Research Article

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Molecular signatures in peripheral blood mononuclear cells with osteoarthritis

Osteoarthritis (OA) is a slow progressive joint disorder in which the joint matrix undergoes dynamic remodeling procedure where the activities of degradative and synthetic enzymes are balanced which in turn maintains the cartilage volume. In OA, the net shift is towards degradation resulting in loss of collagen and proteoglycans from the matrix. However, the etiology of OA remains elusive with inflammation having an important role therefore the present investigation aims to study the gene expression in OA versus control by DNA microarray technology. The study shows upregulated gene expression for apoptotic cell death cascade mediators, inflammatory cytokines, complement components and matrix metalloproteinases (MMPs). Higher expression of anti-inflammatory cytokines and tissue inhibitors of metalloproteinases was observed. Unaltered or lower expression was observed for the components involved in bone metabolism as TGF-β and BMPs. Apoptotic mediators and inflammatory genes are upregulated in OA as compared to genes involved in anabolic and bone turnover pathway, thus shifting the balance towards matrix loss and OA. The higher expression of MMPs, apoptotic death mediators along with cytokines are important to cause cartilage destruction. Therefore therapeutic intervention targeting these may result in better outcome to control the progression of OA.

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Abbreviations: OA, osteoarthritis; TKR, total knee replacement; HTO, high tibial osteotomy; TNF, tumor necrosis factor; IL, interleukin

Introduction

Osteoarthritis (OA) is a common multifactorial disease with unknown etiology characterized by progressive degradation and loss of articular cartilage. Among adults 60 years or above the prevalence of symptomatic knee OA is approximately 10% in men and 13% in women. By 2040, an estimated 78 million Americans ages 18 years or older are projected to have doctor-diagnosed arthritis [1], 49.7% of adults ≥ 65 years reported doctor-diagnosed arthritis from 2010 to 2012 [2]. An estimated 62% of adults with arthritis are <65 years old. An estimated 294,000 children under age 18 have some form of arthritis or rheumatic condition; this represents approximately 1 in every 250 children in the US [3].

OA is generally classified as idiopathic (primary) or secondary. Secondary OA develops in joints with preexisting structural abnormalities. In primary OA, no trauma or other predisposing factors are identified, and intrinsic alterations of the articular tissue, or response to normal cumulative stresses, are presumed responsible.

OA may be a consequence of mechanical and biological events that affects the joints and cause imbalance in the anabolic and catabolic processes within the articular cartilage. The factors which are responsible for increasing susceptibility to OA include obesity, aging, genetics, presence of systemic disorder, trauma etc. In normal people OA might initiate due to minor damage to the joint tissue by physical forces as a single event of trauma or by repeated microtrauma due to altered mechanical loading of the joint [4]. However, pathogenesis of OA is complex where multiple factors are involved in its pathogenesis [5].

Involvement of innate and adaptive immune responses in OA is being accepted in recent epidemiological studies on large number of OA patients because of increased cartilage damage [6], pain and inflammatory synovium/synovitis. Infiltrates of T-cells, B-cells, macrophages [7] and activated complement components [8] are observed in OA synovial tissue.

Immunoglobulins and immune complexes against cartilage components are detected in cartilage, synoium and plasma in OA patients. Thus OA shows the pattern which resembles RA [9] but is less aggressive than RA. Increased mononuclear cell infiltration and overexpression of mediators were found to be present in early OA [10]. However, it is not clear how these proinflammatory cytokines, apoptosis mediators, anti inflammatory cytokines interact and orchestrate the onset of progressive disorder as OA.

Therefore the present investigation aims to study the expression of various components for their involvement in OA versus control. This study would help in understanding the diverse factors which leads to pathogenesis of OA for effective therapeutic management.

Materials & methods

Patient selection for gene expression profiling

Three patients (all females) with knee OA and 3 asymptomatic independent controls (all females) were recruited from OPD of Community Health Centre, India. OA patients were screened according to radiological grading [11]. VAS score and ACR classification was followed for classification of OA [12]. Patients with OA were included who had grade II OA, knee pain (asymmetrical) of more than 6 months, stiffness (<30 min), swelling, crepitation, tenderness on medial side of joint, X-ray had >1/3 decrease in joint space and/or presence of osteophytes and decreased range of motion in their knee joint. Their ligament stability (anterior cruciate, posterior cruciate) was normal. The participants who had trauma, any other disease of joint, smokers and obese were excluded. Participants were matched for sex, age, weight and height (body mass index). None of the controls and patients had any comorbid disease.

