International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 12 Dec. 2022, pp: 549-556 www.ijaem.net ISSN: 2395-5252

Understanding the whole range of pathogenic mechanisms underlying OSTEOARTHRITIS

Submitted: 10-12-2022 Accepted: 23-12-2022

ABSTRACT: The most prevalent degenerative joint disease and a key contributor to pain and disability in adults is osteoarthritis (OA).Injuries to the joints, obesity, ageing, and inheritance are some of the causes of OA. The precise molecular pathways behind the onset and course of OA are still poorly understood, and there are no treatments available to repair damaged cartilage or slow the disease's progression at this time.The diathrodial joint is a complex organ that bears weight, engages in physical activity, and moves with a range of motion unique to the joint. All parts of the joint organ, including the articular cartilage, subchondral bone, synovial tissue, and meniscus, are impacted by the development of OA.The identification of the interplaying mechanisms between the various OA symptoms, such as articular cartilage degradation, osteophyte formation, subchondral sclerosis, and synovial hyperplasia, as well as the signalling pathway(s) governing these pathological processes, is necessary for a complete understanding of the pathological mechanism underlying the development of OA.

INTRODUCTION:-

The most prevalent degenerative joint disease, osteoarthritis (OA), affects more than 25% of people over the age of 18(1) Progressive loss and destruction of articular cartilage, thickening of the subchondral bone, the development of osteophytes, varying degrees of synovial inflammation, degeneration of the ligaments and menisci of the knee, and hypertrophy capsule are some of the pathological changes seen in OA joints. Joint injury, obesity, aging, and heredity are a few of the multiple factors that contribute to the aetiology of OA(2). Because the molecular mechanisms involved in OA initiation and progression remain poorly understood, there are no current interventions to restore degraded cartilage or decelerate disease progression(3).Studies utilising genetically modified mice reveal that signalling molecules like Smad3, -catenin, and HIF-2,6-10 as well as growth factors like transforming growth factor (TGF), Wnt3a, and Indian hedgehog are involved in the development of OA(4).The overexpression of Runx2 is one trait that a number of OA animal models have in common(2). 7,8,11–13 In articular chondrocytes, Runx2 is a crucial transcription factor that controls the transcription of genes encoding matrix degradation enzymesof the joint(5).

Etiology:-

The most common joint condition that causes pain and impairment is OA(6). By 2020, it is expected that more than 50 million adults in the US, or 25% of the adult population, will have this disease, making it the leading cause of morbidity and physical impairment in people over 40(4).Chronic pain, joint instability, stiffness, and radiographic joint space narrowing are among the main clinical symptoms.Post-traumatic OA can result from sports-related traumatic injuries at any age, despite the fact that OA primarily affects the elderly(7). There are currently no effective therapeutic therapies available for OA, with the exception of pain management and end-stage surgical surgery.Therefore, there is an unmet clinical need for research into the causes of and potential remedies for OA(8). Recent research have shown that genetic, mechanical, and environmental variables are linked to the onset of OA utilising the surgically induced destabilisation of the medial meniscus (DMM) model and tissue or cells from human patients(9).OA is defined at the cellular and molecular levels by a shift away from a healthy homeostatic state toward a catabolic state(10).

Aging:-

Age is one of the most prevalent OA risk factors(6). Radiographic abnormalities in one or more joints were found in the majority of persons over 65(1).Aging also has an impact on synovium, subchondral bone, and muscle, which is hypothesised to influence how the joints are loaded(2). Aging also has an impact on cartilage. Studies on articular chondrocytes and other types of cells indicate that oxidative stress levels are higher in ageing cells, which encourages cell

International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 12 Dec. 2022, pp: 549-556 www.ijaem.net ISSN: 2395-5252

senescence and modifies mitochondrial activity(9).The course of Kashin-Back disease, an uncommon form of OA, was linked to cell death and mitochondrial malfunction(6).Reduced repair responses are another sign of ageing chondrocytes, which is partly brought on by changes in the receptor expression pattern(9). The ratio of TGF- receptor ALK1 to ALK5 was elevated in chondrocytes from aged and OA cartilage, down-regulating the TGF- pathway and switching from matrix synthesis activity to catabolic matrix metalloproteinase (MMP) expression(4).Recent research also suggests that the complete genome's DNA methylation demonstrated a distinctive pattern in aged cells(2).The epigenetic modification in OA chondrocytes was further validated by genome-wide sequencing of OA patients, in part because of variations in the expression of the methylation and demethylation enzymes Dnmts and Tets(11).

