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Microarray Analysis Reveals Altered Genes Involved in Apoptosis, Inflammation and Bone Metabolism in Knee Osteoarthritis

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Abstract: *Background*: Osteoarthritis (OA) is a common rheumatic disorder that affects multiple joint tissues and has complex genetic and environmental etiologies. This study explores the genetic basis of OA in the Kanpur District, India. *Methods*: It is examined genes related to metabolism, apoptosis, cytokine signaling, and bone metabolism. Microarray-based gene expression analysis revealed significant differences between OA patients and healthy controls. *Results*: The results indicated the potential involvement of apoptosis and inflammatory pathways, as evidenced by the upregulated expression of caspases, TNF receptors, and ligands. The results also suggested a proinflammatory environment that contributes to cartilage degradation, as shown by the elevated expression of cytokines such as IL-1β, IL-17D, and IL-18. Moreover, the results demonstrated extracellular matrix remodelling and immune activation in OA pathogenesis, as indicated by the increased expression of matrix metalloproteinase (MMP) and complement component genes. Interestingly, bone metabolism-related genes displayed varied expression patterns, with decreased expression of TGFβ isoforms and increased expression of S100 proteins. *Conclusion*: These findings underscore the dysregulation of apoptosis, inflammation, and extracellular matrix homeostasis in OA, offering insights into potential therapeutic targets for disease management. However, the study limitations, such as small sample size and regional specificity, warrant further investigation to confirm and extend these findings. Future research should utilize larger cohorts and diverse methodologies to enhance the understanding of OA's molecular mechanisms and facilitate the development of targeted interventions.

Keywords: Osteoarthritis (OA), Gene Expression, Microarray Analysis, Inflammation, Bone Metabolism, Chemokines

1. Introduction

Osteoarthritis (OA) is a prevalent rheumatologic disorder that affects the joints of the hands, feet, knees, and hips. It is a complex disease that involves all joint tissues, such as cartilage, meniscus, infrapatellar fat pad, subchondral bone, and synovial membrane [1, 2]. The global prevalence of knee OA ranges from 7.5% in China to 25% in North Pakistan, and it is the second most common rheumatologic problem in India, affecting 22-39% of the population [2, 3]. Knee OA can be classified as primary or secondary, depending on whether it is a chronic degenerative disorder, or a late-onset condition induced by other factors or diseases. The pathogenesis of OA remains elusive, but it is likely influenced by both genetic and environmental factors [4]. Genetic risk variants account for more than 20% of OA heritability, and most of them are located in noncoding regions of the genome, where they may regulate the expression of target genes [5]. Some of the genes associated with OA are involved in metabolism, apoptosis,

tumour necrosis factor signalling, BCL family members, cytokines, enzymes (PL CG2, A2G4C and OD1), transcription factors, complement components, growth factors, interferon receptor, toll-like receptors, chemokine ligand, chemokine receptors, matrix metalloproteinases, cytokine suppressor, tissue inhibitor of metalloproteinases, and bone metabolism [6–9]. This study investigated the association of these genes with OA in the Kanpur District of Uttar Pradesh, India. The aim of this study was to explore the genetic factors that contribute to OA and provide insights for the pharmaceutical industry to develop new treatments. Previous studies have reported conflicting results on the correlation between these genes and OA. Therefore, this study attempted to address this discrepancy and answer the question of whether these genes are positively related to OA or not, based on the experimental data collected. Various statistical tools and methods were used to analyse the data and identify the genetic factors associated with OA.

2. Materials and Methods

2.1. Inclusion and Exclusion of Controls and Subjects

The control group consisted of individuals who had no radiological signs of joint pain, crepitus, or joint space narrowing on X-ray, no other comorbidities, normal body mass index, and matched sex, age, weight, and height. Normal healthy individuals and patients were chosen from the medical college, departmental staff, aged between 30 and 60 (50.21 ± 2.10) years with a male (89) to female (131) ratio of ≈ 1:1.47. The patients are recruited from both the outdoor department of the Community Health Centre, Ganesh Shankar Vidyarthi Memorial (GSVM) medical college, Kanpur, and the district hospital of surrounding regions. The patients with knee osteoarthritis were carefully selected based on radiological grading, knee pain (asymmetrical) lasting more than six months, stiffness (less than 30 minutes), swelling, cripitus, tenderness, X-ray (Grade II-78 and III-22), WOMAC score (Difficulty 31.0±8.1; Pain 9.2±2.2; Stiffness 4.2±1.3 and Total 44.2±9.8), VAS score (4.7±1.4), duration of symptoms (3.3±1.49 years), range of movements (0-140/42± 20.2), and VAS pain on movement (4.7±1.4 cm). Only normal weight (BMI 23.72 ± 0.78 for control and BMI 22.91 ± 0.62 for the subject) and similarly normal blood pressure (80-120 mmHg) were considered for this study. Clinical data and treatment history were collected using a health assessment questionnaire (HAQ). Patients who were suffering from smoking, obesity, diabetes mellitus, hypertension, hypersensitivity, cardiovascular disease, thyroid dysfunction, neurological disorders, cancer, or any other form of arthritis were excluded. Finally, this study was conducted with all ethical standards by receiving approval from the Institutional Ethics Committee (CSJMU/BSBT/BT/EC-20), and all individuals, including both controls and OA subjects, provided written informed consent.