RNA extraction and microarray analysis

Total RNA was extracted from whole blood with Qiagen RNA extraction kit according to manufacturer's instructions. Concentration of RNA was measured spectrophotometrically and its integrity was checked by agarose gel electrophoresis. This was used as a starting template to synthesize double-stranded cDNA with random hexamers tagged with T7 promoter sequence. The fragmented DNA was labeled and used for overnight hybridization with Gene ST 1.0 arrays, followed by washing, staining and scanning.

Microarray analysis was done by using Affymetrix Human Gene 1.0 ST arrays. The data QC and RMA normalization was performed for the arrays as recommended by Affymetrix. A fold change of ± 1 was used to select up and down regulated probe sets. The QC analysis was carried out using Affymetrix Expression Console (EC). The statistical analysis was performed using R-programming language and the biological analysis was carried out using GenowizTM software.

Result

The OA patients and controls were matched for age, BMI and sex. None of the participants had any comorbid conditions. The expression profile obtained by DNA microarray analysis revealed differential expression of apoptotic genes, genes for cytokines, complement components, matrix metalloproteases and genes involved in bone metabolism which have known and suspected role in OA pathogenesis. In our findings, most of the caspases like CASP1, CASP4, CASP5, CASP8 were found to be upregulated. Other genes like CRADD, CARD6, CARD16, CARD17 which are members of caspase recruitment domain were found to be upregulated in OA (Figure 1). Apart from these genes there are members of TNF-receptor family, TNFRSF1A, TNFRSF9 and TNFRSF10B and TNF ligand superfamily member 13B were also found to be upregulated (Figure 1).

Anti-apoptotic gene TRAF which interacts with TRADD and inhibit apoptosis was found to be down regulated. BCL2L1, a proapoptotic regulator had significantly high ($p<0.01$) expression in OA as compared to control. The expression of PDCDILG2 and TNFRSF1A was significantly high in OA ($p<0.01$) patients as compared to control. A member of BCL family BAK-1 which is a BCL-anatagonist/killer was also found to be upregulated (Table 1).

Upregulated gene expression was observed for immune system cytokines and complement components. Upregulated expression was observed for IL-1β ($p<0.05$), IL-10, IL-13, IL-17D, IL-18, IL1RA (Figure 2). Downregulated expression was observed for IL-8 and IL-12A. The complement components C1QA, C1QB, C1QC, C2, C3, C4A/C4B, C5, CFP were upregulated (Figure 3). C1QBP and CFH were downregulated.

Prominent upregulation was observed for matrix turnover genes like MMPs of which

gelatinase MMP-9 was highly upregulated ($p<0.01$). Stromelysin MMP11 and MMP17 were upregulated but collagenase MMP8 was downregulated (Figure 4). In Aggrecanases, ADAMTS-5 was upregulated and ADAMTS-1 was downregulated (Figure 4). Upregulated expression was observed for TIMPs particularly TIMP-1, and TIMP-3 (Figure 4).

Interestingly, unaltered expression was observed for many of the candidate susceptibility genes for OA with bone-related functions as COL1A1, ESR1, IGF (data not shown). Bone morphogenetic proteins (BMPs) were all unaltered (data not shown) and BMP6 expression was lower than control (Figure 4). TGFβ1 which plays an important role in bone remodeling as it is a potent stimulator of osteoblastic bone formation, along with TGFβ2 and TGFβ3 which has suppressive effect on IL-2 dependent T-cell growth were also found to be downregulated (Figure 4). S100A proteins which contain calcium binding motif and expressed by macrophages in chronic and acute inflammation were over-expressed. Higher gene expression were observed for vitamin D receptor and S100 protein family as S100P and S100A3, -A4, -A7, -A8, -A11 and -A12 (Figure 4). All the genes and their metabolic pathways along with accession numbers are listed in Table 1.

Discussion

OA is the most frequent disease of muscoskeletal system. Burden of OA is rising consistently due to progressive aging and obesity. Nearly 1 in 2 people may develop symptomatic knee OA by age 85 years [13]. In recent years, there are ever increasing evidences in favor of inflammatory changes in OA. Chronic inflammation has been reported not only in cartilage tissue but also within synovium. Present study shows considerable changes in expression of genes related to apoptotic proteins and inflammatory cytokine, interleukins, and MMPs.