Obesity:-

Obesity has recently spread across the globe and is recognised by an increase in adipose tissue in the body's composition. Obesity and OA have long been linked to one another(11).Obese patients experience OA at an earlier stage, more severe symptoms, an increased risk of infection, and greater technical challenges during total joint replacement surgery(3). Obesity is hypothesised to contribute to low-grade systemic inflammation in addition to increasing the biomechanical strain on the knee joint by the release of adipokines, which are cytokines generated from adipose tissue.In particular, interleukin (IL)-1, IL-6, IL-8, and tumour necrosis factor alpha (TNF-) levels were increased in high-fat diet-induced animal obesity models as well as in obese individuals(8). The nuclear factor-B (NF-B) signalling pathway may be activated by these inflammatory substances, stimulating the breakdown of the extracellular matrix (ECM) by increasing the activity of MMPs(7).

Sport Injury:-

The main cause of OA in young adults is knee injury, which more than four times increases the risk of OA. Recent clinical studies revealed that 41%–51% of participants who had prior knee injuries later developed radiographic signs of knee OA(9).The most frequent clinical injuries that can cause OA are ligament strains and rips, joint dislocations, and cartilage tissue tears. Sport-related trauma can damage bone, cartilage, ligaments, and meniscus, which can all have a negative impact on joint stabilisation(10).Increased cytokine and chemokine production, synovial tissue enlargement, inflammatory cell infiltration, and activation of the NF-B pathway are all indicators of inflammation that have been seen in both people with traumatic knee OA and mice injury models(6).

Inflammation:-

It has been proven that the persistent low-grade inflammation present in OA plays a role in the onset and development of the condition(2). The entire synovial joint, including the cartilage, subchondral bone, and synovium, is affected by inflammation as OA progresses(5).It has been suggested that traditional inflammatory agents including IL-1 and TNF-a, as well as chemokines, play a role in the systemic inflammation that activates NF-B signalling in chondrocytes and synovial cells in elderly and diabetic patients(8).Innate inflammatory signals such as damage-associated molecular patterns (DAMPs), alarmins (S100A8 and S100A9), and complement were also involved in the aetiology of OA(11).According to studies, OA joints contain high levels of DAMPs and alarmins, which communicate through either the conventional NF-B pathway or toll-like receptors (TLR) to modify the expression of MMPs and a disintegrin and metalloprotease with thrombospondin motif (ADAMTS) in chondrocytes(7).DAMPs, ECM fragments, and dead-cell debris can activate complement in OA chondrocytes and synovial cells.Inflammatory mediators from systemic inflammation can re-program chondrocytes toward hypertrophic differentiation and catabolic responses via the NF-B pathway,9,10,79 the ZIP8/Zn+/MTF1 axis,80 and autophagy processes, according to recent research(12). Indeed, current KEGG pathway analyses of OA and control samples show that cytokine-induced mitogen-activated protein (MAP) kinases, NF-B activation, and oxidative phosphorylation are some of the mechanisms through which inflammatory signals contribute to OA pathogenesis(1).

Genetic Predisposition:-

From family-based investigations, it has long been understood that OA is inherited(8). Despite the complexity of OA's genetics, genetics play a major role in the disease(11). Ex vivo research using tissues from OA patients, in vivo studies using surgically generated OA animal models, and genetic mice models have all been used to highlight the significance of genes and signalling pathways in the pathogenesis of OA over the past ten years(4). By primarily triggering

catabolic responses in chondrocytes, changes in the TGF-, Wnt/-catenin, Indian Hedgehog (Ihh), Notch, and fibroblast growth factor (FGF) pathways, for instance, have been demonstrated to contribute to the formation and progression of OA(5).Such responses converge on Hif2, Runx2, and inflammatory mediators, which cause the production of MMPs and ADAMTS activity to increase, degrading the cartilage ECM(10). Over 80 gene mutations or single-nucleotide polymorphisms (SNPs) linked to OA pathogenesis have been confirmed in recent studies of genome-wide association screening (GWAS) conducted on numerous OA and control populations around the world(12).Some of the genes are crucial signalling molecules in the Wnt (Sfrp3), bone morphogenetic protein (BMP) (Gdf5), and TGF- (Smad3) signalling pathways, and some are significant structural and ECM-related factors (Col2a1, Col9a1, and Col11a1)(7). The majority of the genes in this list have already been linked to studies using mouse models of genetically or surgically induced OA(3). A recent genome-wide screen investigation by the arcOGEN Consortium107 discovered novel SNPs in a number of genes, including GNL3, ASTN2, and CHST11. Additional research is required to confirm these results(9).

