"HISTOCHEMICAL STUDY OF MUCOPOLYSACCHARIDES AND MICROSCOPIC STRUCTURE OF OSTEOARTHRITIC MENISCI OF THE HUMAN KNEE JOINT"

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For the award of the degree of

Doctor of Philosophy

In the Faculty of

Medicine

By

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LIST OF ABBREVIATIONS

AB	-	Alcian blue
AB759	-	Antibody759
ACL	-	Anterior cruciate ligament
ACLT	-	Anterior cruciate ligament transection
AF	-	Aldehyde Fuschion
AFM	-	Atomic force microscopy
ANOVA	-	One-way analysis of Variants
BMD	-	Bone mineral density
BMI	-	Body Mass index
BMP	-	Bone morphogenetic protein
BTH	-	Bovine testicular hyaluronidase
CABC	-	Chondroitinase ABC
CAM	-	Cell associated matrix
CEO	-	Chief executive officer
CFC	-	Calcified fibrocartilage
CT	-	Computed tomography
CxOA	-	Characteristic knee Osteoarthritis
d.f.	-	Degree of freedom
dGEMRIC	-	Delayed Gadolinium-Enhanced Magnetic Resonance Imaging of Cartilage
DMM	-	Destabilization of medial meniscus
DNA	-	Deoxyribonucleic acid
DPM	-	Disintegrations per minute
DPX	-	Dibutylphthalate polystyrene xylene
ECM	-	Extracellular matrix
EDTA	-	Ethylene diamine tetra acetic acid
FGF	-	Fibroblast growth factor
GAG	-	Glycosaminoglycans
H & E	-	Haematoxylin and Eosin
HCL	-	Hydrochloric acid
HMDS	-	Hexamethyldisilazane
HPLC	-	High-performance liquid chromatography
IL-1	-	Interleukin-1

IM	-	Inner menisci
JAR	-	Joint area reduction
KC	-	Keratinocytes-derived chemokine
KLES	-	Karnataka Lingayat Education Society
KOA	-	Knee Osteoarthritis
LLA	-	Left leg lateral menisci anterior part
LLM	-	Left leg lateral menisci middle part
LLP	-	Left leg lateral menisci posterior part
LMA	-	Left leg medial menisci anterior part
LMM	-	Left leg medial menisci middle part
LMP	-	Left leg medial menisci posterior part
M.S.S.	-	Mean Sum of Square
MC	-	Mucin carmine
MCA	-	Main constituents analysis
MCP-1	-	Monocyte chemotactic protein-1
MED	-	Mediterranean diet
MFCs	-	Meniscus fibro chondrocytes
MMP	-	Matrix metalloproteinase
MRI	-	Magnetic Resonance Imaging
mRNA	-	Messenger ribonucleic acid
NC	-	Negative control
NCP	-	Non-collagenous proteins
OA	-	Osteoarthritis
OM	-	Outer menisci
ORC	-	Orcein
PAS	-	Periodic acid-Schiff
PGE2	-	Prostaglandin E2
PKO	-	Primary knee osteoarthritis
PUFAs	-	Polyunsaturated fatty acids
RKA	-	Revision knee arthroplasty
RLA	-	Right leg lateral menisci anterior part
RLM	-	Right leg lateral menisci middle part
RLP	-	Right leg lateral menisci posterior part

RMA	-	Right leg medial menisci anterior part
RMM	-	Right leg medial menisci middle part
RMP	-	Right leg medial menisci posterior part
RNase	-	Ribonuclease
ROA	-	Radiographic Osteoarthritis
RT-PCR	-	Reverse transcriptase-polymerase chain reaction
S.S	-	Sum of Square
SH	-	Shore hardness
SL	-	Superficial meniscus layer
TGF	-	Tissue growth factor
TKA	-	Total knee arthroplasty

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ABSTRACT

Background: Osteoarthritis (OA) is a progressively disabling joint disease caused by a pathological imbalance between degenerative and repair processes, for which there is currently no cure. It is one of leading causes of chronic disability. Patients affected by this disease experience pain and loss of function. OA can be caused by a variety of factors, including diet, injury, stress, and genetic abnormalities. However, the molecular mechanisms driving the disease onset and progression are not fully understood. Knowledge regarding changes of mucopolysaccharides (proteoglycans) in human osteoarthritis meniscus may help in understanding development of meniscal degeneration. Therefore, this study is undertaken to estimate a large number of human OA menisci for study of mucopolysaccharides and microscopical structural changes in osteoarthritic menisci by histochemical analysis of different parts of medial and lateral menisci of both legs.

Objectives:

1. To study the histochemistry of mucopolysaccharides in osteoarthritic menisci of a Human knee joint.
2. To study the microscopic structure of menisci in osteoarthritic patients.
3. To compare the age-related changes in histological structure and mucopolysaccharide changes in osteoarthritic menisci.

Methodology: Medial and lateral osteoarthritic menisci were collected from 110 human knee joints of both sexes. Normal meniscal tissue from 8 months old male domesticated ruminant sheep was used as a control. Meniscal samples were stored in 10% formalin for 3 to 5 days. For each meniscus, three separate parts (anterior,

middle, and posterior) were processed. The menisci were sectioned in two places vertically at 45º and 135º angles relative to the sagittal plane. After that, each part was sectioned along the horizontal plane from the inner border to the outer border. Then tissue samples were fixed in 10% buffered formalin for 24 hours. Tissue samples were brought in for routine tissue processing and studied for 10 different histochemical stains with a color intensity, to find mucopolysaccharides in OA menisci and with hematoxylin and eosin (H and E) to find surface integrity, cellularity, fibrous organization and collagen orientation, and mucoid degeneration.

Descriptive statistics were used to generate histochemical scores. Further chi-square test was used to see the association. One-way ANOVA (F-test) was applied to test various parts of different stains staining intensity in medial and lateral OA menisci of both legs. Further, nonparametric method (chi-square test) was used to see the association between the sides and OA. Significance was seen at 5% level. Analyses were performed using MS excel and SPSS version 22.

Results: Decreased staining intensity for mucopolysaccharides (proteoglycans) was observed in different parts of medial and lateral OA menisci of both legs than control meniscus. A significant changes in level of mucins were observed at anterior, middle, and posterior parts of medial and lateral OA menisci of both legs. Tissue fibrillation and tears were first observed at the inner border, spread over time to the articular surface of the meniscus, and progressed to complete destruction or loss of meniscal tissue. Age-related changes in microscopical structure in osteoarthritic menisci of the medial and lateral menisci parts of both legs were observed and it was found that moderate (Grade 3) OA was higher in all 50-59, 60-69, and 70 + years of age groups.

Conclusion: In OA menisci, there was a significant loss of collagen and proteoglycan, indicating that these two substances are more actively involved in the degenerative process and the emergence of OA. According to test statistics used for various histochemical stains, the color intensity of various medial and lateral menisci parts with a side of the legs showed significant variation by F-test ($p < 0.05$). Additionally, the medial and lateral menisci were not significantly associated with each other in the chi-square test correlation of distinct histochemical stain intensities with a side of the legs.

Microscopical structure of OA menisci in both legs was significantly associated with posterior part of the medial menisci ($P < 0.05$), while the anterior and posterior parts of lateral menisci were significantly associated with OA menisci.

The alterations of the proteoglycans (glycosaminoglycans) in human OA menisci give information on the scientific evidence of the progressive nature of OA. Thus, this research will aid medical practitioners in the creation of medications with altered structural properties for the treatment of OA.

Keywords: Histochemical; Mucopolysaccharides; Proteoglycans; Mucins; Osteoarthritic; Microscopical structural; Menisci; Human knee joint.

1. INTRODUCTION

1.1 Background

1.1.1 MENISCI

The term "meniscus" is a diminutive of Greek word "meniskos," which means "crescent" and mene "moon".[1] Menisci are intricate fibrocartilaginous tissues that are crucial for the knee joint's strength, shock absorption, load distribution, and articular cartilage protection. [2]

Each knee joint has two menisci: i.e. i) Medial meniscus and ii) Lateral meniscus.

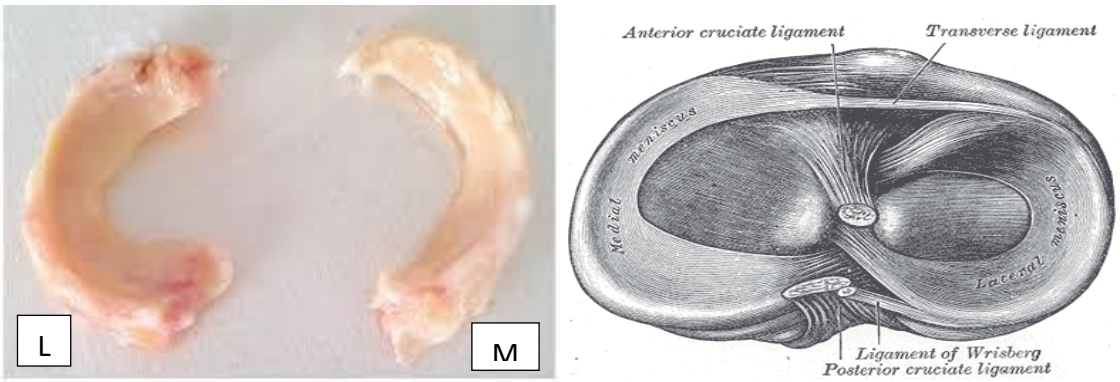


Figure 1: Medial (M) and lateral (L) meniscus tissue

Medial meniscus: - is semicircular and noticeably wider in posterior than the anterior.

Lateral meniscus: - is nearly round and roughly consistent in breadth from the anterior to the posterior. It is more mobile and takes up more of articular surface (80%) than medial meniscus (60%) does.[1]

Between the eighth and tenth weeks of gestation, the lateral and medial menisci develop their distinctive shapes. They originate from the condensation of mesenchymal tissue in an intermediate layer. The rising menisci are densely cellular

and vascular. Menisci gradually lose some of their cellularity as the fetus develops, but their collagen content rises in a circumferential pattern. Just 10% to 30% of the peripheral tissues get blood flow by adulthood. With typically aligned knees, approximately 70% of the stress travels via medial tibiofemoral section whereas 30% through the lateral section.[3]

The main components of extracellular matrix (ECM) are cells sandwiched by water (75%), DNA (0.12%), and collagen (22%). The remaining dry weight is made up of noncollagenous proteins and total glycosaminoglycans.[4]

1.1.2 OSTEOARTHRITIS

Pathological disparity between the deteriorating and healing procedures leads to osteoarthritis, usually referred to as 'OA' a degenerative joint disease. There is no doubt that the meniscus is essential to long-term health of knee and that it also contributes significantly to the intricate biomechanics of knee joint. Individuals at high threat of onset or progression of knee osteoarthritis have no known solution. This also includes those who have undergone incomplete or total meniscectomy or meniscal deterioration. It is characterized by alterations in the meniscus and subchondral (substance) bone as well as articular cartilage degeneration.[3]

Osteoarthritis is one of the main factors in chronic disability. The disease primarily affects middle-aged and older persons and athletes, while it can sometimes afflict younger people due to trauma or overuse. Individuals who have this illness experience discomfort and functional loss.[5]

Many things, such as diet, trauma, strain, and genetic anomalies, might contribute to OA. Unfortunately, our knowledge of the molecular processes behind the disease's genesis and development is still limited. It is regarded as a primary infection of cartilage and is characterized by articular cartilage loss, joint space constriction, and subsequent alterations. The exact mechanism underlying the sickness is unknown despite extensive research on it. OA's etiology is still a mystery.[6]

1.1.3 MUCOPOLYSACCHARIDES

Other names for the mucopolysaccharides include mucins, glycosaminoglycans, and mucosubstance. As an alternative to the names already stated, the term glycoconjugates has been proposed.[7, 8]

Long unbranched polysaccharides made up of repeated disaccharide components are known as mucopolysaccharides or glycosaminoglycans. An amino sugar (N-acetylglucosamine or N-acetylgalactosamine) and a uronic sugar (glucuronic acid or iduronic acid) or galactose make up the repeating unit, with the exception of keratan. Due to their strong polarity, mucopolysaccharides draw water. As a result, they serve as a lubricant or a shock absorber for the body.[9, 10]

Mucopolysaccharides, glycosaminoglycans, and mucosubstance are other names for mucins. Glycoconjugates are now further separated into glycoproteins and proteoglycans.[7, 8, 11]

The word ‘mucin’ has a long history. Histochemically it is categorized into 2 types:-

i) Epithelial mucin

ii) Connective tissue mucin

Epithelial mucin is also referred to as mucins or mucosubstance, while connective tissue mucins are known as mucopolysaccharides or glycosaminoglycans.