2.2. Microarray Analysis

A Qiagen extraction kit was used for the isolation of total RNA from whole blood, and the RNA amount was determined by spectrophotometry. ds cDNA from RNA was generated through random hexamers containing the promoter of the T7 sequence, and DNA fragments were labelled, hybridized overnight by Gene ST 1.0 arrays, washed, stained and scanned. For microarray analysis and QC checks, Affymetrix Human Gene 1.0 ST arrays were used, and probe sets with a fold change of ±1 were selected as up- and downregulated. Affymetrix Expression Console (EC) was used for QC analysis, R programming language for statistical analysis and GenowizTM software for biological analysis. Prior to performing the differential expression analysis, the expression data from each array were tested for quality. The assessment procedures focus on various metrics to identify outlier arrays from the array set, and using Affymetrix Expression Console^{TM} (EC), which supports different metrics along with visualization, the quality of arrays was determined. The probe level intensity distribution was observed through box plots.

3. Results

Note: this presentation is repeated in the manuscript which means the expression status is given as fold change with +/- for upregulated and downregulated gene expression, respectively.

Table 1. *List of study gene and their result.*

Metabolic Pathway	Name of the gene	Expression status	Information about the gene	Gene accession
Apoptosis	CASP1	1.24	Caspase1, apoptosis-related cysteine peptidase (Interleukin 1, beta, convertase)	NM_033292
	CASP4	1.06	Caspase4, apoptosis-related cysteine peptidase	NM_033306
	CASP5	1.43	Caspase5, apoptosis-related cysteine peptidase	NM_004347
	CASP8	1.2	Caspase8, apoptosis-related cysteine peptidase	NM_001228
	PDCDILG2	2.27	Programmed cell death1, ligand 2	NM_025239
	CRADD	1	Adapter with death domain containing CASP2 and RIPK1 domain	NM_003805
	CARD6	1.2	Family member 6 of caspase recruitment domain	NM_032587
	CARD16	1.12	Caspase recruitment domain family member16	NM_052889
	CARD17	1.15	Caspase recruitment domain family member 17	NM_001007232
	CFLAR	1.1	CASP8 and FADD-like apoptosis regulator	NM_003879
Tumour necrosis factor	TNFRSF1A	1	TNF receptor superfamily member 1A	NM_001065
	TNFRSF9	2.29	TNF receptor superfamily member 9	NM_001561
	TNFRSF10B	1	TNF receptor superfamily member 10B	NM_003842
	TNFSF13B	1.33	TNF ligand superfamily member 13b	NM_006573