Mouse model for OA Research:-

DMM model:-

DMM is a surgical OA model for mice and rats that has been around for ten years. It is frequently used in conjunction with transgenic mouse models, ageing and obesity models, to research the onset and progression of OA(8). Transsection of the medial meniscotibial ligament was used in DMM surgery (MMTL)(10).After the initial incision, the medial joint capsule was quickly cut with scissors to reveal the intercondylar region or the MMTL, which fastens the medial meniscus (MM) to the tibial plateau(5). Under a dissection microscope, the MMTL was identified, and it was severed with microsurgical scissors to release the ligament from the tibia plateau and weaken the medial meniscus(8). A continuous 8-0 tapered Vicryl suture was used to close the joint capsule and skin.Sham surgery was carried out by merely exposing the medial side of the knee joint capsule as a control for DMM experiments(9). Greater stress was placed on the posterior femur and central tibia, particularly on the medial side, as a result of the meniscus tissue being displaced medially(12). At four weeks after surgery, histology showed the cartilage surface to be fibrillated and the severity of the OA lesions(5). Eight weeks after surgery, cartilage degeneration and subchondral bone sclerosis appeared, and twelve weeks later, osteophyte formation appeared(11).

Aging Model:-

Aging is a significant risk factor for the most prevalent form of OA in humans, spontaneous OA, because OA is a degenerative disease that always affects senior populations(6). Numerous lab animals have spontaneous OA development, which closely resembles the stages of human OA progression(1). These animal models are useful resources for researching the pathophysiology of OA in the wild(3).The C57/BL6 breed of laboratory mice is the most often used inbred strain; in this strain, knee OA typically appears at around 17 months of age(7). One strain of mice that is prone to developing spontaneous OA is the STR/ort mouse. For STR/ort mice to acquire articular cartilage degeneration, it takes 12–20 weeks(7). They may weigh more than other mouse strains, which may contribute to this in part(8). Although ageing OA models offer several benefits, it often takes mice at least a year to model the disease due to the underlying genetic consistency(9). Because of their relatively quick induction, genetic mouse models and surgically induced OA models are favoured today for use as ageing models for the investigation of OA lesions(2).In addition to the mouse, the Dunkin Hartley guinea pig offers a frequently utilised ageing model to research the emergence of OA(3). Within three months, the Dunkin Hartley guinea pig can manifest an age-related, spontaneous OA phenotype(5). Age-related increases in OA lesion severity can be seen in animals as young as 18 months old(6). According to a histological examination, the spontaneous OA progression in Dunkin Hartley guinea pigs is comparable to that in people(7). As a result, the Dunkin Hartley guinea pig is a useful model for studying the pathogenesis of human OA and evaluating potential treatments(2).

Obesity Model:-

It has become clear that metabolic and inflammatory responses brought on by obesity play a role in a number of musculoskeletal diseases, particularly OA(9). Mouse obesity models are frequently used in conjunction with genetically induced injury and surgically induced injury to investigate the mechanisms of obesity-induced OA(11).A high-fat diet, where 60% of calories come from fat instead of the usual 13%, causes the obese mouse model(2). The high-fat diet has an impact on all joint tissue, but particularly synovium

International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 12 Dec. 2022, pp: 549-556 www.ijaem.net ISSN: 2395-5252

tissue(3). Different laboratories have independently reported a phenotype of synovial inflammation(4).Following DMM surgery, a higher level of systemic inflammation was seen in obese mice(5). Obesity may play a role in the development of post-traumatic OA by increasing serum levels of pro-inflammatory substances such interleukin-12p70,54 interleukin-6, TNF, and several other chemokines (PTOA)(10).