There are several categories for mucopolysaccharides:

i) **Neutral mucopolysaccharides** lack a free acidic group and are made up of hexosamine and hexose units.

ii) **Acidic mucopolysaccharides** consist of hexosamine associated with glucuronic acid, sialic acid, or sulfate radical. Acid mucins are further classified into Sialomucin and Sulphomucin depending on the sialic acid group or ester sulfate group.[12]

1.2 Review of Literature

1.2.1 Review related to socio-demographic variables,

Osteoarthritis by Gender and Age Groups

Vina and Kwoh (2018) conducted cohort research to define radiographic OA deterioration by race and gender over and assess the impact of possibility variables. They examined a total of 1882 patients (786 males and 1096 females) between the ages of 45 and 79 year and discovered that 42% of men and 58% of women had an OA prevalence.[13]

In a cross-sectional investigation, Moyer et al. (2015) quantified sex differences, examined connections between radiographic measurement and assessed sensitivity to medial and lateral joint area reduction (JAR). They examined a total of 2123 individuals, 885 men and 1238 women, who were between the ages of 60 and 65, and discovered that OA prevalence was 42% in males and 58% in women.[14]

In patients with varus knee OA, femoral alignment and deformity were compared between the sexes. Mochizuki et al. (2017) conducted case-control research. They examined 214 patients (54 males and 160 females) between the ages of 65 and 75 years and discovered that 25% males and 75% females had OA prevalence.[15]

Osteoarthritis by religions

From January to March 2007, in a study, Chokkhanchitchai S. et al. (2010) looked at how religious practice affected the prevalence of knee osteoarthritis in Thai individuals. They studied 2,599 people in total, from both Muslim and Buddhist

backgrounds, who were at least 50 years old. They found that the prevalence of OA was lower among Muslims than among Buddhists.[16]

Jordan JM et al. (2007) deliberated a population-based education to examine reporting of current prevalence estimates of knee-related osteoarthritis (OA) effects in African Americans and Caucasians aged ≥ 45 years in North Carolina. 3,018 people participated in total. They discovered that compared to Caucasians, African Americans had a somewhat greater prevalence of knee OA symptoms.[17]

Tangtrakulwanich et al. (2006) conducted a cross-sectional education to assess prevalence, trends, and possible reasons for knee osteoarthritis in Thai monks who resided in southern Thai temples. The study, which included 261 monks over the age of 60 from 85 temples, discovered that radiographic knee osteoarthritis is common among Thai monks and is more prevalent in the elderly, those who were older when they were ordained, and obese subgroups.[18]

Osteoarthritis by family history

A population-based case-control research was carried out by Ding et al. (2005), to determine the heritability of knee cartilage defects in sibling pairs and to compare the variances in knee cartilage faults between themes who had undergone total knee replacement surgery for severe primary knee osteoarthritis (OA) and controls. Participants were 128 siblings from 51 relatives (115 sib-pairs) and 186 matched pairs with a mean age of 45 years. They discovered that the participants have a greater possibility of getting OA from their ancestors. Families can pass this propensity down from one generation to the next, but the pattern of inheritance is unknown. Common cartilage defects in the knee may have a role in the genetic

etiology of osteoarthritis (OA) and involve a gene that is associated with the genetic basis of knee pain and bone density.[19]

To ascertain how much environmental and inherited factors contribute to common forms of osteoarthritis of the hands and knees, Spector et al. (1996) conducted a classic twin research using unselected twins who existed radiographically tested for osteoarthritis. Via a national media campaign and recruitment from a London-based twin register, subjects, 130 matching and 120 non-matching female twins between the ages of 48 to 70 years were chosen. Principal outcome metrics radiographic alterations in the tibiofemoral joint of the knee that are similar in matching twin pairs compared to non-matching twin pairs expressed as intraclass correlations. They discovered that the intraclass correlations of Heberden's nodes and knee discomfort were stronger in same pairs, as were correlations of radiographic osteophytes and constriction at most sites.

They disregarded recognized environmental or demographic confounders and Women's radiographic osteoarthritis of the knee has a known genetic component, with a genetic contribution ranging from 39–65%.[20]

In order to look into patterns of twin and family aggregation of osteoarthritis (OA) for frequently affected joints, Kujala et al. (1999) conducted the Finnish Twin Cohort study. They discovered disparities in the aggregation by joint as well as the twin and family aggregation of OA. Their study found that genetic effects explained a higher fraction of the heterogeneity in women's susceptibility to OA than did genetic effects for men, suggesting that sex and environmental factors may have different impacts on men and women depending on their gender. It is clear from their joint-

specific data of OA that specifically affects the finger and knee joints are the best targets to recognize the role of genetics in development of OA.[21]

In a population-based longitudinal study, Ding et al. (2006) compared children whose parents had total knee replacement surgery for severe primary knee osteoarthritis to controls who had no such history to determine the differences in knee structure and non-knee structural traits. At baseline and roughly two years later, tests were conducted on 163 matched pairs (mean age 45 years). According to their study, the onset of knee OA is likely polygenic but represents a common environment because of knee cartilage injury, changes in cartilage loss, and lower physical performance in addition to hereditary factors. The authors' findings, which suggest that hereditary factors may be implicated in OA, found that independent of structural considerations, knee discomfort is more common in children with a family history of knee OA.[22]

Osteoarthritis by dietary habits

Xu C et al. (2020) conducted prospective cohort study in clinical settings in Maryland to ascertain if food patterns identified by main constituents' analysis (MCA) are related to the development of KOA. 2,757 people with a history of KOA (mean age 62 years) who had their baseline diet examined were monitored for approximately ≤ 72 months. Dietary patterns were developed using Western and sensible methods. They discovered that maintaining a prudent diet was linked to reduced advancement, but adhering to Western food was related to higher radiographic and symptomatic KOA development. A food high in fruits, vegetables, seafood, complete grains, and pulses may generally help persons with KOA slow the progression of their symptoms and radiographic illness.[23]

To look into the potential link between soft drink consumption and the radiographic onset of knee osteoarthritis (OA), Lu B et al. (2013) conducted a prospective cohort study using data from the osteoarthritis initiative (OAI). 2,149 patients with baseline dietary information and radiographic knee OA were followed up to 12, 24, 36, and 48 months. In order to assess the subjects, a baseline Block Short Food Frequency Questionnaire was used. They suggested that guys who regularly consume soft drinks may develop OA more quickly. [24]

Veronese N et al. (2018) conducted a longitudinal cohort study to determine whether higher adherence to mediterranean food is prospectively related to a lower threat of radiographic OA (ROA), radiographic characteristic knee OA (CxOA), and pain aggravation in North American individuals at high threat for or with knee OA. It was assessed using the five-category validated Mediterranean diet score (aMED). There were 4,330 participants (females made up 58.0%; mean age, 61.1 years).

Through the course of a mean follow-up period of 4 years, participants with a higher adherence to mediterranean food reported a reduced threat of pain compared to (ROA), according to multivariable poisson regression analysis. Out of 2994 people who were CxOA-free at baseline, higher adherence to a mediterranean food were associated with 9% decreased risk for CxOA during follow-up. They discovered that higher adherence to the Mediterranean diet is associated with a decrease in knee OA symptoms and pain escalation.[25]

Osteoarthritis by exercise

Manninen P et al. (2001) used case-control study to examine the link between physical activity and the chance of developing severe knee osteoarthritis that requires

arthroplasty. In this study, males (n=55) and females (n=226) between the ages of 55 and 75 had primary osteoarthritis knee replacement surgery at the Kuopio University Hospital. In the province of Kuopio, controls (n=524) were chosen at random from the general population. Exercise over a lifetime was evaluated in the past. Based on the mean (low/high) of the calculated cumulative exercise hours, two groups were created. They found that the likelihood of knee osteoarthritis requiring arthroplasty decreased with cumulative hours of leisure physical exercise. They arrived at the conclusion that moderate recreational physical exercise is associated with a lower risk of developing knee osteoarthritis.[26]

According to the research of the authors (Lane N., 1996; Esser S., Bailey A., 2011), participation in sports that regularly subjected normal joints to high levels of impact or torsional loading, as well as sports that could harm supporting tissues like ligaments, tendons, and menisci, all increased the risk of osteoarthritis (OA). Exercise really slows the progression of knee OA and is clearly effective in managing and treating the pain and functional loss brought on by OA. There is a tonne of proof showing light to moderate exercise does not cause or hasten the onset of knee OA. According to recent studies, regular mild to moderate physical activity has both preventive and therapeutic effects for people with knee OA.[27, 28]

Osteoarthritis by ABO blood group type

In a case-control study from 2020 by Li C. et al, the authors looked at the link between the ABO blood group and primary knee osteoarthritis, as well as the severity of the condition as determined by the Kellgren/Lawrence score and the histopathologic correlation in a subgroup of patients. Retrospective reviews of patients with primary knee osteoarthritis served as the case group, and a random

selection of healthy blood donors served as the control group. In total, there were 30299 controls and 1126 cases. They discovered that the proportion of AB blood type was higher in the case group than in the control group (9.7% vs. 7.8%), and logistic regression showed that the AB blood type was a risk factor for primary knee osteoarthritis.[29]

According to a case-control study carried out by Yaradilmis et al. in 2021 to evaluate the validity of the association between primary knee osteoarthritis and the ABO blood group system in the Turkish population, the ABO blood group system may be a risk factor for early-onset knee osteoarthritis or revision surgery. Between 2011 and 2019, 2752 patients' records who had knee arthroplasty surgery in the clinic were analyzed. 2436 patients with primary knee osteoarthritis (PKO) underwent total knee arthroplasty (TKA) surgery, while 206 individuals underwent revision knee arthroplasty (RKA). The PKO group was found to have an 8:1 female-to-male ratio and an average age of 67.28 years. The blood type with the highest correlation to osteoarthritis was Group A (p 0.001). The relationship in Group AB was significant but thin (p=0.002). Compared to the PKO group, Group A underwent revision knee arthroplasty (RKA) at a statistically significantly higher rate.[30]

In order to determine whether there is a link between primary hip osteoarthrosis and ABO blood groups, Lourie JA (1983) conducted a study. The study sample included 341 individuals with primary OA of the hip who were of both sexes and between the ages of 39 and 86. According to the author, there is a significant group O deficiency among arthritis patients when associated to a control set of blood donors from same region.[31]

Choi and Pai (2004) examined whether the prevalence of osteoporosis varied noticeably according to ABO blood types in one study. They evaluated 227 postmenopausal women's anthropometric characteristics, body composition, and bone mineral density (BMD). They ultimately came to the conclusion that there is a strong correlation between postmenopausal women's ABO blood group status and the prevalence of osteoporosis. The women with blood type AB displayed the lowest BMDs in the proximal femur and lumbar spine among the ABO blood groups.[32]

1.2.2 Review related to Microscopical structure changes of menisci in Osteoarthritis

Animals models

Fetouh and Eman (2008) conducted a study to see how rabbits' aging affected the structural alterations in their knee menisci. Three sets of Egyptian rabbits, aged a month, six months, and two years, were utilized (3-4 rabbits for each). At one month of age, they discovered that the chondrocytes were less compressed and that there were plenty of collagen fibrils in the general matrix in addition to a modest quantity of territorial matrix. The chondrocytes revealed cytoplasmic basophilic and a small number of intracytoplasmic collagen fibrils at 6 months of development. Collagen fibrils were condensed in the matrix, and filaments, fibrils, and protein-polysaccharide particles were found in abundance in the territorial matrix. The chondrocytes also showed a significant shrinkage and size reduction at the age of 2 years. Collagen fibrils with a noticeable range in fibril diameter were discovered in the matrix. Calcium deposits that are electron-dense have partially taken the place of the territorial matrix. They concluded that the anatomy of the menisci in the knee joint is significantly influenced by age, making them more vulnerable to injury as people age.[33]