Metabolic Pathway	Name of the gene	Expression status	Information about the gene	Gene accession
	TRAF5	-0.74	TNF receptor-associated factor5	NM_145759
	BAK1	1.21	BCL2 antagonist/killer 1	NM_001188
BCL family member	BCL2L1	2.31	BCL2 like1	NM_138578
	BCL2L11	1.1	BCL2 like11	NM_138621
	BCL2L13	1.02	BCL2 like13	NM_015367
BCL antagonist	BAK1	1.21	BCL2 antagonist/killer 1	NM_001188
	IL1β	1.6	Interleukin 1β	NM_000576
	IL8	-0.64	Interleukin 8	NM_000584
	IL10	1	Interleukin 10	NM_000572
	IL12A	-1	Interleukin 12A (natural killer cell stimulatory factor1)	NM_000882
Cytokines	IL13	1.54	Interleukin 13	NM_002188
	IL17D	1	Interleukin 17D	NM_138284
	IL18	1	Interleukin 18 (interferon-gamma inducing factor)	NM_001562
	IL1RA	1.2	Interleukin 1 receptor type1	NM_000877
	IL2RA	-0.93	Interleukin 2 receptor alpha	NM_000417
	PLCG2	1	Phospholipase C, gamma 2 (phosphatidylinositol-specific)	NM_002661
Enzymes	PLA2G4C	2.1	Phospholipase A2, group IV C (cytosolic, calcium-dependent)	NM_003706
	PLOD1	1.5	procollagen-lysine1,2-oxoglutarate5dioxygenase1	NM_000302
Transcription factors	JAK1	-0.9	Januskinase 1 (aproteintyrosinekinase)	NM_002227
	STAT1	1.4	Signal transducer and activator of transcription1, 91 kDa	NM_007315
	C1QA	1	Complement component (CC) 1, q subcomponent, A chain	NM_015991
	C1QC	1	CC 1, q subcomponent, C Chain	NM_001114101
	C1QB	1	CC 1, q subcomponent, B chain	NM_000491
	C2	1.27	CC 2	NM_000063
Complement components	C3	1.18	CC 3	NM_00064
	C4A/C4B	1.14	CC 4A/4B	NM_007293
	C5	1	CC 5	NM_001735
	CFP	1.42	Complement factor properdin	NM_002621
	C1QBP	-0.85	CC 1, q subcomponent binding protein	NM_001212
	CFH	-0.65	Complement factor H	NM_000186
	CSF1R	1.4	Colony stimulating factor 1 receptor	NM_005211
Growth factors	CSF2RA	1	Colony-stimulating factor2 receptor alpha, low-affinity (granulocyte-macrophage)	NM_006140
	CSF2RB	1	Colony-stimulating factor2 receptor beta, low-affinity (granulocyte-macrophage)	NM_000395
	CSF3R	1	Colony-stimulating factor3 receptor (granulocyte)	NM_156039
	TLR1	1.2	Toll-like receptor-1	NM_003263
	TLR4	1.2	Toll-like receptor-4	NM_024168
	TLR5	1.24	Toll-like receptor-5	NM_003268
Toll-like receptors	TLR6	1	Toll-like receptor-6	NM_006068
	TLR7	-0.91	Toll-like receptor-7	NM_016562
	TLR8	1.2	Toll-like receptor-8	NM_138636
	TLR9	-0.9	Toll-like receptor-9	NM_017442
	TLR10	-0.6	Toll-like receptor-10	NM_030950
Chemokine receptors	CCR4	-0.8	Chemokine (C-Cmotif) receptor 4	NM_005508
	CCR6	-1	Chemokine (C-Cmotif) receptor 6	NM_031409
	CCR7	-0.95	Chemokine (C-Cmotif) receptor 7	NM_001838
	FCGR1A	1.46	Fc fragment of IgG, high-affinity Ia, receptor (CD64)	NM_000566
	FCGR2A	1	Fc fragment of IgG, low-affinity IIa, receptor (CD32)	NM_001136219
Immunoglobulin and interferon receptor	FCGR3A	1.2	Fc fragment of IgG, low-affinity IIIa, receptor (CD16a)	NM_000569
	FCGRT	1.2	Fc fragment of IgG, receptor, transporter, alpha	NM_004107
	IFNAR1	1.3	Interferon (alpha, beta and omega) receptor1	NM_000629
	IFNGR2	1.1	Interferon-gamma receptor2 (interferon gamma transducer1)	NM_005534
	IFNGR1	1.1	Interferon-gamma receptor1	NM_000416
	CXCL6	-0.7	Chemokine (C-X-Cmotif) ligand6	NM_006564
	CXCL10	2.26	Chemokine (C-X-Cmotif) ligand10	NM_001565
Chemokine ligand	CXCL11	1.13	Chemokine (C-X-Cmotif) ligand11	NM_005409
	CXCL14	1	Chemokine (C-X-Cmotif) ligand14	NM_004887
	CXCL16	1.5	Chemokine (C-X-Cmotif) ligand16	NM_022059
	CXCL17	1.12	Chemokine (C-X-Cmotif) ligand17	NM_198477
	MMP8	-0.7	Matrix metalloproteinases 8 (neutrophil collagenase)	NM_002424
Matrix	MMP9	+3.4*	Matrix metalloproteinases 9 (gelatinase B, 92 kDa gelatinase 92 kDa, typeIV collagenase)	NM_004994
Metallo-proteinases	MMP11	1.12	Matrix metalloproteinases 11 (Stromelysin 3)	NM_005940
	MMP17	1	Matrix metalloproteinases 17 (membrane inserted)	NM_016155
	ADAMTS1	-0.8	ADAM metalloproteinases with thrombospondin type1, motif 1	NM_006988
	ADAMTS5	1.2	ADAM metalloproteinases with thrombospondin type1, motif 5	NM_007038

