-1,3 and 9 with increase in severity of OA. Anti-inflammatory cyto-chemokines showed significant decrease with progression of OA (IL-10, IL-12p70 and IFN-gama).

DISCUSSION

Osteoarthritis results from disruption of homeostasis between synthesis and degradation of extra cellular matrix (ECM) of cartilage. Aging, trauma either directly from injury or indirectly from deformity (biomechanical disadvantage) are the main inciting events. As age advances chondrocyte function and matrix changes. Oxidative insult and decreased level of growth factor leads to decreased matrix synthesis [34]. Structural disadvantage in form of malalignment or loss of meniscal tissue / ligament laxity ultimately leads to unequal load distribution and consequently OA. This seems to be oversimplification of its pathogenesis. In the last decade extensive research has evolved the significant role of inflammatory cyto-chemokines in the propagation cascade leading to OA. This is not well understood that how do chondrocytes respond to mechanical stimuli? There may be changes at transcription, translation and post-translation level that lead to degradation of matrix [7,11,37]. It has been observed that increased mechanical stress can lead to increased production of free oxygen radicals and decreased synthesis of proteoglycans thus speeding senescence of chondrocytes [22].

Using modern techniques many researchers have now established role of inflammatory mediators in OA. Association of C-reactive protein (CRP) with progression of knee OA has been suggested [35,26]. Many authors have reported synovial inflammation in the early phase of OA [12,6]. Here it should be understood that although synovitis plays a key role in setting up of OA but inflammation also affects chondrocytes and other parts of joint like menisci and cartilage. Whatever be the initial event (trauma, aging etc.), the inducing event sets in pathological inflammatory response that causes severe joint damage. Now question arises that what are the pathways and driving forces that lead the inducing event to advanced changes of OA. Fotini Kostopoulou et al [9] proposed central role of sterol regulatory element binding proteins (SREBPs) in the pathogenesis of OA. They suggested TGF-beta induces SREBP-2 pathway activation through phospho-phosphoinositide -3-kinase (PI3K) and integrin alfa V (ITGAV), which plays a key role in OA. Anna Koskinen [4] studied role of adiponectin in OA and proposed that adiponectin mediates cartilage damage and induces production of pro-inflammatory and catabolic factors through mitogen activated protein kinase pathways. This is now clear that many pathways that lead to progression of OA are mediated by cyto-chemokines like IL-1beta, TNF alfa, Nitric oxide (NO), and complements [39,1,27,5].

Association of IL-1beta with joint narrowing [10] and that of uric acid with synovial fluid IL-1beta and IL-18 [8] also indicates role of inflammation and symptoms of OA. Ling et al [20] demonstrated high level of IL-1alfa, IL-2, IL-15 and MIP-1alfa (Macrophage inflammatory protein-1 alfa) and MMP-7 in early OA as compared to control. Role of cyto-chemokines as mediators of OA has been reported by many workers, notably are IL-6 and TNF-alfa [36], IL-7[30], IL-6 [16], IL-15 [32], TNF-alfa [31], RANTES [15], IL-18 [25] and IL-1 [28]. Role of complement in OA was suggested by Qian Wang et al [29]. Proteomic and transcriptomic analysis of synovial fluid of OA patients showed expression and level of complement is abnormally high in OA patients. In this study using ultrasensitive MSD multiplex ELISA we demonstrated increase in pro-inflammatory cyto-chemokines and decrease in anti-inflammatory cyto-chemokines in synovial fluid with increasing severity of OA (confirmed by arthroscopy and histology). One interesting finding was elevated level of pro-inflammatory cyto-chemokines from early stage of OA, particularly those associated with toll like receptors-3 (TLR-3) activation (TNF-alfa, IL-6, IL-1 beta etc). Our finding indicates vital role of TLR-3 in development and progression of OA. Another interesting finding was increased level of MMP 1,3 and 9 in synovial fluid. Thus there can be a close interplay of destructive enzymes MMP 1,3,9 and pro and anti-inflammatory cyto-chemokines in synovial fluid of OA.

TLR are pattern recognition receptors that confer the innate immunity and respond to conserved pattern introduced by bacteria, fungi and viruses.[17] Apart from pathogen associated molecular pattern (PAMP) another group known as damage associated molecular pattern (DAMP) activate toll like receptors. Extra cellular matrix (ECM) derived DAMP includes fibronectin,[13] hyaluronic acid and tenascin c.[21,23] Mollen et al,[24] proposed the theory of damage signaling. During stress injury many damage associated molecules are released from degradation of ECM. Fibronectin can induce the production of pro-inflammatory cyto-chemokines including TNF-alfa, IL-beta, MMP-1 and 3.[13] These observations support our findings of role of pro-inflammatory chemo-cytokines in OA. Kuroki et al [19] demonstrated up regulation of TLR-2 and 4 at sites of osteoarthritic cartilage lesions. Many recent studies also support role of these receptors in inducing catabolic responses in chondrocytes [14,18,19,21,23]. In our study we found important role of ligands that act upon Toll like receptors mainly TLR-3 receptors. Thus development of OA is complicated interplay of cyto-chemokines and TLRs. These cyto-chemokines and Toll like receptors can be target of therapeutics in early OA to prevent its progression.

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