We have found increased expression of apoptotic genes in PBMCs. In the recent studies, increased cell death has been reported to be a feature of OA cartilage in humans and in animal models [14-16]. Cell density is found to be reduced in aging and OA cartilage [17]. In OA cartilage cell death and matrix degradation correlate with one another [18] as matrix degradation results in the loss of survival mechanisms [19] and cell death can contribute to matrix degradation [20] and calcification. It has been reported that cell death in OA cartilage has certain features of apoptosis [14] which is mediated by aspartate specific cysteine proteases or caspases [21]. They must be playing an important role in OA pathogenesis as in *in vitro* studies caspase inhibitors are able to prevent cell death and maintain chondrocyte function [22].

The study be Lima *et al.* [23] showed that the broad spectrum caspase inhibitor was able to reduce the size of OA cartilage lesions, however, it was not able to bring improvement in grade of OA lesion. In our study high gene expression was observed for some caspases and positive regulators of apoptosis. These support the role of cell death mediators contribute to OA pathogenesis. Lima *et al.* [23] have reported high CASP1 and CASP3 activities in animal model.

However, pathophysiology of OA is complex in which many biochemical factors, enzymes and immune mediators are involved. Different cytokines are produced by activated synoviocytes, mononuclear cells or by articular cartilage which in turn upregulate gene expression of many metalloproteinases. In our study IL-1β and IL-18 are upregulated which have a role in promoting joint inflammation and cartilage degradation [24]. Thus OA can have pathologic changes which may involve inflammation with varying degree of severity. As reported by Wojdasiewicz *et al.* [25] that interactions between pro-inflammatory and anti-inflammatory process occur which are driven by cytokine networks.

Minor wear and tear of the joint may be due to obesity, systemic disease, postural causes or infections. Therefore minor wear and tear response in load bearing joint may release chemotactic factors which attract immune cells at the target site. Regular tear response due to mechanical load bearing of the joint might result in release of cytokines and further many other components are involved which may result in OA. The cytokines disrupt the catabolic and anabolic processes, which are important in mechanical load bearing joints [26] resulting in progressive degeneration of articular cartilage. These collectively are mediators of inflammatory, degradative and production processing leading to gradual loss of joint function and onset of pain.

Inflammatory cytokines involved in disease pathogenesis include IL-1β, TNF-α, IL-6, -15, -17 and -18 whereas cytokines with antagonistic effects involved are IL-4, -10 and -13. The proinflammatory cytokines upregulated in our study as IL-1β, IL-17D, IL-18 along with TNFSF