Metabolic Pathway	Name of the gene	Expression status	Information about the gene	Gene accession
Cytokine suppressor	SOCS5	-0.8	Suppressor of cytokine signalling 5	NM-014011
	SOCS4	1.01	Suppressor of cytokine signalling 4	NM_199421
Genes involved in bone metabolism	BMP6	-0.8	Bone morphogenetic protein 3	NM_001718
	TGFB1	-0.95	Transforming growth-factor beta-1	NM_000660
	TGFB2	-0.8	Transforming growth-factor beta-2	NM_001135599
	TGFB3	-0.95	Transforming growth-factor beta-3	NM_003239
	COL1A1	-	Collagen type I alpha 1	NM_000088
	ESR1	-	Estrogen receptor-1	X_74439
	IGF1	-	Insulin-like growth factor 1 (somatomedin C)	NM_0011111283
	SFRP1	1	Secreted Frizzled related protein	NM_003012
	S100A3, A4, A7, A8	1.2	S100 Calcium binding proteinA3, A4, A7, A8	NM_002960, 019554, 002963, 002964
	S100A11, A12	2	S100 Calcium binding proteinA11, A12	NM_005620, NM_005621
Metalloproteinases tissue inhibitor	TIMP1	1.2	TIMP metalloproteinases inhibitor 1	NM_003254
	TIMP3	1.2	TIMP metalloproteinases inhibitor 3	NM_000362

3.1. Apoptosis and Anti-Apoptosis Gene Expression

We mostly found upregulated caspases (such as CASP 1, 4, 5, and 8). OA also upregulated other genes belonging to the caspase recruitment domain (such as CRAD D, 6, 16, and 17). Upregulation of the TNF- receptor family members TNFRSF 1A and 10B and the TNF ligand superfamily members 13B and 9 was also observed. The anti-apoptotic gene TRAF, which interacts with TRADD and inhibits apoptosis, was downregulated. BCL2L1, a proapoptotic regulator, had very high expression in OA compared with the control. Upregulation of BAK-1, a BCL family member and BCL antagonist/killer, was also recorded. Programmed cell death 1, ligand 2 was highly upregulated in OA patients (see Figure 1).

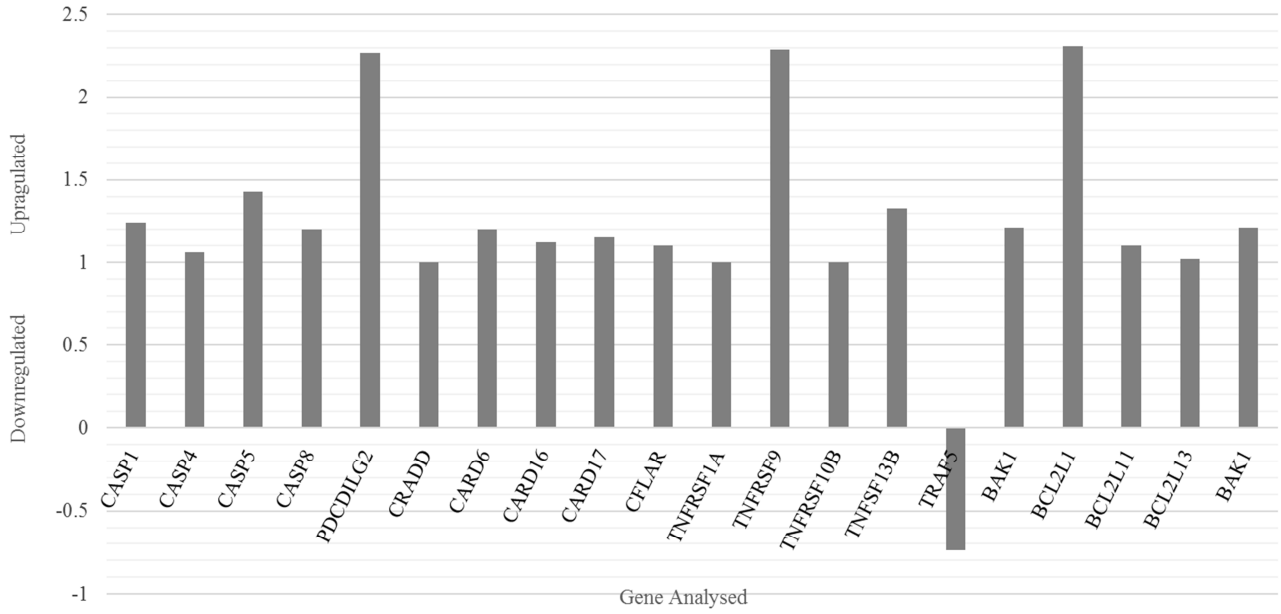

Figure 1. *Up and Down regulation analysis of apoptosis and anti-apoptosis gene.*

3.2. Immune Cells, Complement Components and Receptor Gene Expression

We found upregulated gene expression for immune system cytokines, signalling components, complement components and their receptors. We observed upregulated expression of IL-1β IL-17D, IL-18, and IL1RA. PLOD1, which hydroxylates lysyl residues in collagen-like peptides [10], was significantly upregulated. The complement components C1QA, C1QB, C1QC, C2, C3, C4A/C4B, C5, and CFB were upregulated. Certain immune receptor genes, such as CSF1R CSF2RA, CSF2RB, CSF3R, FCG-R1A, -R2A, -R3A, FCGRT, IFNγR1 and IFNγR2, were upregulated (). All other members of the suppressor of cytokine signalling (SOCS) genes were either downregulated or unaffected, but SOCS4 was upregulated in OA patients.