Genetic Mouse Models:-

Recent years have seen a rise in the use of genetic mouse models to study the cellular and molecular pathways underlying the development of OA. Mutant mouse strains were created bearing either mutant genes or SNPS based on the GWAS investigations of human patients(9). For instance, the collagen II gene was altered in Del1+/- mice. Del1+/- mice and Col9a1/ mice both spontaneously developed OA(12).Conventional gene deletion techniques have the problem of resulting in embryonic mortality or severe skeletal deformity since cartilage serves as a skeletal architect(2). Inducible conditional KO technology has been extensively employed to get around constitutive gene knockout (KO) limitations and avoid embryonic lethality(3). This typically combines tissue-specific Cre-loxP gene targeting with tamoxifen-induced nuclear translocation of CreER fusion protein(4).The transgenic mice Col2a1-CreERT2, Agc1-CreERT2, and Prg4-CreERT2 have developed into effective instruments for focusing on joint tissue to research the mechanism of OA development(5). Both Col2a1 and Agc1 can effectively target chondrocytes in the growth plate cartilage, articular cartilage, and temporomandibular joint based on the gene expression pattern(1). Agc1 is anticipated to more effectively target chondrocytes in adult mice because it is expressed more robustly than Col2a1 in cartilage tissue(10).Agc1 has also been discovered to target the nucleus pulposus tissue in the intervertebral disc in addition to chondrocytes(9). Only the top layer of articular chondrocytes is targeted by 123 Prg4(12). It must be underlined that the role of cartilage tissue in the development of OA is addressed by all of these genetic methods(2). It is necessary to create more CreER transgenic mice to effectively target meniscus, synovial tissue, and subchondral bone(8).The role of particular genes in OA has been analysed in chondrocyte-specific research using these transgenic mice(5). In vivo research using mutant mice suggests that OA development may be influenced in some way by pathways including I receptor ligands like TGF-1, Wnt3a, and Indian hedgehog, (ii) signalling molecules like Smads, -catenin, Runx2, and HIF-2, and (iii) peptidases like MMP13 and ADAMTS4/5.Important roles in the pathophysiology of OA are played by TGF- and its downstream components. In patients with early-onset OA, mutations of Smad3, a crucial molecule in TGF- signalling, have been discovered(1). Since many years ago, it has been understood that TGF- suppresses chondrocyte hypertrophy while encouraging the development of mesenchymal progenitor cells and the production of matrix proteins(4). During the development of OA, TGF- signaling may have diverse effects on the tissues of the joints(3). For instance, chondrocyte enlargement and OA-like damage to the articular cartilage result from the total loss of Smad3.An OA-like phenotype was also produced by the deletion of the genes encoding the type II TGF- receptor Tgfbr2 or Smad312 in articular chondrocytes(9). On the other hand, OA-like lesions were also brought on by the stimulation of TGF- signalling in subchondral bone mesenchymal progenitor cells(11). According to these findings, TGF-signaling may play diverse functions in different joint tissues, and therapeutic approaches that target it may call for a tissue-specific strategy(8).

Techniques for OA Studies:-

In Vitro Studies:-

Isolation and cultivation of articular chondrocytes in vitro Primary human articular chondrocytes will be extracted from surgically discarded cartilage tissues to study signalling systems in articular cartilage(3). Sections of cartilage are removed from the subchondral bone in full thickness(6). Using a digestion buffer, the cartilage fragments will be digested for roughly 15 hours(7). Following collection, filtering out undigested tissue and debris, and washing with Hanks' buffered salt solution, the isolated cells will be collected(4). Once again suspended in chondrocyte basal media, the cells will be plated in high density monolayer cultures(9).It is also possible to grow human articular chondrocytes in three dimensions. In order to create beads, 4 106 freshly isolated human articular chondrocytes will be re-suspended in alginate solution and added drop-by-drop to 102 mmol L CaCl2(10). The chondrocytes encapsulated in alginate beads will be cultivated in three dimensions using basal medium after washing the beads with 0.15 mol L NaCl and basal media(5).Human articular cartilage explant cultured in vitro Osteochondral tissues from joints that are anatomically and radiographically normal will be taken from patients who have undergone

International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 12 Dec. 2022, pp: 549-556 www.ijaem.net ISSN: 2395-5252

various surgeries, including meniscal repair, complete knee replacement, and oncologic surgical procedures(1). First, sterile phosphate-buffered saline will be used to wash the obtained osteochondral tissues (PBS). A 6 mm diameter biopunch will be used to remove fresh cartilage samples from the femoral condyle. Chondrocyte basal medium will be used to cultivate the cartilage explants(12).