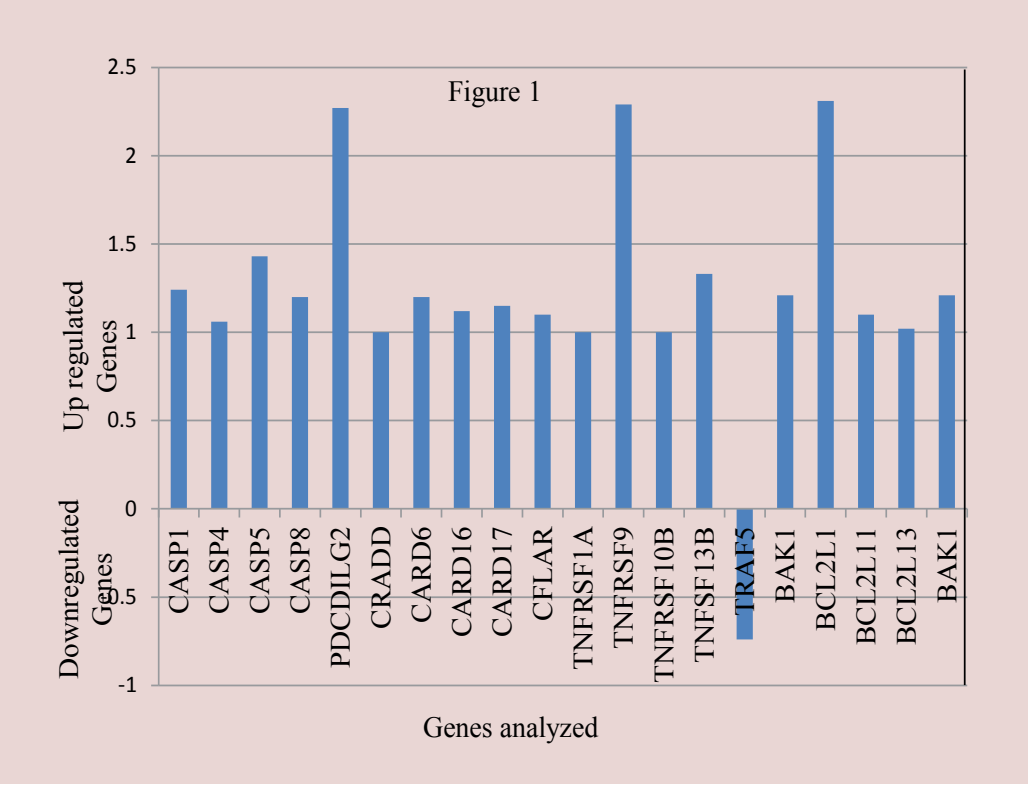

Figure 1: The figure shows the differential expression of genes related to apoptotic cell death cascade in osteoarthritis. The expression of PDCDILG2, TNFRSF1A and BCL2L1 was significantly high in OA ($p<0.0.1$) patients as compared to control. TRAF5 showed downregulated expression as compared o control. The value >0 indicate up-regulation, value <0 indicate down-regulation.

Table 1. The table shows the genes along with their accessions and their information along with their respective metabolic pathway in which they are implicated. Differential expression is observed for genes involved in apoptosis, regulators of apoptosis, BCL family, cytokines, complement components, matrix metallo proteases, tissue inhibitors of metallo-proteases and genes involved in bone metabolism in OA subjects as compared to control.

Component	Name of the Gene	Gene accession	Information about the gene
Apoptosis Tumor necrosis factor	CASP1	NM_033292	Caspase1,apoptosis related cysteine peptidase (Interleukin 1,beta,convertase)
	CASP4	NM_033306	Caspase4,apoptosis related cysteine peptidase
	CASP5	NM_004347	Caspase5,apoptosis related cysteine peptidase
	CASP8	NM_001228	Caspase8,apoptosis related cysteine peptidase
	PDCDILG2	NM_025239	Programmed cell death1, ligand 2
	CRADD	NM_003805	CASP2 and RIPK1 domain containing adapter with death domain
	CARD6	NM_032587	Caspase recruitment domain family member 6
	CARD16	NM_052889	Caspase recruitment domain family member16
	CARD17	NM_001007232	Caspase recruitment domain family member 17
	CFLAR	NM_003879	CASP8 and FADD like apoptosis regulator
	TNFRSF1A	NM_001065	TNF receptor superfamily member 1A
	TNFRSF9	NM_001561	TNF receptor superfamily member 9
	TNFRSF10B	NM_003842	TNF receptor superfamily member 10B
	TNFSF13B	NM_006573	TNF ligand superfamily member 13b
	TRAF5	NM_145759	TNF receptor associated factor5
	BAK1	NM_001188	BCL2 antagonist/killer 1
BCL family member	BCL2L1	NM_138578	BCL2 like1
	BCL2L11	NM_138621	BCL2 like11
	BCL2L13	NM_015367	BCL2 like13

BCL antagonist	BAK1	NM_001188	BCL2 antagonist/killer 1
Cytokines	IL1β	NM_000576	Interleukin 1β
	IL8	NM_000584	Interleukin 8
	IL10	NM_000572	Interleukin 10
	IL12A	NM_000882	Interleukin 12A (natural killer cell stimulatory factor1)
	IL13	NM_002188	Interleukin 13
	IL17D	NM_138284	Interleukin 17D
	IL18	NM_001562	Interleukin 18 (interferon gamma inducing factor)
	IL1RA	NM_000877	Interleukin 1 receptor type1
Complement components	C1QA	NM_015991	Complementcomponent1,qsubcomponent,Achain
	C1QC	NM_001114101	Complementcomponent1,qsubcomponent,Cchain
	C1QB	NM_000491	Complementcomponent1,qsubcomponent,Bchain
	C2	NM_000063	Complementcomponent2
	C3	NM_00064	Complementcomponent3
	C4A/C4B	NM_007293	Complementcomponent4A/4B
	C5	NM_001735	Complementcomponent5
	CFP	NM_002621	Complement factor properdin
	C1QBP	NM_001212	Complement component 1, q subcomponent binding protein
	CFH	NM_000186	Complement factor H
Matrix metalloproteinases	MMP8	NM_002424	Matrix metallopeptidase 8 (neutrophil collagenase)
	MMP9	NM_004994	Matrix metallopeptidase 9 (gelatinase B,92kDa gelatinase 92kDa, typeIV collagenase)
	MMP11	NM_005940	Matrix metallopeptidase 11 (Stromelysin 3)
	MMP17	NM_016155	Matrix metallopeptidase 17 (membrane inserted)
	ADAMTS1	NM_006988	ADAM metallopeptidase with thrombospondin type1, motif 1
	ADAMTS5	NM_007038	ADAM metallopeptidase with thrombospondin type1, motif 5
Tissue inhibitor of metallopeptidase	TIMP1	NM_003254	TIMP metallopeptidase inhibitor 1
	TIMP3	NM_000362	TIMP metallopeptidase inhibitor 3
Genes involved in bone metabolism	BMP6	NM_001718	Bone morphogenetic protein 3
	TGFB1	NM_000660	Transforming growth factor β1
	TGFB2	NM_001135599	Transforming growth factor β 2
	TGFB3	NM_003239	Transforming growth factor β 3
	VDR	NM_001017535	Vitamin D (1,25-dihydroxy vitamin D3) receptor
	S100A3,A4 A7,A8	NM_002960,019554 ,002963,002964	S100 Calcium binding proteinA3,A4,A7,A8
	S100A11,A12	NM_005620,NM_005621	S100 Calcium binding proteinA11, A12