To determine the relationship between pathological meniscus alterations in OA knees, Zhao et al. (2014) conducted a study. They included female Chinese rabbits aged 20 months who either underwent sham procedures on one knee at random or knee-damaging operations using the articular cartilage scratch technique. They came to the conclusion that changes in the biomechanical and biochemical ecology around the meniscus are altered when OA occurs. OA would be more severe if the meniscus displayed signs of degeneration, subluxation, and dysfunction.[34]

Hellio et al. (2001) examined the medial and lateral menisci from the knees of rabbits with anterior cruciate ligament (ACL) operations to determine changes that occurred throughout the early stages of OA development. They used histology and immunohistochemistry on the meniscal tissues obtained from control and experimental rabbits to study the structure and makeup of the matrix. Their results demonstrated that extracellular matrix deposition, altered matrix architecture, and altered cell distribution occur early in the medial meniscus after ACL transection.[34] The authors in part II examined the changes in the medial and lateral menisci from the knees of rabbits with transected ACLs at 3 and 8 weeks following surgery. Reverse transcriptase-polymerase chain reaction (RT-PCR) and molecular examinations of rabbit meniscal tissues, particularly the medial meniscus, showed that extensive molecular modifications, including death, take place early after ACL transection.[35, 36]

In 1987, Cheung HS published a study on the distribution of collagen types I, II, III, and V in pepsin-solubilized bovine menisci. IM makes up about 10% of the wet weight of the entire meniscus and is composed of 70% collagen, of which 34% is pepsin soluble, 60% type II, and 40% type I collagen. He was examined for 1/3 of the inner menisci (IM) and 2/3 of the outer menisci (OM) of both the lateral and medial

menisci. Collagen makes up 80% of OM, and 17% of it is pepsin soluble. Type I collagen predominates, with traces of types III and V.[37]

Shapiro & Glimcher carried out a study in 1980 on the induction of osteoarthrosis in rabbit knee joints. Due to medial meniscectomy and emergence of "bucket-handle" lesions of the medial meniscus, mature rabbits' knee joints exhibit mild to moderate osteoarthritis changes. Gross examination and histological sections were used to study the osteoarthritic lesions. According to their findings, the pathologic changes start at the surface and progress to deeper layers. These alterations include peripheral osteophytes, peripheral cloning, hypocellularity, loss of cartilage shine, structural abnormalities in the matrix, and decreased glycosaminoglycan levels.

In healthy persons, the menisci are regularly injured, and the torn or lost fragments may be enough to compromise knee function that a partial or complete meniscectomy is required. Yet, experimental data suggests that even normal menisci removal can result in osteoarthrosis of knee.[38]

In a 2005 study, Melrose et al. immunolocalized perlecan in meniscal tissues and demonstrated how its localization in the ovine meniscus in relation to aggrecan and type I, II, and IV collagen varied with aging. They found that the middle and inner meniscal zones contained perlecan, which is expressed by cells with an oval or rounded shape. Almost little perlecan, other from tiny blood vessels were visible in the peripheral outer meniscal zones. All meniscal zones had a high concentration of type I collagen, the inner meniscal zone was home to type II collagen, and blood vessels were strongly associated with type IV collagen. Eventually, they came to the conclusion that this composite fibrocartilaginous tissue's key weight-bearing qualities

are provided by proteoglycans rather than the meniscus' complicated arrangement of collagen fibers.[39]

Research on the morphology of rabbit semilunar cartilages under light and transmission electron microscopes was conducted by Ghadially et al. in 1978. Four 6- to 8-month-old rabbits of each sex were utilized. They found that the tissue's cells are more similar to chondrocytes than fibroblasts, and collagen fibrils are contained within smooth membrane-bound tubular structures, evidently inside cells, using transmission electron microscopy on tissue from the contralateral knee of each animal. The main component of the menisci were collagen fibrils, which formed fibers, fiber bundles, and lamellae. The interfibrillar matrix and thin regional matrix next to chondrocytes both included protein-polysaccharide particles. Among the collagen fibrils in the general matrix were many juvenile elastic fibrils. A few adult elastic fibers were also found.[40]

The canine menisci's structural makeup, biosynthetic activity, and material characteristics were assessed in a 1988 study by Arnoczky et al. The menisci were cryopreserved using a controlled rate freezing method after being incubated in 4% solution of dimethyl sulfoxide in a physiological medium. Then kept in liquid nitrogen for a range of times, from 0 to 12 weeks (-196 degrees C). Also, properties of cryopreservation procedure on meniscal tissue's flexible strength and modulus were evaluated. A biosynthetic activity, Although cryopreservation and short-term storage did not appear to influence the morphological appearance or biomechanical character of the menisci, autoradiography analysis of these tissues revealed that only around 10% of the meniscal cells were metabolically active. The level of overall metabolic activity decreased over the course of storage.[41]

As degeneration was taking place, López-Franco and Gómez-Barrena (2018) evaluated and emphasized alterations in the meniscal cells and matrix. Differential meniscal anatomy and metabolism in the degenerative knee were examined using both surgical specimens and experimental animal models. They found that menisci were connected to extracellular matrix disturbance, variations in collagen and non-collagen protein synthesis, and expression, in addition to knee degradation and cell population loss. These alterations were distinct from those brought on by aging and were thought to be disease-specific.[42]

Wildey et al. (2001) investigated how meniscus cells respond to joint damage in the early phases of post-traumatic osteoarthritis by finding the changes in matrix gene expression in menisci. In this study, which employed dogs three and twelve weeks following surgery, the anterior cruciate ligament (ACL) was torn in one joint while the other unoperated joint served as a control. Absolute amounts of mRNA for the COL1A1 gene, which produces type 1 collagen, major fibrillar collagen of the meniscus, and the COL6A3 gene, which produces type VI collagen, an important repair molecule, were measured using a quantitative ribonuclease (RNase) protection test. They discovered that the concentration of total RNA in medial and lateral menisci increased from 40 to 60 microns RNA/g wet wt. in unoperated, normal joints to 200-350 microns RNA/g wet wt. in ACL-deficient joints. The DNA concentration showed no significant variations. Low quantities of COL1A1 and COL6A3 mRNA transcripts were identified in normal menisci. ACL deficiency led to elevations in COL1A1 and COL6A3 mRNA concentration of 20–38 fold at 3 weeks and 11–19 fold at 12 weeks following surgery in the medial menisci but not in the lateral menisci.[43]

Gigante et al. (1994) examined the distribution and histochemical properties of elastic fibers in the knees of newborn, young, adult, and old New Zealand white rabbits. Growing rabbits have elastic fibres, notably oxytalan fibers, which are consistently distributed in tendons, ligaments, and menisci as well as plentiful in the perichondrium and the fibrous layer of the periosteum. The epiphyseal cartilage, growth plate, and location at the enthesis are devoid of them. In mature rabbits, elastic fibers are abundant in the peritenon, periosteum, perimysium, synovial membrane, articular capsule, and perivascular connective tissue. At last, they discovered that the type of elastic fiber in different tissues varies with age; young animals contain more oxytalan fibers than adult animals. As a result, it can be inferred that the distribution of elastic fibers is likely connected to the diverse functional roles and biomechanical characteristics of each tissue.[44]

In a 2005 study, Kambic and McDevitt examined the distribution of type II collagen in canine meniscus and its spatial relationship to type I collagen using immunohistochemistry and confocal microscopy. Ten skeletally mature dogs weighing about 25 kg each had medial and lateral menisci from their knee joints predigested with Streptomyces hyaluronate lyase and bacterial protease enzyme XXIV. They employed anti-type II collagen polyclonal antibody (AB759), type I and type II collagen monoclonal antibodies (CP17L and II-IIBB3, respectively). They found that the fibrocartilage of the meniscus was stained as a neat network of type II collagen. The only part of the meniscus devoid of type II collagen is the outer zone that houses the blood vessels. Coronal and dorsal staining revealed bundles of type I circumferential fibrils localizing with type II collagen in the meniscus. Confocal overlays showed a superimposition of types I and II collagens and confinement of cells to types I and II collagen fibrils that surrounded the bundles. The radial tie

fibers were composed of patches of localized type II collagen. They eventually discovered that meniscus's fibrocartilage has a distinct type II collagen network that serves to distinguish fibro- and hyaline cartilage morphologically.[45]

Liang et al. (2020) used a rabbit anterior cruciate ligament transection model to perform the study to clarify micro biomechanical features of meniscus following degeneration and to look at corresponding macroscale histology and chemical composition. Twenty white bunnies were used. Menisci were taken from the knee joints of both the corresponding control groups and those that had undergone ACLT four and eight weeks earlier. Atomic force microscopy (AFM), histology, and an energy-dispersive spectrometer were used to explore the core regions of medial and lateral menisci. When compared to intact meniscus, they found that 4 weeks following surgery, the dynamic elastic modulus at the micro level considerably increased at both the top and bottom layers ($P = 0.021$). All locations experienced stiffness at 8 weeks following surgery ($P = 0.030$). The medial meniscus showed a larger alteration than lateral meniscus. All of these minute biomechanical changes took place before macroscale histological findings. After degeneration, GAG content increased according to histological analysis; 4 weeks after ACLT, however, there was no noticeable change in GAG content.[46]

In mice joints after medial meniscus (DMM) destabilisation, Huang et al. (2017) examined how age effects cartilage and subchondral bone modifications used to assess disease-modifying OA. DMM was administered to male C57BL/6 mice at ages of 4, 12, and 19+ and to females at ages of 12 and 18+. Contralateral knees that had had surgery and those that had not were harvested two months after the operation and assessed using cartilage histology scores, a CT scan, and measurements of

osteophyte production and subchondral bone plate thickness. In comparison to 4 M males, 12 M, and 19 M+ male mice had larger subchondral bone plates and greater cartilage erosions after DMM. Age-related increases in osteophyte size were seen, although the 19 M+ groups had considerably greater bone volume fractions. Additionally, 12 M females had milder OA than males, as evidenced by reduced osteophytes, subchondral bone plate sclerosis, and cartilage degradation.[47]

In a study by Ribitsch I. et al. (2018), arrangement and organization of equine knee meniscus were examined in connection to putative location-precise biomechanical features as well as site and age-specific differences. A histological investigation was done into the meniscus architecture. The menisci's stiffness, shore stiffness, and vitality loss were all assessed by biomechanical testing. The tibial meniscus surface was shown to have a generally greater SH than the surrounding bone, and the SH increased with aging. There were no significant site- or age-related variations in stiffness or energy loss. Finally, they suggested that, as was previously hypothesized for human menisci, superficial meniscus layer (SL) might contribute to meniscus SH and may be essential for meniscus function. The histologic architecture of equine SL is comparable to that of human menisci. The SH of horse menisci varies depending on both age and site-specific parameters, with a generally greater SH of the tibial surface and an increase in SH with age. During the gross mechanical testing, whole menisci showed no discernible changes in stiffness or energy loss, independent of location or age.[48]

Humans study

In 2012, Sun Y et al. reviewed the study to examine collagen and proteoglycan modifications in menisci of individuals with osteoarthritis. Meniscal samples were

obtained from eight consecutive unselected OA patients who received complete joint replacement surgery and three osteosarcoma patients who underwent lower limb amputation surgery. These tissues were surgical trash, after all. Collagens were examined using picrosirius red, hematoxylin, and eosin. Types I and II of collagen and aggrecan were assessed using immunochemistry. Their results showed that OA menisci had significant collagen loss, particularly in intermediate and inner sections, and that the collagen linkages were less organized than in normal menisci. Immunohistochemistry revealed co-localization of type I and type II collagens and suggested that decrease of type I collagen in OA menisci was more pronounced in middle and deep regions than in surface regions. But in all three zones, there was a significant reduction of type II collagen. Moreover, the OA menisci showed increased aggrecan staining. They came to the conclusion that these findings collectively show that OA menisci exhibit substantial collagen loss, intrameniscal degeneration, and extracellular matrix degeneration.[49]