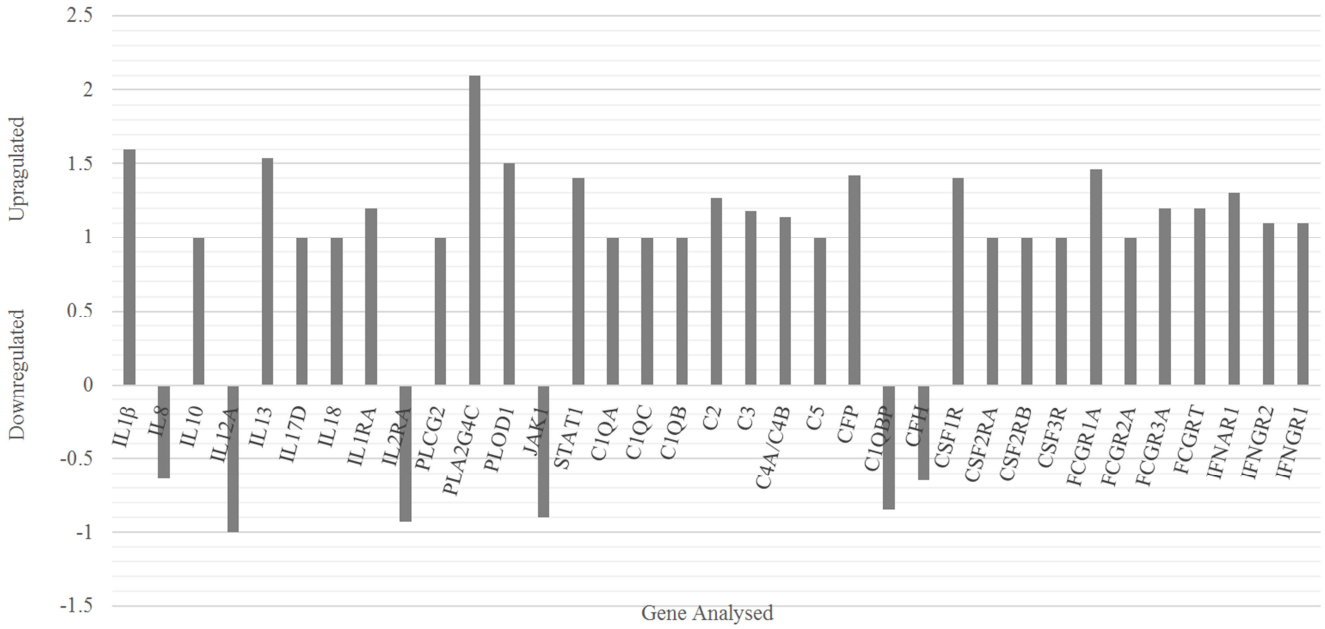

Figure 2. *Up and Down regulation analysis of immune cells, complement components and receptor gene.*

3.3. TLR, Chemokine Ligand and Receptor Gene Expression

Toll-like receptors (TLRs) (TLR1-TLR10 in humans) are a group of pattern recognition receptors. TLR-1, -4, -5, -6, and -8 were upregulated, whereas TLR-7, -9, and -10 were downregulated. Members of the chemokine C-X-C motif family, such as CXCL10, 11, 14, 16 and 17, were upregulated. However, chemokine CXCL6 and chemokine receptors such as CCR4, CCR6, and CCR7 were downregulated.