Histology:-

Knee cartilage samples will be fixed in 10% neutral buffered formalin (NBF), decalcified in 14% EDTA for 10 days, and embedded in paraffin before being utilised for histological and histomorphometric analysis(2). To identify changes in the architectures of cartilage, bone, and synovial tissues throughout OA progression, the paraffin-embedded samples will be cut into 5 m slices and stained with Safranin O/Fast green or Alcian blue/Hematoxylin-Orange G (ABH)(6).Using a Visiopharm analysis system, quantitative histomorphometric examinations of ABH-stained sections can be carried out(12). This method allows for the creation of high definition digital photographs of histology slides(7). The femoral and tibial condyles' middles will serve as the measurement point for cartilage thickness(3). Both articular cartilage surfaces will be used to trace the cartilage region. The top and deep zones of the articular cartilage will be separated using the tidemark(1).

OARSI score system:-

Using a Visiopharm analysis system, quantitative histomorphometric examinations of ABH-stained sections can be carried out(11). This method allows for the creation of high definition digital photographs of histology slides. The femoral and tibial condyles' middles will serve as the measurement point for cartilage thickness(10). Both articular cartilage surfaces will be used to trace the cartilage region(9). The top and deep zones of the articular cartilage will be separated using the tidemark(10).

Nanoidentation:-

Because they directly represent functional changes in cartilage throughout degradation, alterations in the mechanical characteristics of OA cartilage over various length scales must be understood(2). Nanoindentation based on atomic force microscopy (AFM) is ideally suited for assessing changes at nm-to-m scales that are comparable to the sizes of matrix molecules and cells(12). A microspherical or pyramidal tip is programmed to indent the sample tissues, cells, or tissue slices to a pre-set force or depth for AFM-nanoindentation measurement(9).By fitting the loading section of each indentation force versus depth curve to the elastic Hertz model, an effective indentation modulus may be derived(4). Over the past ten years, the use of nanoindentation has shown numerous novel links between cartilage structure and mechanics and the pathomechanics of OA(7). Highlights from this list include the mechanics of individual chondrocytes, micromechanical anisotropy and heterogeneity of healthy and OA cartilage or meniscus, cartilage weakening in spontaneous and post-traumatic OA, and quality assessment of created neo-tissues(9).Notably, the mechanical properties of murine cartilage have been studied thanks to AFM-nanoindentation(11). Such endeavours were previously thwarted by the 100-m thickness of mouse cartilage(1). Nanoindentation serves as a vital link between the two key areas of OA research—biology and biomechanics—because in vivo OA studies are largely dependent on murine models(2).A number of recent studies have shown the value of nanoindentation for research using mouse models(3). For instance, the moduli of cartilage were abnormally greater in animals lacking collagen IX (Col9a1/) and lower in mice lacking lubricin (Prg4/) or chondroadherin (Chad/)(4). The development of macroscopic indications of OA in Col9a1 and Prg4 mice clearly highlights the strong association between defects in cartilage biomechanics and OA (8). recently showed that the mouse meniscus can benefit from nanoindentation(5). Nanoindentation has the potential to be used further to clinically relevant OA models, such as the DMM model110, to assess OA as a whole joint illness by biomechanical symptoms in various murine synovial tissues(1).Two other recent technology developments offer avenues for deeper research(12). First, type VI collagen and perlecan, the pericellular matrix signature molecules, were used by Wilusz et al.161 to dye cartilage cryosections(10).Nanoindentation was used to distinguish the mechanical properties of the cartilage pericellular matrix and ECM using immunofluorescence guidance, and to identify the function of type VI collagen in each matrix using Col6/ mice(3).As a result, it is now able to directly evaluate the connections between the biochemical composition and the biomechanical characteristics of cartilage, the meniscus, or other synovial tissues in their natural settings(2). Second, the AFM was transformed into a high-bandwidth nanorheometer by Nia et al. This method made it possible to

International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 12 Dec. 2022, pp: 549-556 www.ijaem.net ISSN: 2395-5252

separate the intrinsic viscoelasticity of macromolecular friction from the fluid flow-driven poroelasticity that controls the energy-dissipative mechanics of cartilage(5). The characteristic that controls poroelasticity, known as hydraulic permeability, was discovered to be primarily governed by aggrecan rather than collagen and to change more dramatically than modulus following aggrecan depletion(11). This new tool offers a thorough method for evaluating cartilage functional changes in OA that goes beyond elastic modulus(1).