Under the assumption that patient age is a predictor of the recovery of meniscus tissue, Senan, V. et al. (2011) conducted a prospective study on the histological characteristics of meniscal injury. Thirty patients' torn menisci were collected, and ten control menisci were conducted. To assess tissue healing, the histologic scoring system was employed. Patients over 40 showed significantly less meniscus cells than those under 40, which meant that age had a significant impact on a patient's capacity to mend a meniscus. They came to the conclusion that older patients' menisci may be more susceptible to degeneration and re-tear after repair than are menisci from younger patients.[50]

DeHaven et al. (1995) study set out to document long-term clinical and radiological results of open meniscal repair. This study evaluated 33 open repairs on 30 consecutive patients. A typical follow-up time of 10.9 years was used. There weren't any lost patients to find. They found seven confirmed meniscal tears (21%). Three of the 21 acute repairs (14%) re-tore, compared to four of the 12 chronic repairs (33%). Standing radiographs in compartments with successful repairs revealed no degenerative changes in 22 of 26 (85%), compared to 3 of 7 (43%) compartments with re-torn menisci. The long-term survival rate of repaired menisci was revealed to be 79 percent, unstable knees saw greater rates of re-tear, and radiographs provided evidence of biomechanical function of effective meniscal repairs.[51]

Pauli C. et al. presented the research in 2011. Its goals were to (1) establish standardized protocols for representative macroscopic and microscopic research, (2) improve the scoring techniques, that were being used then (3) deploy them on a sizable number of human menisci. They used 107 human knees for this investigation, cutting central region, anterior and posterior horns, of medial and lateral menisci in two distinct planes (cross and horizontal) sections as well. Each segment's surface integrity, cellularity, matrix/fiber organization, collagen alignment, and Safranin-O staining intensity were all graded. The most notable age-related changes they saw in menisci in joints without or with mild OA included increased Safranin-O staining intensity, decreased cell density, the creation of acellular zones, and evidence of mucoid degeneration with some loss of collagen fiber structure. Menisci from OA joints exhibited calcification, widespread fraying, fissures, and significant fibrocartilaginous separation of the matrix. Reduced cellularity, generalized hypercellularity combined with cellular hypertrophy, and aberrant cell clusters were

examples of atypical cell configurations. In general, OA and aging had less of an impact on anterior horns of medial and lateral menisci.[52]

Menisci feature three collagen fiber layers that are particularly arranged, according to Bullough PG. et al. (1970), to transform compressive loads into circumferential or "hoop" stresses. Radially organized fibers that serve as ‘ties’ to prevent shearing or splitting are seen in the topmost layer. To endure hoop stress during weight bearing, the middle layer's fibers are arranged parallel or circumferentially. The collagen fibres are arranged in a thick layer and are parallel to the edge. Researchers found that collagen fibers organized themselves in a parallel way in majority of locations to form thick collagen bundles, which may be observed lengthwise or in cross-section. It appears that the radial fibers’ job is to prevent longitudinal cracks from forming in the tissue as a result of too much compression.[53]

According to Englund M. et al. (2012), osteoarthritis is one of the top ten primary causes of disease burden in high-income countries and most common cause of musculoskeletal disability in underdeveloped countries. Knee deterioration and functional failure are possible outcomes of slow-moving knee OA. Aching, restricted movement and pain are most typical symptoms, and these frequently lead to weakened joints and incapacity.[54]

Clark and Ogden (1983) analyzed prenatal and postnatal cadaver knees to comprehend the developmental changes that occur in the menisci prior to skeletal maturity. The medial and lateral menisci were extracted from 109 fetuses with gestational ages between 14 and 34 weeks and 28 cadavers with postnatal ages ranging from 3 months to 14 years. It involves analyzing the general morphology, determining the ratios of medial to lateral menisci's area to that of their respective

tibial plateaus, and histologically inspecting the menisci and ligaments. They found that early in fetal development, both medial and lateral menisci evolved into their separate forms, but there were also incremental modifications, including a decline in the vascularity of the developing meniscus' central to peripheral margins and its biochemical makeup, which is responsible for its resistance to injury. They came to the conclusion that the developing menisci might be more capable of healing than the mature menisci.[55]

In order to compare samples from end-stage osteoarthritis (OA) with healthy standards, Kestila et al. (2019) devised and carried out ex vivo 3D imaging of meniscus posterior horn microstructure using micro-computed tomography. In this study, human medial and lateral menisci from ten deceased donors without knee OA and ten patients who underwent complete knee replacement surgery for medial compartment OA were both employed. They discovered that traditional histology and 3D visualization with CT qualitatively indicated comparable microstructural alterations in the posterior spines. In comparison to medial reference menisci and lateral menisci from OA knees, medial menisci from OA knees showed a higher mean histopathological score. Both the lateral menisci from OA knees and the medial and lateral comparison menisci had similar ratings. Lastly, they found that a special 3D visualization of meniscus microstructures is possible using a CT procedure based on hexamethyldisilazane (HMDS). The posterior horns of medial menisci from medial compartment OA knees had greater histopathological results than both the lateral posterior horns from the same OA knees and medial reference menisci, suggesting a strong association among meniscus degeneration and unicompartmental knee OA.[56]

In order to comprehend structural alterations, Battistelli et al. (2019) compared the complicated geometry, meniscus has a different ultrastructure and tissue composition in both healthy and pathological conditions. They examined meniscus samples taken from three healthy multi-organ donors, five patients who had suffered traumatic meniscal tears, and three patients who were having total knee replacements for last-phase osteoarthritis; their ages ranged from 66 to 72 years on average. Electron microscopy and histology to examine extracellular matrix's organization, existence and distribution of calcified areas, and alterations to cellular organization and structure. Researchers discovered that individuals with late-stage OA and traumatic meniscus tears had similar ECM structures, with increased proteoglycan concentration and collagen fiber disarray. Organelles were well-preserved and dispersed chromatin was the predominant feature of healthy menisci cells. Additionally, traumatic and OA menisci displayed elevated chromatin condensation, organelle breakdown, and cytoplasmic vacuolization, some of which contained signs of lysosomal vacuoles. Together with apoptotic-identical characteristics, areas of calcification were also seen. These features were more noticeable in samples of severe meniscal tears. Conclude that acute meniscal injury patients' meniscal tissue exhibits pathological changes that are typical of older individuals having TKR.[57]

For the purpose of examining articular cartilage tissue under typical, pathological, and experimental circumstances, Musumeci G. et al. (2014) reviewed and discussed the merits and drawbacks of conventional histochemical techniques. They demonstrated how histological and immunohistochemical factors underlie current knowledge of cartilage tissue and came to the view that adult articular cartilage has very little ability to repair its own, meaning even slight trauma may

result in progressive damage and osteoarthritic joint degeneration, causing significant pain and disfunction.[58]

According to Verdonk PC. et al. (2005), little investigations have been done on the biology of the human meniscus cell. The objective of this work is to assess human meniscus cell survival and proliferation in diverse growing environments, as well as extracellular matrix that these cells make in these artificial habitats. From visibly intact lateral and medial knee menisci, human meniscus cells were enzymatically extracted for this study. Hoechst 33258 dye was used to measure cell proliferation after cells were grown in monolayer or alginate gel conditions. They discovered that monolayer cultivated meniscus cells had a cell-associated matrix (CAM) made up primarily of type I and type II collagen and very little aggrecan. On the other hand, meniscus cells grown in alginate produced a CAM rich in aggrecan, with low levels of type II collagen and large levels of type I collagen. CD44+CD105+CD34-CD31- describes this group. Contrarily, a small cell population in the alginate culture was primarily CD34+ and did not collect ECM. In monolayer culture conditions, meniscus cells multiply more rapidly. In the alginate culture, cell counts marginally decline. It was concluded that different cell types can be distinguished from one another in the human meniscus by a distinctive CAM composition and membrane marker expression. When phenotypically stable articular cartilage chondrocytes are grown in the same alginate matrix, their CAM composition differs noticeably from this one.[59]

1.2.3 Histochemistry of mucopolysaccharides in Osteoarthritic menisci

Animals model

A study on the metabolism of glycosaminoglycans in experimental osteoarthritis brought on by immobilizing the rabbit knee in extension was conducted by Videman et al. in 1979. Right knees were examined in 18 rabbits older than 9 months. Following immobilization for 2, 6, 10, 17, 30, and 87 days, samples of the medial meniscus, medial collateral ligament of the knee, and hip joint capsule were collected and analyzed. Control tissue was taken from the mobile left leg. Hexosamine and uronic acid measurements were utilized to assess the tissue quantities of glycosaminoglycans after purification and previous papain proteolysis. The rate of synthesis of sulfated glycosaminoglycans was measured by measuring the absorption of 35S-sulphate (DPM/pg hexosamine). In comparison to controls, they found that samples from immobilized joints had significantly higher tissue content of hexosamine and uronic acid in meniscus. Ultimately, in both early and late immobilization osteoarthritis, they discovered that production rate and content of glycosaminoglycans increased in all tissues.[60]

In a 1998 study, Djurasovic et al. looked at how joint immobilization affected expression of aggrecan gene in meniscus. Animals had the front and posterior sections of their medial and lateral menisci surgically removed at 4 weeks. Following their inquiry, they found that menisci from immobilized knees had decreased aggrecan gene expression as seen by quantitative PCR analysis. They ultimately came to the conclusion that joint immobilization can considerably influence meniscal cellular activity and composition, which can subsequently potentially affect meniscal

function. With immobilization, the meniscus composition was likewise shown to have less proteoglycan and more water.[61]

Adams ME et al. in 1983 looked at meniscal glycosaminoglycans in both artificial and real osteoarthritis. In their investigation, after tearing the anterior cruciate ligament in one knee to cause osteoarthritis, 5-7-year-old beagles had their menisci evaluated at various points. The unoperated knee was used for the control. They discovered that operated joint showed signs of inflammation within the first month of osteoarthritis induction. The glycosaminoglycan content decreased and the water content increased after one week. The amount of hyaluronic acid did not alter consistently, However, keratan sulfate decreased in quantity more than chondroitin sulfate. The corresponding levels of chondroitin sulfate, chondroitin sulfate-4, and chondroitin sulfate-6 all remained unchanged. The levels of glycosaminoglycan returned to normal after 3 to 18 months, and they were high after 15 to 18 months.[62]

To lay the groundwork for upcoming research on the modifications to glycosaminoglycans in experimental osteoarthritis, Adams and Muir (1981) examined the content and composition of glycosaminoglycans in several locations of normal canine menisci. Both sexes of five foxhounds between the ages of 7-9 were employed. One of them had moderate to severe osteoarthritis in one knee, and the other had mild. The study did not include the osteoarthritic joints in either case. They discovered that compared to the articular cartilage of these animals, the menisci contained nearly eight times as much glycosaminoglycan and 10% less water. Despite the fact that the glycosaminoglycan composition was the same throughout the menisci, the overall quantities varied greatly. Chondroitinase can digest chondroitin 6-sulfate, chondroitin

4-sulfate, 25% chondroitin, 10% chondroitin, and 5% dermatan sulphate, in that order. Hyaluronic acid made up approximately 6% of all uronic acid.[63]

In order to identify noncollagenous proteins of the menisci, Fife RS (1985) conducted a study. 7 mature, healthy mongrel dogs were killed with a sodium pentothal overdose, and their medial and lateral menisci were removed from both knees. Several noncollagenous proteins and meniscal cartilage were isolated with 4 M guanidinium chloride, such as a 116,000-Da component protein and link proteins. They found that protein extracted from the meniscal cartilage comprises 3.8% of the 116,000-Da subunit protein. They ultimately came to a decision that these matrix proteins comprise 116-kDa proteins with unidentified roles and link proteins that stabilize proteoglycan hyaluronic acid aggregates. High-buoyant-density proteoglycans settle near the bottom of an associative cesium chloride density range, where the link proteins also settle. This protein appears to be a disulfide-bonded complex with a high molecular weight that is embedded in a matrix.[64]