and its helper components may contribute to disease response. Of these IL-1β is considered key cytokine involved in pathogenesis of OA. Patients with OA have an elevated levels of IL-1β in both synovial fluid, synovial membrane, cartilage and subchondral bone layer [27,28]. IL-1β is produced as precursor (pro IL-1β) which is cleaved by caspase -1 (IL-1β converting enzyme). Once IL-1β is bound to its receptor it increased various effector expressions.

Study by Kubota *et al.* [29] showed that IL-1β levels in synovial fluid of temporomandibular joints have positive correlation with OA changes. Shlopov *et al.* [30] have reported high IL-1β binding due to increased expression of its receptor on chondrocyte located in cartilage proximal to macroscopic OA site. There is a possibility that upregulation of IL-1R1 on the chondrocytes makes these cells sensitive to the effects of IL-1β. Higher IL-1R1 has been reported in OA synovial cells [31]. IL-1 ligand cluster also increases susceptibility to knee OA [32]. IL-1β has been reported to enhance expression of MMPs as MMP1, -3, -13 and ADAMTS-4 in OA chondrocytes [33]. Bondeson *et al.* reported ADAMTS-4 and ADAMTS-5 were upregulated by IL-1β in human OA synovial fibroblasts [34]. Dai *et al.* reported upregulation of IL-

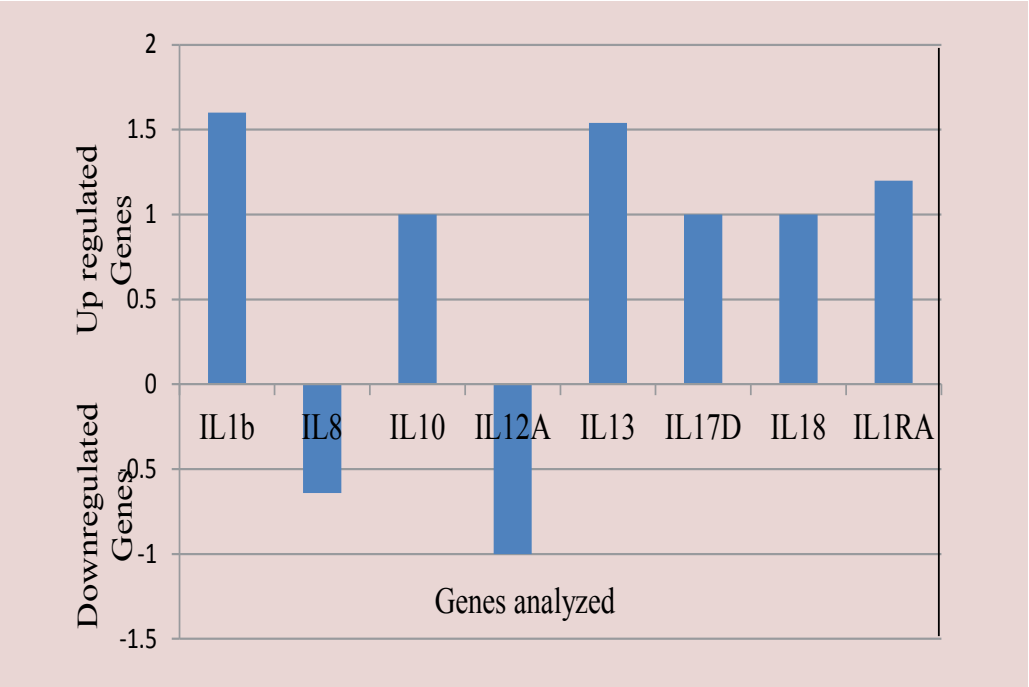

Figure 2: The figure shows the differential expression of transcripts of cytokines in osteoarthritis. The expression of IL1b (β) was significantly high in OA ($p<0.0.5$) patients as compared to control. IL8 and IL12A showed down regulated expression as compared o control. The value>0 indicate up-regulation, value<0 indicate down-regulation.