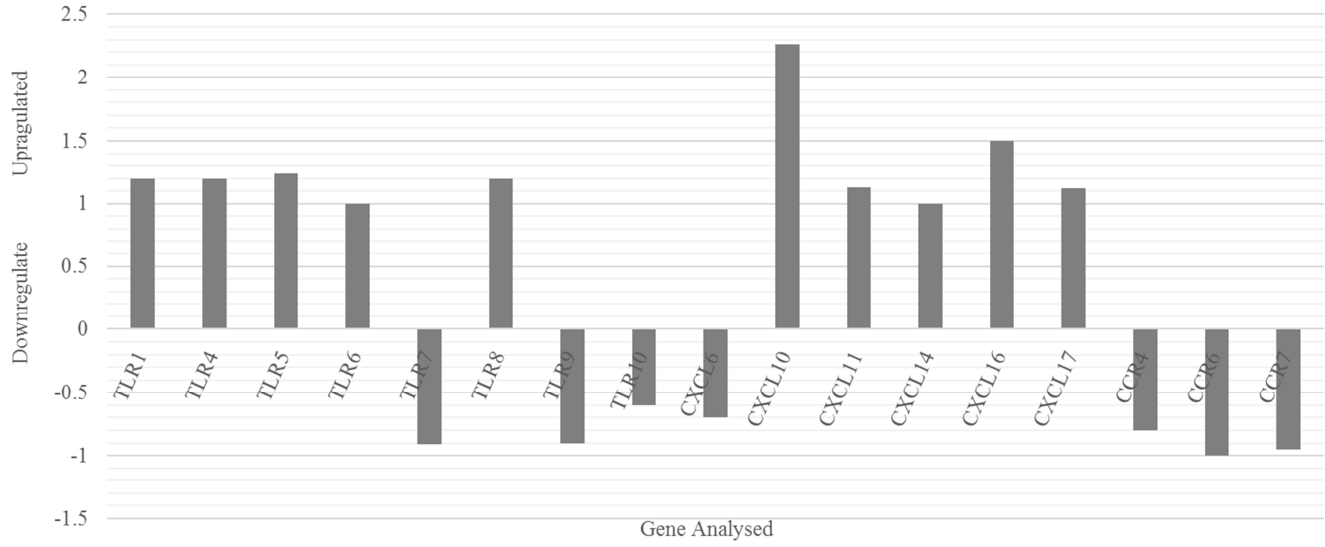

Figure 3. *Up and Down regulation analysis of TLR, chemokine ligand and receptor gene.*

3.4. Gene Expression of MMPs and TIMPs

Genes involved in matrix turnover, such as MMPs, were prominently upregulated. Gelatinase MMP-9 showed high upregulation. Upregulation of stromelysin MMP (11 and 17) and downregulation of collagenase MMP8 were observed (see Figure 4). Upregulation of ADAMTS-5, an Aggrecanase, and downregulation of ADAMTS1 were observed (Annexture 1). TIMP expression was upregulated, especially for TIMP (1 and 3).

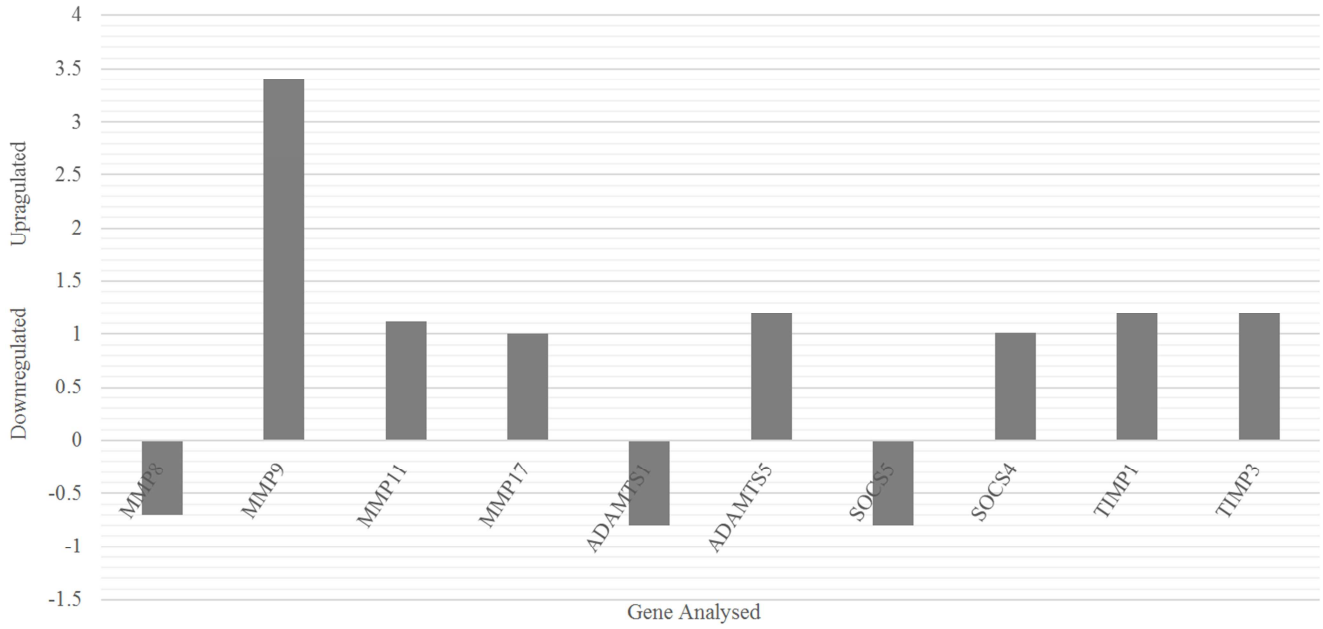

Figure 4. *Up and Down regulation analysis of gene expression of MMPs and TIMPs.*