Sanchez-Adams et al. (2011) investigated the material properties of the inner, middle, and outer meniscus to understand structure-function relations in the knee meniscus using compressive and tensile mechanics. In this investigation, medial menisci from 2-week-old bovine knees were used. Chondroitinase ABC (CABC) is used to remove GAGs from each meniscus region before comparing the material properties of reduction and control samples. Different geographic effects of the GAG reduction on the material characteristics of the meniscus are anticipated. Compressive experiments revealed that GAG deficiency drastically reduced the relaxation modulus in inner and middle meniscus areas decreases, respectively, as well as viscosity significantly decreased in all locations. The outer and middle meniscus specimens'

tensile characteristics were unchanged by CABC treatment, whereas the inner meniscus showed a considerable increase following GAG depletion. There was an increase in Young's modulus and ultimate tensile stress. Their findings demonstrate that GAGs enhance tissue resistance to compressive stresses in middle and inner meniscus, where GAGs are most numerous while increasing tissue viscosity in outer meniscus. The presence of GAGs in inner meniscus also contributes to a reduction in circumferential tensile properties of tissue, possibly as a result of pre-stress on collagen network caused by the ECM's increased hydration.[65]

In a 2015 study, Levillain et al. looked at initial biomechanical changes of the menisci during the onset of osteoarthritis (OA) and related them to chemical composition and matrix modification. Collagen fibre architecture and pathological changes in glycosaminoglycan (GAG) content have drawn the most interest. The menisci (n = 24) were removed 6 weeks after anterior cruciate ligament transection surgery (ACLT) on rabbit knee joints. The anterior and posterior regions of medial and lateral menisci were separately identified using indentation tests, Raman microspectroscopy, bi-photonic confocal microscopy, and histology. They observed mechanical and matrix alterations in medial and lateral menisci. In operated menisci compared to opposite menisci, microstructural investigations revealed less compact and organized collagen bundles, as well as a reduction of fiber tension. Mechanical properties significantly decreased in OA menisci. In OA menisci, particularly in damaged regions, GAG content was higher. Eventually, it was determined that ACLT caused the collagen framework to become disorganized at the initial stages of the development of OA, which reduced the menisci's mechanical resistance. This degradation results in an increase in GAG content.[66]

Cook et al. (2018) conducted a study to ascertain the possible mechanisms of action for meniscal destruction in reaction to joint discomfort and the potential functions of the meniscus in the initial stages and progression of osteoarthritis. Nitric oxide, matrix metalloproteinase generation and action, and related cytokine production were expected to increase significantly after interleukin-1 (IL-1) stimulation of meniscal explants in their investigation. Canine meniscal explants (4 mm) were cultured for 21 days with (IL-1) or without (negative control (n = 6/group) and analyzed for MMP activity, ADAMTS-4 activity, MMP-1, MMP-2, MMP-3, MMP-9, MMP-13, NO, prostaglandin E2 (PGE2), IL-6, IL-8, monocyte chemotactic protein-1 (MCP-1), and keratinocyte-derived chemokine (KC) concentrations. When compared to the NC group, the GAG content was shown to be considerably lower in the IL-1 group. In conclusion, proinflammatory mediators appear to directly affect the degenerative processes of the meniscus, which in turn contribute to the beginning and progression of OA.[67]

In a 2017 investigation, Levillain et al. evaluated the impact of early osteoarthritis (OA) on the viscoelastic characteristics of rabbit menisci and connected the mechanical changes to the microstructural abnormalities. In their investigation, the anterior cruciate ligament was severed in six male rabbits with right knee joints. Six normal rabbits served as controls. Menisci were removed and macroscopically examined 6 weeks following ACLT. The anterior and posterior parts of medial menisci underwent indentation-relaxation testing. By using biphotonic confocal microscopy and histology to analyse the collagen fibre structure and glycosaminoglycan (GAG) content, it was shown that OA brought on by ACLT has a significant impact on the surface integrity of rabbit menisci on both macroscopic and microscopic level. Reduced GAG content in the anterior area changed how the

circumferential collagen bundles slipped past one another. This alteration to the microstructure resulted in a significant reduction in the OA menisci's stiffness and a loss of elasticity in the posterior region. They came to the conclusion that early on in the formation of OA, all of these mechanical and microstructural alterations took place.[68]

Otsuki S. et al. (2010) undertook an experiment to determine how Sulfs influence articular cartilage homeostasis and regulate bone morphogenetic protein (BMP)/Smad and fibroblast growth factor (FGF)/Erk signaling. In order to compare spontaneous cartilage degeneration in Sulf-1/ and Sulf-2/ mice on C57BL/6 to wild-type mice, safranin O staining was utilized in this investigation. They discovered that Sulf-1 and Sulf-2 mice developed far more significant spontaneous cartilage deterioration and surgically induced OA than wild-type mice. Col2a1 and aggrecan were decreased whereas MMP-13, ADAMTS-5, and the BMP antagonist noggin were increased in chondrocytes and cartilage from Sulf-1/Sulf-2 mice. Sulf-1/Sulf-2 mice had lower Smad1 protein expression and Smad1/5 phosphorylation, but enhanced phosphorylation of Erk1/2 in articular cartilage and cultured chondrocytes. Sulfs siRNA decreased Smad phosphorylation in human chondrocytes but increased FGF-2-induced Erk1/2 signalling. They concluded that Sulfs could preserve cartilage homeostasis by simultaneously enhancing BMP and inhibiting FGF signalling in chondrocytes. In OA, methods to correct aberrant Sulf expression may help to prevent cartilage deterioration and encourage cartilage healing.[69]

Stone AV et al. (2015) proposed the theory that degenerative menisci from older adult vervet monkeys would secrete matrix metalloproteinases and pro-inflammatory cytokines that contribute to the development of osteoarthritis and that

these animals would exhibit knee osteoarthritic changes in their critique of the study. They examined healthy young adult (9–12 years) and old adult (19–26 years) female vervet monkey knees using computed tomography (CT) imaging, and joint tissues were morphologically graded at necropsy. In order to assess meniscal MMP and cytokine release, meniscus explants were subsequently cultured. Meniscus explants were then grown to evaluate the release of cytokines and MMP from the menisci. They observed that significant bone osteoarthritic changes, including an increase in the frequency of osteophytes and meniscal calcification, were visible in 80% of the older monkeys on CT scans. Meniscus and cartilage degradation scores were greater and more strongly correlated in older animals. Older animals with osteoarthritic defects generated significantly more MMP-1, MMP-3, and MMP-8 compared to young monkey menisci which were in normal condition. While older, osteoarthritic menisci released more IL-7 and granulocyte-macrophage colony-stimulating factor than younger, healthy menisci, older menisci without significant osteoarthritic changes secreted more of these substances. Finally, the conclusion was drawn that knee osteoarthritis, including meniscus involvement, develops naturally in old vervets. Matrix-degrading enzyme secretion and inflammatory cytokine production were significantly elevated in degenerative menisci. These elements might influence the meniscus tissue and other joint tissues, ultimately promoting the onset of osteoarthritis.[70]

Humans study

In 2012, Sun Y et al. reviewed the study to examine collagen and proteoglycan modifications in the menisci of individuals with osteoarthritis. Meniscal samples were obtained from 8 consecutive unselected OA patients who received complete joint

replacement surgery and 3 osteosarcoma patients who underwent lower limb amputation surgery. These tissues were surgical trash, after all. Proteoglycans were examined using the safranin-O and alcian blue staining methods. They observed that the OA meniscus was stained with proteoglycan more significantly in the intermediate and deep regions than the meniscus in the normal control group.[49]

In a research conducted by Herwig JU et al. in 1984, the water, collagen, and glycosaminoglycan levels of menisci from human knee joints in different stages of degeneration were studied. The researchers discovered that typical menisci comprised 72% water, 12% Deoxyribonucleic acid, 22% collagen, and 8% glycosaminoglycans, with a distribution of these glycosaminoglycans being as follows: 40% chondroitin 6-sulfate, 10-20% chondroitin 4-sulfate, 20-30% dermatan sulfate, and 15% keratan sulfate. Water content increased as degeneration progressed, but collagen and glycosaminoglycan contents decreased as chondroitin 6-sulfate levels rose.[4]

In their investigation, Lindahl U and Hook M from 1978 polysaccharides called glycosaminoglycans are typically present in covalent interaction with proteins in mammalian tissues. There are seven varieties that are well known; six of them share a structural similarity in that their carbohydrate backbones alternate between uronic acid and hexosamine remains. Hyaluronate is a lone sulfate-containing compound. These polysaccharide types can be identified by the composition of their monomers, their sulfate substituents' number and placement, as well as the position and shape of their glycosidic links, It was also noted that glycosaminoglycans and proteoglycans give cartilage its compressive stiffness by retaining water in the tissue. They interact with collagen, affecting it’s in vivo characteristics, and they regulate the solutes' ability to penetrate the cartilage.[71]

McAlinden et al. (2001) examined the study to determine whether the individual's age has an effect on the biosynthesis of aggrecan and decorin in human meniscus and capacity of the cells to produce these mRNA, and if so, whether those modifications differ from those observed in articular cartilage removed from the same joint. Radiolabeling tissue explants, anion-exchange chromatography, and agarose-polyacrylamide electrophoresis gels were used to analyze the newly generated proteoglycans. They found that young donors had increased proteoglycan production in the meniscus compared to adult tissues. During this period, decorin dominated proteoglycan synthesis. Age-related increases in the meniscus's percentage of aggrecan synthesis were also seen. However, levels of decorin mRNA expression in articular cartilage were mostly unchanged with aging, but levels of aggrecan mRNA expression in the meniscus rose. As a result, investigators came to the conclusion that human meniscal aggrecan and decorin production and turnover differ from those of articular cartilage and are affected by an individual's age.[72]

According to Mcdevitt and Webber (1990), sticky glycoproteins are a matrix glycoproteins subgroup. These macromolecules either possess the capacity to attach to various matrix macromolecules or cell surfaces, or they possess amino acid sequences or protein domains that suggest the likelihood of interactions. These molecules operate as crucial adhesion molecules, allowing cells to adhere to or move through their extracellular matrix. The meniscus contains three of these adhesions, or putative adhesion molecules: Type VI collagen, Fibronectin, and Thrombospondin. The fibro chondrocytes of the meniscus appear to have a large potential for reacting to growth and different modifying factors during the tissue's repair or reproduction.[73]

Ingman et al. (1974) investigated the chemical makeup of 50 menisci from knee joints belonging to individuals ranging in age from 0-86. 12 of them displayed degenerative regions that underwent individual analysis. They discovered that 77.9 % of collagen, 8.1 % of non-collagenous proteins, and 1.0 % of hexosamine were present in normal adult tissues. Collagen levels rise with age, while NCP levels fall. Degenerate regions displayed a statistically significant reduction in collagen and a rise in NCP and hexosamine when compared to normal menisci of similar age. It was determined that collagen content rose from birth to age 30 and was steady until age 80, at which point a drop was seen. Non-collagenous protein in neonate meniscus made up nearly 22% of its dry weight. This decreased to around 8.0% between the ages of 30 and 70, at which point it dramatically rose to about 11.6%. Hexosamine levels declined from infancy through adulthood but were slightly elevated after age 70.[74]

McNicol and Roughley (1980) conducted a study that included (1) removing proteoglycan under dissociative circumstances from the human meniscus, (2) examining how this proteoglycan changes in abundance and structure as it ages, and (3) contrasting their findings with those for the proteoglycan found in the articular cartilage of humans. Despite the significantly decreased tissue amounts, they found that proteoglycan molecules with cartilage-like size and glycosaminoglycan content were present. In addition, regarding the presence of keratan sulfate and the sulphation of chondroitin sulphate chains, both tissues showed age-related alterations. The meniscus proteoglycan preparation also contained dermatan sulfate, and the core proteins displayed some variations. Finally, it was shown that this type of proteoglycan structure would be consistent with its role in the meniscus' flexibility, especially considering that the material is constrained to a specific area.[75]

In comparative research conducted by Ghosh et al. in 1975, the contents of the menisci nitrogen, collagen, non-collagenous proteins, and hexamine levels in osteoarthritic and rheumatoid knee joints were analyzed. Separate regions that have degenerated were examined. NCP and hexosamine levels were greater and collagen levels were lower in the degenerative regions than in the control tissue. The rheumatoid menisci displayed reduced collagen and hexosamine levels in areas of localized degradation. However, in contrast to the same-age normal tissue, the residual tissue similarly contained less collagen and hexosamine. Eventually, they came to the conclusion that in osteoarthritic menisci compared to controls, collagen content decreased, but proteoglycan and matrix glycoprotein concentration increased. However, there was a decrease in the collagen and proteoglycan concentrations in rheumatoid arthritis menisci.[76]