Molecules Mediating OA Pain:-

The perception of OA pain is a dynamic and complex process that involves changes to the joint's structure and biochemistry as well as to the peripheral and central nervous systems(11). Although there have been many studies on the mediators of OA joint degeneration, studies on the biochemical influences on and in the peripheral and central nerve systems in OA have just lately started(4). In this sense, OA seems to exhibit both parallels and distinctions from other painful disorders(9). Numerous signalling pathways have been connected to joint degeneration and/or pain(7). In this section, we'll talk about three recently discovered and extremely important routes that shed light on the mechanics behind OA pain(5).

Chemotactic cytokine ligand 2/chemokine (C–C motif) receptor 2

Chemotactic cytokine ligand 2 (CCL2), also known as monocyte chemoattractant protein 1 (MCP-1), is widely recognised for signalling through chemokine (C-C motif) receptor 2 to mediate the migration and infiltration of monocytes and macrophages (CCR2). CCL2 encourages joint inflammation in arthritis(10). Additionally, evidence points to CCL2 as a key modulator of neuroinflammation(1). In the dorsal root ganglia (DRGs), where CCL2 can then be transported to and released into the central spinal nerve terminals, CCL2 expression is raised in microglia and sensory neurons during neuropathic pain(3). Increased CCL2/CCR2 signalling has been linked to chronic allodynia and hyperalgesia as well as microglial activation and direct excitability of nociceptive neurons(11).CCL2 and CCR2 levels in DRGs were increased in a DMM mouse OA model at 8 weeks after surgery, which was correlated with an increase in OA-related pain behaviours(4). According to previous research, elevated levels of CCL2 and CCR2 in the DRG were thought to mediate pro-nociceptive effects by boosting sensory neuron excitability both directly through CCL2/CCR2 signalling in DRG sensory neurons and indirectly through CCL2/CCR2-mediated recruitment of macrophages in the DRG(5). Ccr2-null animals had diminished pain responses after DMM with comparable amounts of joint injury in comparison to wild-type mice(2). Although CCR2 antagonists are now being evaluated in clinical investigations, neither CCL2 nor CCR2 have been the focus of any clinical studies on OA pain(1).

Nerve growth factor/tropomyosin receptor kinase A

Targeted suppression of nerve growth factor (NGF) and its corresponding receptor, tropomyosin receptor kinase A (TrkA), decreased OA pain in both human and animal investigations. In healthy human volunteers, systemic treatment of NGF led to chronic whole-body muscle hyperalgesia whereas anti-NGF antibody therapy with tanezumab dramatically reduced OA pain(12). NGF could mediate pain via a variety of different methods.The DRG can be reached retrogradely by overexpressed NGF that binds directly to TrkA at sensory neuron nerve terminals. The mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) signalling is triggered in sensory neurons there(7). The brain-derived neurotrophic factor (BDNF), substance P, calcitonin gene-related peptide (CGRP), and nociceptor-specific ion channels, such as Cav 3.2, 3.3, and Nav1.8, are among the molecules associated with pain that are upregulated when the NGF-MAPK/ERK axis is activated(4).In addition to directly signalling sensory neurons, NGF also targets other cell types to induce analgesic effects(3). For instance, NGF/TrkAsignalling in mast cells results in the release of pain and pro-inflammatory mediators, such as prostaglandins and histamine, in addition to NGF(11). Pro-inflammatory factors increase NGF signalling, and NGF encourages leukocyte chemotaxis and vascular permeability, further promoting inflammation(6). Angiogenesis and nerve development are additionally aided by NGF/TrkAsignalling(8). Angiogenesis is not only an inflammatory process; it also acts as a pathway for nerve development into the joint(4).It is of great interest to further define NGF/TrkA pain signalling mechanisms and to find additional therapeutic targets in this pathway given the high efficacy of targeting NGF in a clinical study on reducing OA pain(8). Recent research shows that decreased PKC signalling is linked to increased MAPK/ERK signalling at the innervating DRGs, which results in an increase in NGF and TrkA levels in the