3.5. Bone Metabolism-Related Gene Expression

Unaltered expression of OA-susceptible genes, such as ESR1, COL1A1, and IGF, was observed for many candidates responsible for bone-related functions (see Figure 5). The gene expression of all BMPs was unaltered, and only BMP6 expression was lower in the patient than in the control. TGFβ-1, which promotes osteoblastic bone formation, and TGFβ (2 and 3), which restrains T-cell growth in an IL-2-dependent manner [11], were downregulated (Annexture 1). S100A proteins with calcium-binding motifs and expressed in inflammation by macrophages were overexpressed. The S100 protein family, such as S100P and S100A (3-4, 7-8, and 11-12) showed higher gene expression.

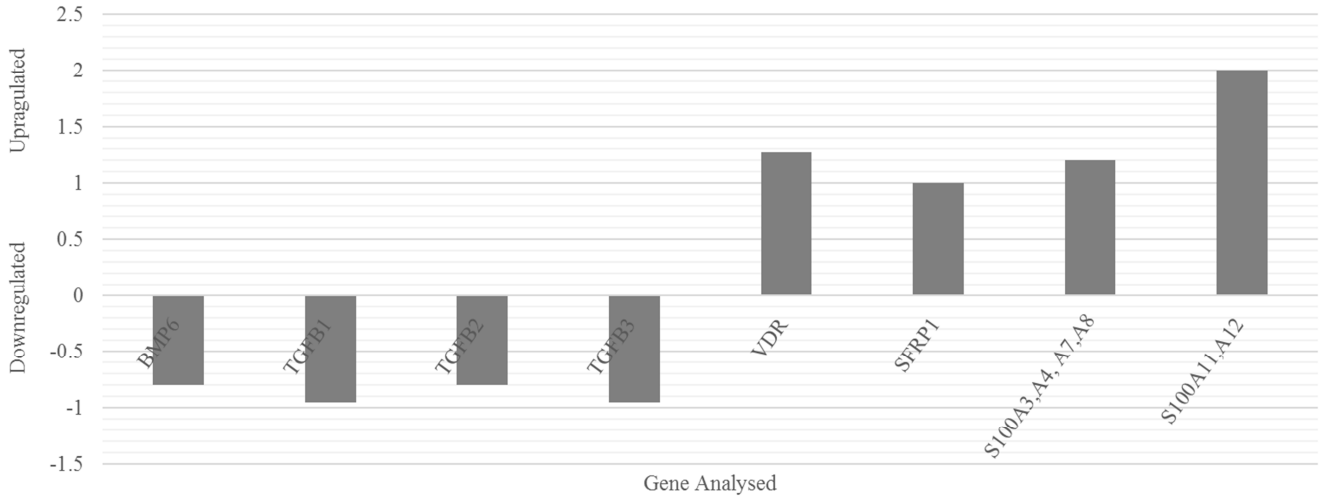

Figure 5. *Up and Down regulation analysis of bone metabolism-related gene.*

4. Discussion

Osteoarthritis (OA) is a common musculoskeletal disease that is becoming more prevalent due to factors such as obesity and a sedentary lifestyle. Our study investigated the gene expression changes associated with OA and revealed significant alterations in genes related to inflammatory cytokines, apoptotic proteins, interleukins, and matrix metalloproteinases (MMPs). Cell death mediated by caspases was observed in our study, with an upregulation of caspase expression. This suggests that caspases may play a key role in the pathogenesis of OA, as inhibiting caspases could prevent cell death and maintain chondrocyte function. In OA, various cytokines produced by activated synoviocytes, articular cartilage, or mononuclear cells can enhance the gene expression of MMPs [9]. Our study showed high expression of IL-1β and IL-18, which are involved in promoting joint inflammation and cartilage degradation. Our study also observed high expression of proinflammatory cytokines such