According to Jerosch J. (2011), chondroprotective nutrients like glucosamine and chondroitin sulfate, as well as other foods like antioxidants and PUFAs, can modify osteoarthritis. A balance between catabolic and anabolic processes was corrected in osteoarthritis patients by the chondroprotective, an essential component of cartilage metabolism and a stimulant of vital cartilage regeneration processes. In a rat animal model, the study showed promising effects of additional dietary components or phytochemicals, including those found in ginger extracts, demonstrating a variety of anti-osteoarthritic actions and even intra-articular displaying chondroprotective effects. A future 'nutraceutical' approach to OA should include glucosamine sulfate, hyaluronic acid, collagen hydrolysate, and multiple other nutrients that have been shown to have positive effects on joint cartilage, synovial fluid, and overall clinical outcome in OA patients.[77]

Williams A. et al. (2004) used the delayed gadolinium-enhanced MRI of cartilage approach to describe a variety of in vivo studies of glycosaminoglycan distribution in knee cartilage. 23 subjects were scanned for this investigation utilizing the dGEMRIC method. They noticed that the index of glycosaminoglycan distribution, T1Gd, might be less than 300 msec, with focal areas as low as 240 msec, or it could reach 500 msec (indicating high glycosaminoglycan). Patients with persistent osteoarthritis or damage to the knee's ligaments and menisci showed compartmental differences as well as specific deficiencies inside the knee.[78]

Using delayed Gadolinium-Enhanced Magnetic Resonance Imaging of Cartilage, Ericsson et al. (2009) examined the relationship between meniscectomy patients' epidemiologic risk factors for knee osteoarthritis (OA) and cartilage integrity. The BMI of 45 patients (16 female) with an average age of 46 years who underwent an arthroscopic medial meniscectomy 1-6 years ago was assessed in this research. The glycosaminoglycan (GAG) content of the cartilage was assessed using the dGEMRIC Index, and tests of isokinetic strength and functionality were also conducted. The BMI ranged from 20.0 to 34.3. The medial index compartment showed a 14.4% lower dGEMRIC Index compared to the lateral reference compartment (437+/-59 ms, mean+/-SD). Knee flexor and knee extensor strength in relation to body weight, the one-leg hop test, and the dGEMRIC Index of the medial diseased compartment all showed favorable relationships. Additionally, a negative correlation between the medial compartment dGEMRIC Index and BMI was found. Following medial meniscectomy, the lower dGEMRIC Index of the medial compartment displays reduced cartilage GAG content, indicating an early-stage OA. There were no discernible associations in the lateral reference compartment. Additionally, the results

suggest that obesity contributes to cartilage deterioration, whereas strong, coordinated thigh muscles may have a preventive impact on cartilage integrity.[79]

In a 2005 research, Tiderius et al. investigated the glycosaminoglycan (GAG) content of cartilage and synovial fluid in individuals with acute anterior cruciate ligament (ACL) injuries to determine whether there was any association between the two. They used delayed gadolinium-enhanced magnetic resonance imaging of the cartilage and 24 healthy volunteers to study 24 patients with an average age of 27 years, 14 of whom were male. The patients' synovial fluid was removed soon before the MRI, and GAG was evaluated by dye precipitation with Alcian blue. The lateral femoral condyle in 15 of the 24 patients had a single bone bruise, and the cartilage T1 (Gd) there was shorter than it was in the controls, indicating a lower GAG concentration. The T1 (Gd) was similarly decreased in the medial femoral cartilage, despite the absence of significant bone contusion. The mean +/- SD synovial fluid GAG concentration in patients was 157 +/- 86 mg/ml (Gd), and the T1 was favourably linked with this value. They concluded by adding that cartilage quality should be considered when interpreting cartilage indications of metabolism since cartilage with a high GAG content releases more GAG into the synovial fluid.[80]

Abraham et al. (2014) did a study to find mechanical and structural changes in meniscal materials after the onset of OA. In this work, the histo-morphological features, mineralization, and mechanical properties of meniscal materials from osteoarthritic and healthy individuals were compared. Researchers discovered that the calcium content and anterior meniscal materials with osteoarthritis had significantly thicker GAG in calcified fibrocartilage (CFC) zone. Tidemark integrity was significantly diminished in OA tissue in the medial anterior enthesis. The mineralized

area of osteoarthritic meniscal materials was thicker than normal materials and showed reduced bone mineral density. Osteoarthritic tissue exhibited higher compliance, according to an analysis of viscoelastic mechanical characteristics. Lastly, it was found that the development of OA causes considerable alterations at meniscal materials locations.[81]

Mine T. et al. (2013) examined variations in acid mucopolysaccharides and collagen expression throughout meniscal degeneration, tearing, and recovery using menisci extracted during knee joint operations. In this research, the menisci from 23 patients (15-80) years old who underwent meniscal surgery for flap and bucket handle cuts (n=11) and total knee arthroplasty for osteoarthritis (n=12) were analyzed histologically. Acutely injured menisci and menisci with and without degeneration (from people with osteoarthritis) were compared for acid mucopolysaccharides and collagen types I, II, and III expressions. They observed that acid mucopolysaccharides, collagen types I and II were expressed throughout the whole meniscus with the exception of the circulation area in menisci without degeneration. At outer marginal border and on surface, collagen type III was highly expressed. As meniscal degeneration progressed, acid mucopolysaccharides were expressed more frequently while collagen types I, II, and III were expressed less frequently. Collagen types II and III were the first to disappear in acutely wounded menisci, tracked by collagen type I, which led to a cessation of fiber formation. Finally, meniscal function was preserved and acid mucopolysaccharides and collagen types I, II, and III were in a balanced state in normal menisci.[82]

Liang Y. et al. (2017) looked into the impact of oxygen tension and mitotic divisions on adult human meniscus fibro chondrocytes (MFCs) plasticity. Their

evaluation process comprised biochemical, histological, and immunofluorescence studies in addition to gene expression. TGF-1 and FGF-2 coupled (T1F2) were used to develop MFCs in monolayer culture while maintaining normoxia (21% O2). The differentiation of the trilineage (adipogenesis, chondrogenesis, and osteogenesis) was carried out in both normoxic (21% O2) and hypoxic (3% O2) environments. They discovered that MFCs can go through adipogenesis and chondrogenesis with a mean total population doubling of 10. Under hypoxic conditions, this skill was improved. Osteogenesis did not occur in the MFCs. The ability of significantly expanded human MFCs to create tissues with avascular meniscus-like functional matrix properties was finally determined.[83]

Shrikanth CB. et al. (2018) revised research to evaluate the possibility of recovering isolated sulfated GAGs (sGAG) determined by metachromasia for more investigation. In this study, the sGAG-DMMB complex developed following the quantification of sGAG by DMMB dye-binding test and recovery of sGAGs. After being fluorescently labeled, prior to being used for HPLC analysis to determine their disaccharide composition, recovered sGAGs underwent cellulose acetate membrane electrophoresis examination. All samples, ranging in purity from low to high, indicated that sGAGs had recovered well after metachromasia. In a second investigation utilising cellulose acetate membrane electrophoresis, the two types of sGAGs, chondroitin/dermatan sulphate and heparan sulphate, were successfully separated, with hyaluronic acid, a non-sulfated GAG, interfering with the separation just slightly. Heparan sulfate, an improved sGAG, had a distinctive disaccharide composition, according to an HPLC analysis.[84]

1.3 Justification

Severe musculoskeletal damage from meniscal injuries is well established. Meniscal treatment and repair are challenging for patients, surgeons, and physical therapists because of their unique and complex architecture. Long-term injuries can also cause degenerative joint changes such as osteophytes, articular cartilage loss, reduction of joint spaces, and symptomatic osteoarthritis.[1, 85 – 87]

Osteoarthritis (OA) is a degenerative joint disease that affects the cartilage and several surrounding tissues. Even though the condition usually progresses gradually, it can eventually cause joint failure, pain, and disability. The group is often most affected by OA of the knees because discomfort and stiffness in large weight-bearing joints usually cause significant impairment and call for surgical intervention. It is difficult to define symptoms and radiographic changes for research purposes since they are not well connected.[88, 89]

There is currently no treatment for OA. Nonpharmacological, pharmacological, and surgical treatments can all be used to control OA. Surgery is typically only used if medical treatment has failed and functional incapacity has a negative impression on a patient's value of life.[5, 90 – 92]

However, as OA progresses, the collagen matrix becomes more disordered, and the content of mucopolysaccharides in the cartilage changes. Water content rises overall as a result of collagen fiber breakdown. This rise is brought on by a general decline in mucopolysaccharides (and thus a decreased osmotic pull). In comparison, collagen loss outweighs it. The collagen fibers in the cartilage are susceptible to

deterioration without the mucopolysaccharides' protective properties, which can worsen degeneration.[84, 93 – 95]

The matrix has an essential role in maintaining softness and serving as a shock absorber. Age-related fibrocartilage alterations play an important role in health and disease. Understanding pathophysiological changes in normal and aging adults as well as OA requires recent discoveries in glycobiology and the classification of mucopolysaccharides.

Regarding the classification of mucopolysaccharides in health and disease, very little information is available. This research may be useful for understanding OA and its relationship to molecular changes in histochemistry. This work may contribute to the understanding of how carbohydrate moieties and their alteration and age-related changes may help in the prevention and design of a futuristic plan to reverse the process of degenerative changes. Understanding the stages of the disease and morpho-chemically categorizing it may be helpful. For the creation of novel, structure-modifying drugs for the treatment of osteoarthritis and for a deeper comprehension of the disease process, this study will be essential.

Additionally, this research will aid in understanding the microscopic configuration of cells, collagen fibres, and glycosaminoglycan alterations in healthy and osteoarthritic menisci.

1.4 Objectives of the study

1. To study the histochemistry of mucopolysaccharides in osteoarthritic menisci of a human knee joint.
2. To study the microscopic structure of menisci in osteoarthritic patients.
3. To compare the age-related changes in histological structure and mucopolysaccharide changes in osteoarthritic menisci.

2. MATERIALS AND METHODS

2.1 *Source of data:*

The study was carried out in the Department of Anatomy, J. N. Medical College, KLE Academy of Higher Education & Research, Belagavi. Menisci samples were obtained from osteoarthritis (OA) patients who had undergone total knee replacement (TKR) or lower limb amputation surgery from the Orthopedics Department unit of KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi, Karnataka, India.

2.1.1 *Study design:*

Case-series study

2.1.2 *Study population:*

From 110 human knee joints of both sexes (male and female), medial and lateral osteoarthritic menisci were collected. 65 females and 45 males, ages 50 to 84 years.

Normal meniscal tissue from 8-month-old male domesticated ruminant sheep was used as a control and showed no signs of knee-related musculoskeletal disease. It was obtained from a commercial source at Nehru nagar in Belagavi and dissected after 4 hours of defeat.

2.1.3 Sample size:

The following formula was used to determine the sample size;

$$n = \frac{Z^2_{1-\propto/2} \times SD^2}{(0.2 \times SD)^2} \times 1.1 = 110$$

Where,

$Z^2_{1-\propto/2}$ = 1.96 with 95% C.I.

SD = Standard deviation

d = 20% of estimate = (0.2 x SD)

Sample size at 95% CI, 20% tolerable error, and 10% lost to data entry/data collection or outliers

2.1.4 Inclusion and exclusion criteria:

Inclusion criteria:

Menisci were collected from patients who were diagnosed with osteoarthritis and underwent total knee replacement surgery (TKR), and who had undergone lower limb amputation surgery.

Exclusion criteria:

Patients were disqualified if they had meniscal cancer, torn menisci, or other meniscal injuries.

2.1.5 Administrative and ethical considerations:

The institutional ethics committee's prior consent (ref. no. KLEU/EC/17-18/D-97, dated 16/5/2017) was obtained. Before starting the process of collecting the data, the participants provided their informed, written consent. They were informed of their

right to freedom of choice and the privacy and confidentiality of their information was preserved.