synovium and DRGs, as well as an increase in OA hyperalgesia(9).A small subset of patients receiving systemic anti-NGF medication, however, showed fast OA progression and an increased risk of bone fractures in recent clinical investigations(5). Understanding the precise functions of the NGF/TrkA pathway in distinct joint tissues in OA and OA-associated pain is of great interest given the analgesic effects of anti-NGF therapy on OA-associated pain(7).

ADAMTS5

By using Adamts5 KO mice and therapeutic anti-ADAMTS5 antibody treatment on wild-type mice, it is possible to decrease ADAMTS5 signalling and expression in the DMM model, which lessens joint degeneration and pain(9).Since ADAMTS5 is a significant aggrecanase and aggrecan is a crucial part of the cartilage's proteoglycans that provide compressive resistance, it is believed that ADAMTS5 is a key mediator of cartilage degradation during the development of OA(10). The use of Adamts5 KO mice and the direct prevention of joint degeneration with anti-ADAMTS5 antibody may help to shed light on how joint degeneration causes OA pain, even if variations in pain signalling can be independent of the severity of joint degeneration(8). For instance, it has been proposed that hyalectan fragments produced by ADAMTS5 directly trigger nociceptive neurons as well as glial activation, increasing pain perception(7). Additionally, suppression of ADAMTS5 after DMM caused CCL2 levels in DRG neurons to decrease, indicating a function for CCL2 in OA-specific pain(2).

Pain-related behavior tests

The most frequent cause of medical visits is pain, which is also a key sign that a joint replacement is necessary(1). Therefore, it is crucial to assess pain in pre-clinical animal models in order to comprehend its causes and create effective treatments for OA pain(10). Animals' OA pain is assessed using both indirect and direct methods(11).Effective OA pain management requires research that acknowledges pain as a clinical indicator and quantifies pain intensity(4). Because of the comparatively low cost of animal upkeep, the accessibility of historical data for comparison, and the lesser doses of medications needed for experimental trials, rodent animal models are frequently employed for basic and pre-clinical research(8). Rodents offer an advantage over other tiny animal models for pain measures, such as rabbits, which make it difficult to get a pain response and become immobilised if frightened by an unfamiliar observer(3). To enable molecular understanding of OA progression and pain in vivo, genetically modified strains are typically developed using mice(12). Larger animals like dogs, sheep, goats, and horses are also occasionally used to simulate the pain of OA.In small animal models of OA, a variety of direct and indirect measures of pain are employed(4). Static or dynamic weight bearing, foot position, gait analysis, spontaneous activity, as well as sensitivity to mechanical allodynia, mechanical hyperalgesia, and thermal, and cold stimuli, are all examples of indirect and/or direct measurements of pain(9). Mechanical stimulation may be the indirect test that has the strongest correlation with OA pain among those that involve pain-evoked actions(7). The von Frey test for mechanical allodynia utilising filaments to quantify referred pain is a widely used indicator of indirect pain(1).The paw pressure pain threshold is measured using an analgesiometer to perform direct mechanical hyperalgesia(9). The hind limb withdrawal test, the struggle reaction to knee extension, the vocalisation produced by knee compression on the afflicted knee, the ambulation and rearing spontaneous movements are additional direct indices of OA discomfort(8). Because these tests are also used to evaluate clinical OA pain, weight-bearing and gait analyses may have significant translational significance for measuring OA pain(7). However, since OA pain is a dull pain as opposed to, say, acute inflammatory pain, eliciting unambiguous pain responses from weight bearing or gait when utilising the unilateral DMM mouse model is difficult(4).Testing for pain behaviour in large animals is more difficult, and the most effective way to measure pain is still up for debate(1). However, it has been proposed that dogs, the most popular large animal, offer the best predictive modelling for OA pain transferred into the clinical context. Large animals can only be assessed for pain using methods that measure lameness, gait analysis, and subjective rating scales that measure human-like pain descriptors(6).Overall, both small and big animal models can be used for a variety of pain-behavior tests(7). Although there is no exact animal model or pain behaviour test that can accurately predict OA-associated pain in patients, these tests provide important insight into the mechanisms underlying OA pain and enable for the evaluation of potential treatments(2). Although rodents are still frequently used in basic OA pain research, large animals are still crucial because of their superior ability to simulate clinical OA pain(9).