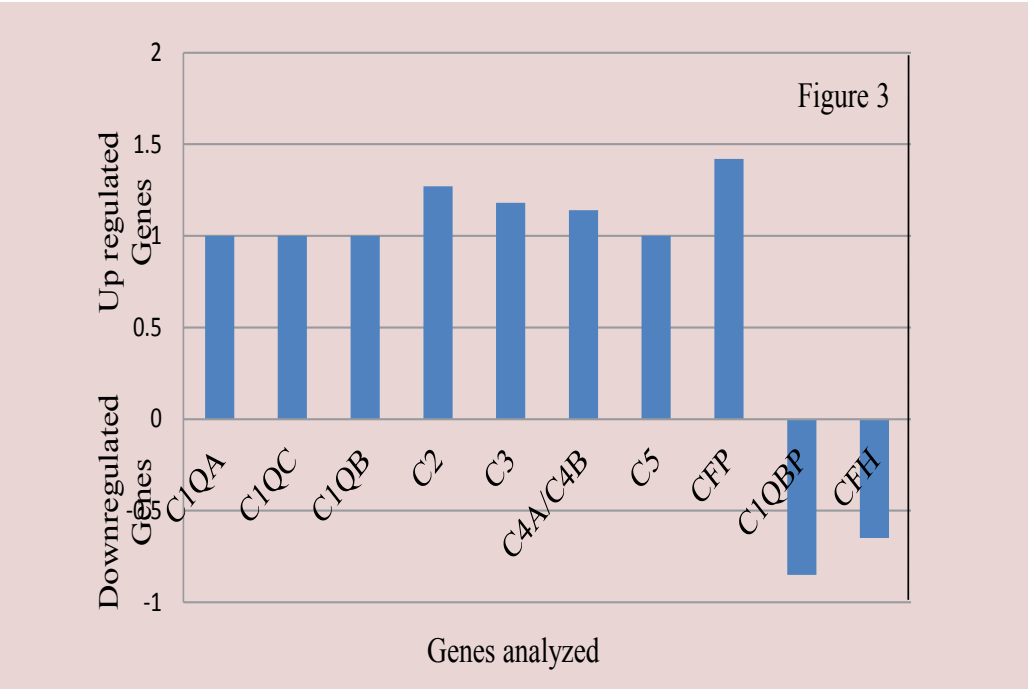

Figure 3. The figure shows the differential expression of transcripts of complement components in osteoarthritis. The value >0 indicate up-regulation, value <0 indicate down-regulation.

18 by IL-1β and upregulation of ADAMTS-5 by IL-18 [35]. Thus IL-1βmay mediate its catabolic effects through other factors in the system. ADAMTS are responsible for proteolysis of aggrecan molecule, one of their member ADAMTS-4 production is stimulated by both IL-1β and TNF-α. However in our study ADAMTS-4 expression did not showed any variation and ADAMTS-5 and ADAMTSL-1 were upregulated and ADAMTS-1 expression was downregulated.

Collagen, the major articular joint protein

is another target whose synthesis is suppressed by IL-1β. In our study also the gene expression involved in anabolic metabolism of bone as BMP6, TGFβ1, β2 and β3 are downregulated and other members do not show any variation as compared to control. Yudoh *et al.* [36] have reported significant reduction in the production of type-II collagen by rabbit chondrocytes incubated in the presence of 10 ng/mL of IL-1β. Similar inhibition of type II collagen mRNA was observed in human chondrocyte cell lines [37].

IL-1β has also an important role in apoptotic cell death. In our study key apoptotic family members along with their accessory proteins showed higher expression as compared to control (Figure 1). Lopez-Armada *et al.* reported depolarization of mitochondria and upregulation of proapoptotic Bcl-2 family proteins in human articular chondrocytes treated with IL-1β [38]. Heraud *et al.* reported 18–21% of human OA cartilage chondrocytes exhibit apoptotic features and IL-1β is capable of increasing the percentage of apoptotic cells in both normal and OA cartilage in a dose dependent manner [39]. Thus IL-1β has multiple effects on the cartilage whereby it inhibits its resoration possibility, increases its deterioration by other factors and has direct adverse effects on chondrocytes. Studies have reported that IL-1β stimulation of articular cells such as chondrocytes leads to expression of tumor necrosis factor-a (TNF-α), IL-8, complement factors, and prostaglandin E2, each having the capacity to induce hematopoietic cell infiltration and propagate local inflammation and tissue damage.