2.1.6 Tools and techniques for data collection:

Permission was taken from the Head of the Department of concerned Orthopedics units, the Principal of J.N. Medical College, the medical director, the chief executive officer (CEO), and the superintendent of KLES Dr. Prabhakar kore hospital and medical research centre, belagavi, joint replacement unit and In charge of the Hi-tech operation theater. After getting the approval letter from ethical committee, the study commenced.

The collection of meniscal tissue samples was started on 01st October 2017 and continued until reached the sample size i.e. 110, which was completed on 15th February 2020. Meniscal tissue specimens were collected immediately after the total knee joint replacement surgery of osteoarthritis patients from the surgery unit.

Subsequently, the medial or lateral meniscal tissue was identified. Then menisci were washed thoroughly to remove the blood and fat deposits. Then along with identification labels tissue was transferred into sterilized plastic containers containing 10% buffered formalin for fixation. Then samples were brought to the Department of Anatomy in the histology research laboratory for further tissue processing.

2.1.7 Collection of history of patients:

History was collected after a patient was shifted to the ward on pre-designed proforma regarding socio-demographics, food habits, height, weight, and lifestyle.

2.1.8 Materials required for data collection and sample processing:

Slides labeling stickers, coverslips, glass slides, small and large blades, forceps, ruler, fluid – resistant dissection surface pad, cassettes of varying sizes and colors, specimen collections containers, hand gloves, microtomes (rotary microtome), microtome knife or blades, forceps with fine, curved points, scalpel, slide rack, teasing needle, ice tray, chemical-resistant pencil or pen, sable or camel hair brush, binocular microscope with camera, ocular micrometer (graticule scale), stage micrometer, fixative reagent, dehydrating reagent, clearing reagent, paraffin wax, staining reagents.

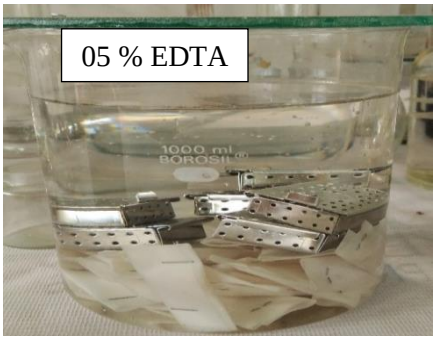
2.1.9 Sample process: [96]

Tissue processing and block preparation:

Meniscal samples were stored in 10% formalin for 3 – 4 days. After that menisci were cut in a standardized way. For each meniscus, three separate parts (anterior, middle, and posterior) were processed. The menisci were sectioned in two places vertically at 45º and 135º angles relative to the sagittal plane. After that, each part was sectioned along the horizontal plane from the inner border to the outer border. Then tissue of medial and lateral meniscal samples was processed as follows:-

1. Tissue was kept in 05 % EDTA solution for 2 – 4 days for decalcification.
2. Then the tissue was washed for 2 hours in running tap water.
3. **Dehydration** of the tissue was done by following concentration of alcohol:-
    - In 70 % alcohol kept overnight.
    - In 80 % alcohol kept for 3 hours.
    - In 90 % alcohol kept for 3 hours.
    - In absolute alcohol 3 changes each of 1 hour.

4. **Clearing** of the tissues was done with:
    - In acetone for 1 hour.
    - In xylene 2 changes each of 30 minutes.
5. **Infiltration** was done with liquid paraffin wax for 2 – 3 hours with 60-65 ºC
6. **Embedding:** The tissues were embedded in a paraffin block
7. **Prepared the tissues block**

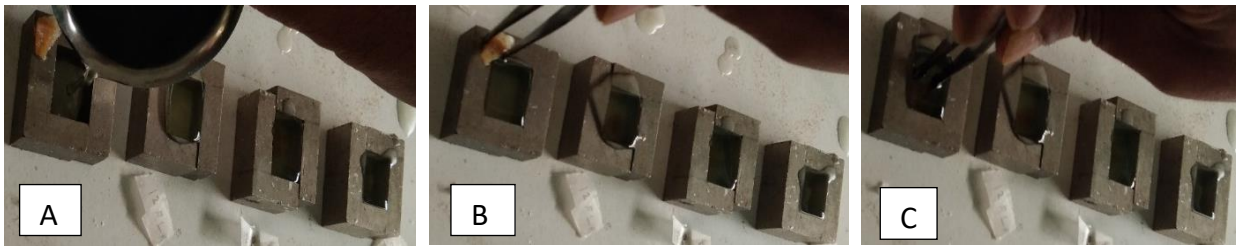


05 % EDTA solution

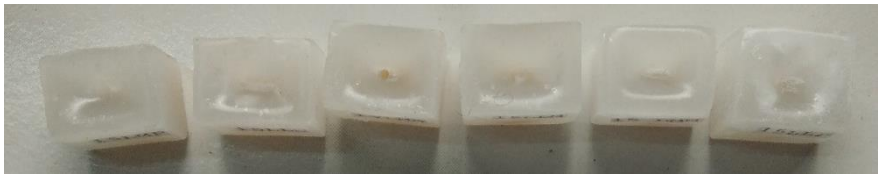
Dehydrating process in different graded Alcohol

Tissue clearing process

Infiltration process in liquid paraffin wax bath



The tissues embedded in L blocks with paraffin wax A, B, and C



Prepared Paraffin tissue blocks

Figure 2: tissue processes and preparation of paraffin blocks

8. **Sectioning and fixing the tissue on slides**

Tissue blocks were prepared from 3 different regions (anterior, middle, and posterior) of medial and lateral menisci and processed as follows.

- Tissue block was taken and fixed at the tissue holder with the help of a heating process.
- Tissue holder was fixed at the rotary microtome and adjusted the microtome blade at the proper position.
- The microtome was fixed at 5µm thickness for section cutting.
- Sections were cut with the help of a rotary hand wheel.
- After that sections were kept in the watch glass contained with absolute alcohol then the sections were transferred to a 60 ºC hot water bath.
- Then tissue section was fixed onto the egg albumin-coated glass slide.
- Slide was dried with the help of a slide warmer.

9. Staining and mounting

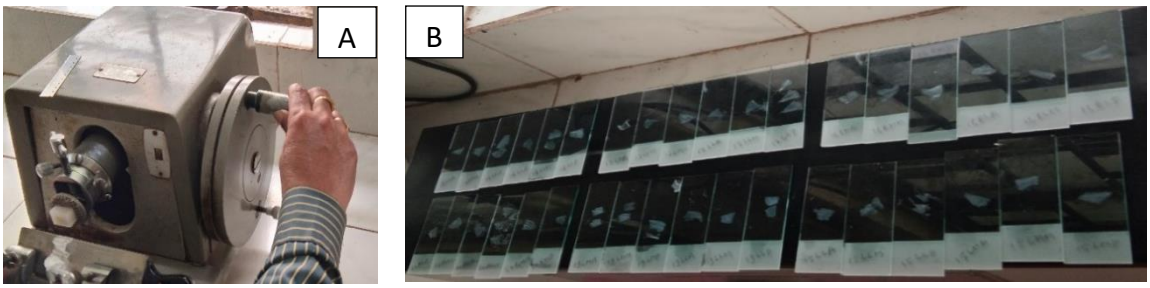


Figure 3: Microtome with tissue block (A) and tissue sections fixed at glass slide (B)

2.1.10 Different histological and histochemical techniques: [96]

Tissue sections were stained with these 10 histochemical stains:

1. Haematoxylin and Eosin (H & E)
2. Alcian blue pH 1.0 (AB – 1)
3. Alcian blue pH 2.5 (AB – 2.5)
4. Periodic acid-Schiff (PAS)
5. AB2.5 +PAS (combine technique)
6. Aldehyde fuchsin (AF)
7. AF + AB2.5 (combine technique)
8. Orcein + AB2.5
9. Mucin carmine
10. Hyaluronidase enzyme labile technique

Methods followed for different histological techniques:

Method for preparation of stain

1. **Haematoxylin and eosin (H & E): [96]**

Haematoxylin - 2.5 gms

Absolute alcohol - 25 ml

Potassium alum – 50 gms

Distilled water -500 ml

Mercuric oxide - 1.25 gms

Glacial acetic acid – 20 ml

Alum was dissolved in warm distilled water, and hematoxylin was added after it had been completely dissolved in pure alcohol. The mercuric oxide was added after the liquid was quickly heated to a boil. The flask was then quickly submerged in ice

water to quickly cool the stain. The acetic acid was added when the solution had cooled, and the stain was then ready for use.

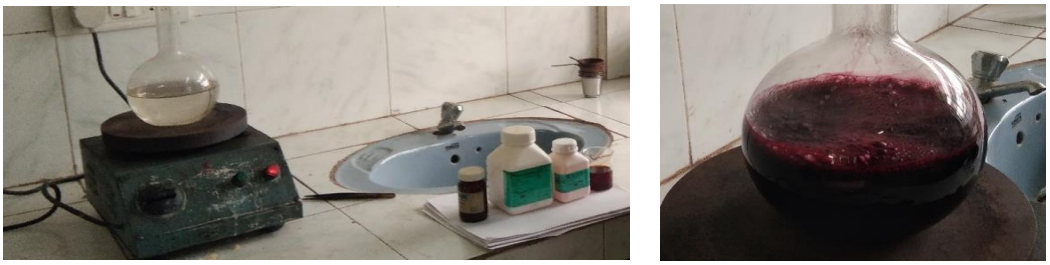


Figure 4: Preparation of H&E stain

Steps of staining

1. The tissue-fixed slides were put onto the slide warmer for 5 minutes at 65 ºC after that they were deparaffinized two times with xylene for 5 to 10 minutes each.
2. The deparaffinized tissue fixed slides were then kept in descending graded alcohol like in 100%, 95%, 70%, and 50% at 5 minutes respectively for rehydration.
3. Next, they were washed gently in the tap water for 5 minutes.
4. After that they were stained with Harris's hematoxylin – for 1 minute.
5. Next, the slides were washed well in running tap water until sections appear 'blue'- for 5 minutes or less.
6. They were then stained with 1% eosin Y- for 30 seconds.
7. Next, they were washed in running tap water for 2-3 dips.
8. After that the slides were dehydrated through alcohols – 1 dip.
9. Next, they were cleared with xylene
10. Finally, they were mounted with DPX.

Figure 5: Processing for deparaffinization, stain, and mount of H&E stain

Methods for interpretation of H and E stained slides:

Each part of medial and lateral menisci was stained with H &E to assess surface integrity, cellularity, fibrous organization, and collagen alignment. Alcian blue pH 2.5 stain was applied for evaluation of mucoid degeneration.

Development of histological grading/scoring system

After examining the slides of patients with OA of various ages, the grading system described in this study was created. A total number of 660 slides were observed and graded. The criteria included (i) characteristics of the tissue surface (smoothness or degree of fibrillation, indentations, and undulations); (ii) cellularity patterns (normal, hypercellular, hypocellular, and acellular); (iii) organization of the collagen matrix and fibers including hyalinization, cyst formation, chipping, and tears; (iv) Intensity of Alcian blue pH 2.5 staining for mucoid degeneration. A total score was determined after each category was evaluated. Grade 1 is normal tissue, with scores ranging from 0 to 3, Grade 2 is mild degeneration, with scores ranging from 4 to 6, Grade 3 is substantial degeneration, with scores ranging from 7-9, and Grade 4 is severe degeneration, with scores ranging from 10 to 12 (Table X)

The "C. Pauli" microscopic grading method was used to determine the degeneration grade for the histological examination of meniscus specimens. The four grades G1 = 0 - 3, G2 = 4 - 6, G3 = 7 - 9, and G4 = 10 - 12 were created from the expected total score range of 0 to 12 (Table X). [52]

- G1 – represents – Normal
- G2 – represents – Mild degeneration
- G3 – represents – Moderate degeneration
- G4 – represents – Severe degeneration

Table X: Histological scoring/grading system

1. Surface	**Score**
▪ Smooth or linear	0
▪ Slight abrasion or cell cluster	1
▪ Moderate abrasion or markedly undulation	2
▪ Severe abrasion or disruption	3
2. Cellularity	
▪ Normal	0
▪ Diffuse hypercellularity	1
▪ Diffuse hypo/acellular regions	2
▪ Hypo cellular (lacuna, pycnotic cells)	3
3. Collagen fiber organization	
▪ Collagen fibers organized, homogenous of extracellular matrix.	0
▪ Collagen fibers organized, diffuse foci of hyalinization.	1
▪ Collagen fibers unorganized, confluent foci or band of hyalinization.	2
▪ Collagen fibers unorganized, fibrocartilaginous separation and tears.	3
4. Mucoid degeneration (Alcian blue ph.2.5)	
▪ None	0
▪ Slight	1
▪ Moderate	2
▪ Severe	3

Note: Criteria and scores for histological assessment of menisci developed by C. Pauli (2011), have been used to evaluate: 1) Surface, 2) Cellularity, cellular morphology, 3) Collagen fiber organization 4) mucoid degeneration.