as IL-1β, IL-12A, IL-17D, IL-18, and TNFSF, as well as their accessory factors, and these factors may contribute to the pathogenesis of OA. Additionally, we found higher expression of IL-1R1 receptor in chondrocytes and fibroblast-like synoviocytes (FLS) of OA patients compared to the treatment group. IL-1β and IL-18 were found to increase the expression of MMPs [12], while our study did not observe changes in ADAMTS4 expression. However, ADAMTS5 and ADAMTSL1 were found to be high, and ADAMTS1 expression was low compared to the control. Key apoptotic members and their assistant proteins also showed increased expression in our study compared to the control. IL-17, which reduces the production of proteoglycans by chondrocytes and increases MMP synthesis, was found to be highly expressed in our study. Similarly, IL-18 expression was high in our study and stimulated the expression of MMP1, MMP3, and MMP13. In our study, we observed changes in the expression of members of the CXCL family and lower expression of CCR4, CCR6, and CCR7, while the expression of others was unchanged. Complement effectors C2, C4A, C4B, CFP, and C5 showed increased transcript levels in OA, while complement inhibitor clusters such as C1QBP, factor H, and C1 inhibitor showed decreased transcript levels. These results align with previously published reports on complement components in the synovial membrane of healthy and OA patients [13]. TLR-2 and TLR4 were detected in OA synovial membranes and were upregulated in damaged cartilage in patients with advanced OA. High expression of TLR-1, -4, -5, -6, and -8, as well as S100 proteins, which function as DAMPs, was also found. In contrast to other studies, our study did not observe differences in MMP2 and MMP1 gene expression between OA patients and the control group. However, MMP9 expression was very high, and MMP17 and MMP11 expression was high in OA patients compared to the controls. Osteoclast-derived MMPs might contribute to osteoclast lacunar resorption in OA. We observed reduced expression of TGFβ1, TGFβ2, TGFβ3, IGF1, and BMP6, with unaltered expression of IGF1 in OA subjects compared to the control. Additionally, high expression of anti-inflammatory cytokines such as IL-13 and IL-10 was detected. Preliminary gene expression analysis of OA patients with different grades revealed high expression of MMP9, TNFβ, and IL8 in grade II OA [14]. MMP9 expression remained high in all grades, while TGF-β expression varied, and TIMP-1 expression peaked in grade II OA, indicating the role of inflammation in OA progression.

5. Conclusion

The study revealed higher proportions of genes associated with apoptosis, immunoreceptors, inflammation (including chemokines, cytokines, complement components, and matrix metalloproteases), and bone metabolism. The upregulation of apoptotic, immune receptor, and inflammatory genes suggests a dysregulated cellular response and immune activation in OA. Additionally, the increased expression of matrix metalloproteases and bone metabolism-related genes highlights the significance of extracellular matrix remodelling and bone changes in OA progression. Targeting the identified genes, especially those involved in apoptosis, immunoreceptors, inflammation, and bone metabolism, holds promise for controlling OA progression. However, the study's limitations, such as a relatively small sample size potentially not representative of the general population, should be acknowledged. Future research should address these limitations by employing larger sample sizes and using multiple platforms and methods for gene expression analysis.

Abbreviation

OA	-	Osteoarthritis
DNA	-	Deoxyribonucleic Acid
F	-	Female
M	-	Male
IL	-	Interleukin
TNF	-	Tumor Necrosis Factor
MMP	-	Matrix Metalloproteinase
TIMP	-	Tissue Inhibitor of Metalloproteinase
BMI	-	Body Mass Index
HAQ	-	Health Assessment Questionnaire
X-ray	-	X-radiation
QC	-	Quality Control
EC	-	Expression Console
SOCS	-	Suppressor of Cytokine Signalling
TLR	-	Toll-like Receptor
CXCL	-	Chemokine (C-X-C motif) Ligand
CCR	-	Chemokine (C-C motif) Receptor
BMP	-	Bone Morphogenetic Protein
TGF	-	Transforming Growth Factor
IGF	-	Insulin-like Growth Factor
ADAMTS	-	ADAM Metallopeptidase with Thrombospondin Motif
DAMP	-	Damage-Associated Molecular Pattern

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Declaration(s)

Competing Interests

The authors report no conflicts of interest.

Ethical Approval and Consent to Participate

Ethical standards were followed throughout the study, which obtained approval from the Institutional Ethical Committee (CSJMU/BSBT/BT/EC-20). Moreover, written consent was provided by patients and OA controls.

Guarantor

The article’s full responsibility lies with PY, who is the corresponding author and the third author in the list.

Authors' Contributions

Dr. VC was responsible for manuscript conceptualization, writing - original draft, ethical approvals, consent, and sample collection. VR participated in writing - review & editing. PY contributed to manuscript writing, formatting, revision and communication with all authors. Dr. TA supervised all authors and wrote, reviewed and edited the final manuscript.

Availability of Data and Materials

Data and materials are available upon request.

Consent for Publication

All authors consented to manuscript publication.

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