Future perspective

Even while OA research has advanced significantly in recent years, very little is still understood about the molecular pathways underlying OA development(11). OA is a complex illness brought on by a number of reasons. Runx2, which is increased in a number of OA mice models and in cartilage samples taken from individuals with the illness, is a significant potential contributor to the development of OA(5).Two important questions that need to be answered are: (1) Can Runx2 expression be altered to treat OA disease? and (2) Is Runx2 a key molecule mediating the development of OA in joint tissue? The entire joint, including the articular cartilage, subchondral bone, synovial tissues, and menisci, is affected by OA(3). It is currently unknown which of these joint tissues experiences OA damage initially after disease onset; this is significant since it has a direct bearing on OA treatment(9). Additionally, more research is needed to understand the interactions between other OA symptoms as articular cartilage loss, osteophyte development, subchondral sclerosis, and synovial hyperplasia(1). The creation of cutting-edge therapeutic approaches for OA will be accelerated by our growing understanding of the molecular mechanisms underlying these problems(12).

REFERENCES:-

[1]. Pei Y, H. A. Y. X. et al. (2006). Differential regulation of cytokine-induced MMP1 and MMP13 expression by p38 kinase inhibitors in human chondrosarcoma cells: potential role of Runx2 in mediating p38 effects. Osteoarthritis Cartilage, 749–758.

[2]. Hirata M, K. F. F. A. et al. (2012). C/EBPβ and RUNX2 cooperate to degrade cartilage with MMP-13 as the target and HIF-2α as the inducer in chondrocytes. .Hum Mol Genet ,1(21), 1111–1123.

[3]. Chen CG, T. D. C. E. et al. (2012). Chondrocyte-intrinsic Smad3 represses Runx2-inducible matrix metalloproteinase 13 expression to maintain articular cartilage and prevent osteoarthritis. .Arthritis Rheum ,1(64), 3278–3289.

[4]. Kamekura S, K. Y. H. K. et al. (2006). Contribution of runt-related transcription factor 2 to the pathogenesis of osteoarthritis in mice after induction of knee joint instability. Arthritis Rheum, 1(54), 2462–2470.

[5]. Yang S, K. J. R. J. et al. (2010). Hypoxia-inducible factor-2alpha is a catabolic regulator of osteoarthritic cartilage destruction. .Nat Med ,1(16), 687–693.

[6]. Saito T, F. A. M. A. et al. (2010). Transcriptional regulation of endochondral ossification by HIF-2alpha during skeletal growth and osteoarthritis development. Nat Med, 1(16), 678–686.

[7]. Lin AC, S. B. B. J. et al. (2009). Modulating hedgehog signaling can attenuate the severity of osteoarthritis. Nat Med, 1(15), 1421–1425.

[8]. Zhu M, T. D. W. Q. et al. (2009). Activation of β-catenin signaling in articular chondrocytes leads to osteoarthritis-like phenotype in adult β-catenin conditional activation mice. J Bone Miner Res ,1(24), 12–21.

[9]. Yang X, C. L. X. X. et al. (2001). TGF-β/Smad3 signals repress chondrocyte hypertrophic differentiation and are required for maintaining articular cartilage. J Cell Biol, 35–46.

[10]. Loeser RF. (2009). Aging and osteoarthritis: the role of chondrocyte senescence and aging changes in the cartilage matrix. Osteoarthritis Cartilage, 1(17), 971–979.

[11]. Krasnokutsky S, S. J. A. S. (2007). Osteoarthritis. Bull NYU Hosp Jt Dis, 1(65), 222–228.

[12]. Goldring MB, G. S. (2007). Osteoarthritis. J Cell Physiology, 1(213), 626–634.

[13]. Felson DT. (2006). Clinical practice. Osteoarthritis of the knee. N Engl, 1(354), 841–848.

[14]. Loeser RF, G. S. S. C. et al. (2012). Osteoarthritis: a disease of the joint as an organ. Arthritis Rheum, 1(1), 1697–1707.