In our study TNFRSF1A, TNFRSF10B and TNFSF13B are upregulated (Table 1). TNF may bind to membrane receptor located on every nucleated cell and mediate its effects. Another cytokine IL-17 is high in the serum and the synovial fluid of patients with OA and is positively correlated with the radiographic image of lesions in OA [40]. IL17 is upregulated in our study also. It inhibits the synthesis of proteoglycans by chondrocytes and promotes the production of enzymes of the MMP group [41].

Like IL1β, IL18 stimulates the expression of MMP1,-3, -13 [35] and is upregulated in our study. It increase the concentration of cartilage degrading enzymes, and inhibition of production of proteoglycans, aggrecan and type II collagen and these chondrocytes exhibit morphological changes of apoptotic cells [42].

In our study high expression were observed for complement components C1QA, QB, QC, C2, C3, C4A/B, C5, CFP and factor D in OA patients. Study by Wang *et al.* [43] showed

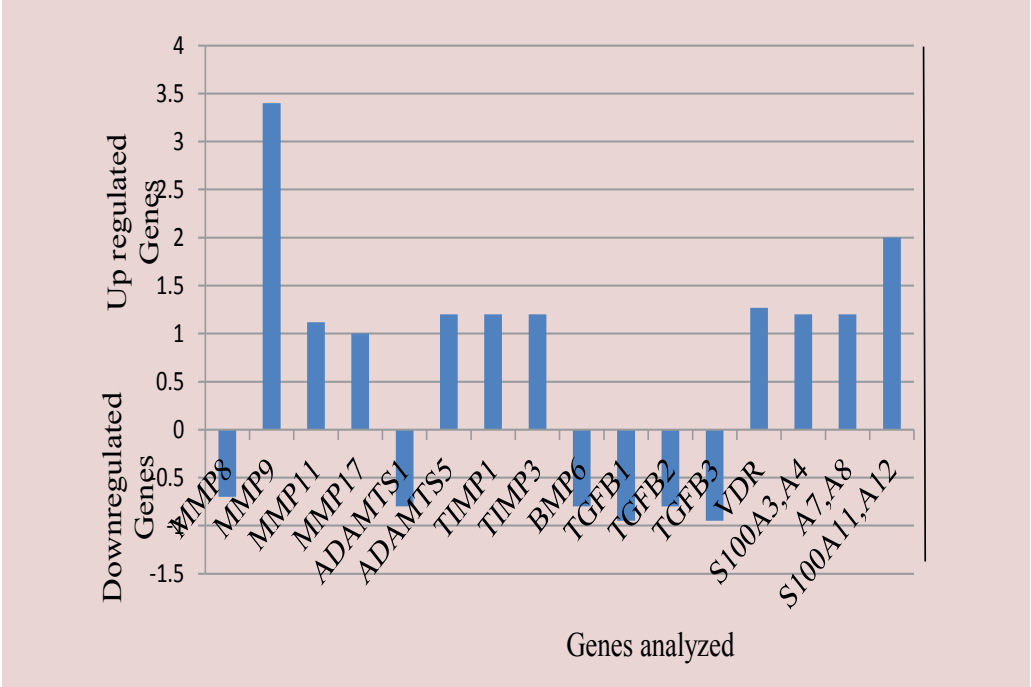

Figure 4: The figure shows the differential expression of transcripts of matrix metalloproteases (MMP), tissue inhibitors of metallo proteases (TIMP), TGF, S100 proteins in osteoarthritis. The expression of MMP9 was significantly high in OA ($p<0.0.1$) patients as compared to control. TGFβ, BMP6 showed downregulated expression as compared o control. The value >0 indicate up-regulation, value <0 indicate down-regulation.

higher transcript for complement effectors C7, C4A, factor B, C9 and C5 and markedly lower expression of the transcripts encoding the complement inhibitors clusters as factor H, C4-BP and C1 inhibitor in OA as compared to healthy synovial membrane. They reported that effector complement component membrane attack complex (MAC) was able to induce expression of proinflammatory and degradative enzymes in OA [43]. Cultured chondrocytes coated with sublytic levels of MAC, increased the expression of multiple genes as those encoding cartilage degrading enzymes [44] (MMPs and ADAMTS) and inflammatory cytokines [45] which are implicated in OA. MAC deficient mice were protected against (OA). Thus complement cascade is crucial to the pathogenesis of OA. Where dysregulation of gene expression in joint tissues may contribute to a local preponderance of complement effectors over inhibitors in OA. Complement activation in turn results in the formation of MAC on chondrocytes, which kills the cells or triggers release of MMPs, inflammatory mediators etc.

The expression of S100A3, S100A4, S100A7, S100A8, S100A11, S100A12 is upregulated in our patients. Articular ECM has been reported to be a target of catabolic activities of cytokines especially IL1β. IL1β upregulated the major extracellular proteolytic enzymes in cartilage degradation, as matrix metalloproteinases (MMPs) and ADAMTS. The protein levels of MMP-1, MMP2 and MMP9 were higher in patients with OA than those in the control group [46]. In our study we could not observe differences in gene expression of MMP1 and MMP2 in OA versus control group but observed significantly high expression of MMP9 and high MMP11 and MMP17 in OA patients than control. High MMP9 protein along with others is implicated in pathogenesis of OA. MMP9 along with MMP1 and MMP2 is very efficient in the turnover of the extracellular matrix due to their protease activities against their target proteins and contribute to the process of tumor invasion and metastasis in some diseases through tissue remodeling [47]. Osteoclasts constitutively express MMP2 and synthesize MMP9, MMP3 and TIMP1 in response to IL1 stimulation, and during OA the increased levels of osteoclast-derived MMPs might contribute to osteoclast lacunar resorption [48]. Masuhara *et al.* [49] have demonstrated higher plasma levels of MMP-9 in patients with rapidly destructive hip OA in comparison with patients with OA or normal controls. There is a possibility of enhanced production of MMP9 by synovial cells of patients with destructive OA [49]. A direct route into the bloodstream via the subchondral microcirculatory system and an indirect route from synovial fluid into circulation may account for high MMP9 in OA [50]. Regulatory mechanism of gelatinase expression has been proposed. The increase of gelatinases in OA may be due to abnormal mechanical pressure applied to the articulation. The cyclic compression on osteoblasts from OA subchondral bone increases the expression of genes coding for MMP9. TGFβ1 protects articular cartilage by downregulating the expression of MMP9 of chondrocytes and synoviocytes in OA, which may delay the biological behavior of this disease. Negative correlation was observed between MMP9 expression and TGFβ1 protein [51]. In our study TGF1, β2 and β3 are downregulated along with BMP6. For other members of these families no difference in the gene expression was observed. We also observed high expression of anti inflammatory cytokines as IL-10 and IL13. These anti-inflammatory cytokines modulate an inflammatory response and act protectively on joint tissue. Chondrocytes express both the cytokine IL10 and the receptor IL10R [52]. It has been reported that IL10 is involved in stimulating the synthesis of type II collagen and aggrecan. Following the administration of IL10 in *in vitro* conditions, both healthy articular cartilage and one in the course of OA demonstrated an increase in proteoglycan synthesis. IL10 has also been shown to inhibit MMP levels [53] and apoptosis of chondrocytes. The anti-inflammatory and chondroprotective effects of IL13 on the cells of the immune system, articular cartilage and synovium in OA have been well documented [54]. IL13 affects the levels of IL1β, TNFα, MMP3 and increases the levels of IL1Ra [25].

In our preliminary study on analysis of gene expression conducted on OA patients with differing grades, MMP9, IL13, TNFα, CCR5 were highly upregulated in grade II OA (data not shown). Expression of MMP9 remained high throughout (data not shown). The expression of TGF-β was inconsistent and TIMP-1 was expressed in all grades of OA (data not shown).

In conclusion inflammation along with apoptotic death mediators deteriorates articular cartilage in the joint. However, simultaneous anabolic changes are not sufficient to control the process of degradation. Therefore management

with appropriate inflammatory controls may reduce the morbidity of OA with prolonging time for HTO or TKR.

Compliance with ethical standards

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional ethical committee and written informed consent was obtained from all the participants.

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Conflict of interest

Authors declare that there are no conflicts of interest. Author 1 declares that he has no conflict of interest. Author 2 declares that he has no conflict of interest. Author 3 declares that she has no conflict of interest. Author 4 declares that he has no conflict of interest. Author 5 declares that he has no conflict of interest. Author 6 declares that he has no conflict of interest. Author 7 declares that she has no conflict of interest.

Author contribution

Authors 1, 2, 3, 5, 7 planned and did all the experimental work. Authors 4, 5, 6, 7 contributed to work planning and finalization of the manuscript.

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