2. Alcian blue pH 1.0 (AB-1) stain :[96]

Alcian blue is a positively charged cationic dye that interacts electrostatically with certain polyanion tissues that include carboxyl or sulfate groups. To separate and distinguish the various acid mucins, it was advantageous to employ an alcian blue solution with a range of pH. Esters react to sulfate in general at a lower pH than carboxylated.

Technique for AB pH 1.0

0.1M hydrochloric acid (pH 1.0) with 1gm of alcian blue in 100 ml

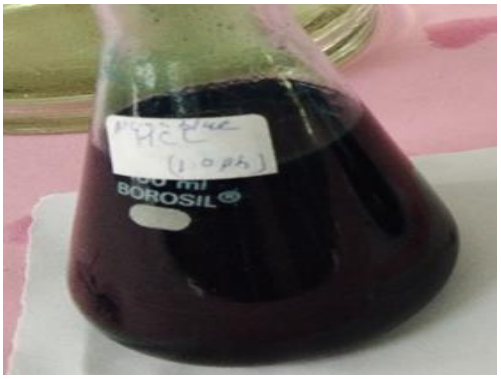


Figure 6: pH 1.0 Alcian blue stain

Steps of staining:

1. The tissue-fixed slides were put onto the slide warmer for 5 minutes at 65 ºC after which they were deparaffinized (De-wax) 2 times with xylene for 5 to 10 minutes each.
2. The deparaffinized tissue fixed slides were then kept in descending graded alcohol like in 100%, 95%, 70%, and 50% at 5 minutes respectively for rehydration.
3. Then they were washed gently in tap water – for 5 minutes.

4. Next, they were stained with Alcian blue pH -1 for 5 -7 minutes.
5. After that they were washed in water.
6. Next, the slides were dehydrated through alcohols – 1 dip.
7. The slides were cleared with xylene
8. Next, they were mounted with DPX.

Results: AB- pH -1- Sulfated acid mucins are stained blue.

3. Alcian blue pH 2.5 (AB-2.5) [96]

Technique for AB-2.5

3% acetic acid in 100 ml with 1gm of Alcian blue (pH 2.5)

Figure 7: pH 2.5 Alcian blue stain

Steps of staining:

1. The tissue-fixed slides were put onto the slide warmer for 5 minutes at 65 ºC after that deparaffinized (De-wax) 2 times with xylene for 5 to 10 minutes each.
2. The deparaffinized tissue fixed slides were then kept in descending graded alcohol like in 100%, 95%, 70%, and 50% at 5 minutes respectively for rehydration.

3. Soon, after that the slide was washed gently in the tap water – for 5 minutes.
4. After that the slides were stained with pH -2.5 Alcian blue for 7 -10 minutes.
5. Soon, they were washed in water.
6. They were then dehydrated through alcohols – 1 dip.
7. After that the slides were cleared with xylene
8. Finally, they were mounted with DPX.

Results: - AB-pH 2.5- Acid mucins are stained blue.

4. Periodic acid Schiff technique (PAS):[96]

The PAS reaction is a useful indicator of the presence of tissue carbohydrates, and particularly so for glycogen when the technique incorporates a diastase digestion stage. The principle of the reaction is that, periodic acid causes the carbon bond in 1, 2, glycols or their amino or alkylamino derivatives to oxidative cleave, resulting in dialdehydes. The basic pararosaniline and the aldehyde will combine to generate a magenta-colored molecule after the reaction with the fuchsin sulfuric acid. The ideal oxidant for the reaction is periodic acid because it prevents the produced aldehydes from being gradually over-oxidized to carboxylic acid, which would cause a weak schiff reaction.

PAS technique:-

Periodic acid solution

Periodic acid - 1gm

Distilled water - 200 ml

Schiff's chemical

Dissolve 1 gm basic fuchsin in 200 ml of boiling distilled water, and the water flask was taken out of the Bunsen burner shortly before the basic fuchsin was added. The solution was allowed to cool to 50 °C before adding 2 gm of potassium metabisulfite and stirred continuously. After allowing it to reach room temperature, 2 ml of strong hydrochloric acid was added and the mixture was stirred. Then 2 gms of activated charcoal was added and it was left overnight at room temperature. The mixture was then filtered through a No. 1 Whatman paper. The solution was characterized as either clear or light yellow. Finally, it was stored at 4°C in a dark container.

Steps of staining:

1. The sections were dewaxed and brought into distilled water for 2 – 3 min.
2. They were treated with periodic acid for 5 – 7 min.
3. Then they were washed with distilled water for 3 - 4 times, 1 – 2 min. each.
4. The sections were covered with Schiff's solution, for 15 min.
5. They were again washed in running tap water, for 2 – 3 min.
6. Then the sections were rinsed in absolute alcohol for 1 dip.
7. Next, they were cleared in xylene and mounted with D.P.X.

Results: Glycogen and other periodate-reactive carbohydrates are stained with magenta color.

5. Combined (Alcian blue pH 2.5 + PAS stain):[96]

This method, which is also valuable as a standard demonstration approach, allows for the distinct separation of acid mucins and neutral mucins. The idea is that by first staining all acid mucins with Alcian blue, those acid mucins that are also PAS-

positive will not respond in the second PAS test; only the neutral mucins will. This makes it possible to distinguish between acid and neutral moieties by color.

Following solutions were prepared:

1. Alcian blue solution of pH 2.5
2. 1% aqueous periodic acid.
3. Schiff's reagent

Steps of staining:

1. The sections were dewaxed and dipped into water for 4- 5 min.
2. They were treated with the Alcian blue solution for 5 -7 minutes.
3. Then they were washed well in distilled water.
4. After that they were treated with the periodic acid solution for 4 – 5 minutes
5. Next, they were washed in distilled water. Then they were treated with Schiff's reagent for 10 minutes.
6. Next, they were washed in running tap water for 10 minutes.
7. Soon after, they were dehydrated, cleared, and mounted with D.P.X.

Results:

1. Acid mucins are stained blue.
2. Neutral mucins are stained magenta.
3. Mixtures of neutral and acid mucins are stained purple.

The color will vary from blue-purple to purple depending on the dominating entity.

6. Aldehyde fuchsin Technique :[96]

Aldehyde Fuchsin Solution:

Basic fuchsin	- 1gm
Paraldehyde	- 2 ml
Concentrated HCL acid	- 1 ml
Ethanol	- 60 ml
Distilled water	- 40 ml

Basic fuchsin was dissolved in ethyl alcohol and distilled water. The HCL acid and paraldehyde were added. Then they were allowed to ripen at room temperature for 2 to 7 days, and then filtered and stored at 4 ºC.

Figure 8: Preparation of Aldehyde Fuschion (AF) stain

Steps of Staining:

1. The sections were dewaxed and dipped into water for 5 min.
2. Then sections were rinsed in 70 % alcohol
3. After that they were stained with aldehyde fuchsin solution for 20 minutes
4. Again, the sections were rinsed in 70 % alcohol and then in water for 2 – 3 min.
5. Soon after they were dehydrated, cleared, and mounted with D.P.X.

Results:

1. Sulfated mucins stain deep purple
2. Non-sulfated acid mucins stain purple, weakly to moderately.
3. Neutral mucins are unstained.

7. Combined aldehyde fuchsin –Alcian blue pH 2.5 stain:[96]

Using this method, sulfated and carboxylated mucins may be distinguished with reliability. The reasoning is based on aldehyde fuchsin's stronger affinity for sulfated mucins, which causes them to become purple when stained with this solution initially and only turn blue when counterstained with alcian blue.

Aldehyde fuchsin solution:

Basic fuchsin	- 1 gm
Paraldehyde	- 2 ml
Concentrated HCL acid	- 1 ml
Ethanol	- 60 ml
Distilled water	- 40 ml

Alcohol and distilled water were used to dissolve the basic fuchsin. The paraldehyde and HCL acid were added and allowed it 'to ripen' for 2 to 7 days at room temperature. Then it was filtered and stored at 4 °C.

Alcian blue pH 2.5 solution:

Figure 9: Combine AF + AB 2.5 stain

Steps of Staining:

1. The sections were dewaxed and dipped into water for 5 min.

2. Then sections were rinsed in 70 % alcohol.

3. After that they were stained with the aldehyde fuchsin solution for 20 minutes.

4. Again, the sections were rinsed in 70 % alcohol and then in water for 2-3 min.

5. After that, sections were stained with alcian blue solution for 7-10 minutes.

6. Soon after they were washed in water for 30 sec.

7. Next, they were dehydrated, cleared, and mounted with D.P.X.

Results:

1. Sulfated mucins were stained purple.
2. Carboxylated mucins were stained blue.

8. Combine orcein - AB 2.5 stains:[96]

Solutions preparation:

1. 0.25 % potassium permanganate in 0.25% concentrated sulphuric acid.
2. 2 % Oxalic acid
3. Orcein solution (1gm Orcein in 100 ml, 70% alcohol, and added 1ml concentrated HCL)
4. AB - 2.5
5. 1% acid alcohol

Stapes of staining:

1. The sections were dewaxed and kept under water for 5 min.
2. They were oxidized in a solution of 0.25% potassium permanganate and in 0.25% concentrated sulphuric acid for 1 minute.
3. The sections were decolorized in 2% oxalic acid and rinsed with water.
4. Then, the sections were stained with orcein solution for 4 hours.
5. Next, they were washed well in tap water and differentiated by acid alcohol for a few seconds.
6. The sections were then washed in water and stained with AB 2.5 for 7 minutes.
7. Finally, they were dehydrated, cleared and mounted with D.P.X.

Results: - Sulphomucins were stained brown.

Sialomucins were stained blue.

9. Mucin carmine:[96]

This technique is one of the histochemical methods for the visualization of mucins specimens.

Solution preparation:-

Carmine (alum lake)	- 1gm
Aluminum hydroxide	- 1 gm
50% ethanol	- 100 ml

500ml of Pyrex flask was filled with an aforementioned components and 50% ethanol. Then, 0.5 g of anhydrous aluminum chloride was added after thorough shaking. After that the solution was made to boil in the flask while it was submerged in a pot of boiling water bath for 2 to 3 minutes. It was stirred while the water was

boiling. Then it was cooled under running tap water. Finally, it was cooled in a flask and filtered and stored at 4°C.

Mucicarmine working solution:

Southgate's Mucicarmine stock solution	10 ml
Distilled water	90 ml

***Alcohol hematoxylin*:**

Hematoxylin	1gm
Ethanol	100 ml

Acidified ferric chloride stock solution:

Ferric chloride ($FeCl_3.6H_2O$)	2.48 gm
Distilled water	97 ml
Concentrated hydrochloric acid (HCL)	1 ml

Wiegert's iron hematoxylin working solution:

This solution should be mixed just before use.

Alcoholic hematoxylin	50 ml
Acidified ferric chloride stock solution	50 ml

Metanil yellow working solution:

Metanil yellow	0.25 gm
Distilled water	100 ml
Glacial acetic acid	0.25 ml

Mix and store in a brown bottle.

Method:

1. The sections were dewaxed and dipped into water for 5 min.
2. Then the sections were stained in Wiegert's iron hematoxylin working solution for 10 min.

3. After that, they were rinsed in running tap water for 5 min.
4. Then again, sections were stained in the mucicarmine working solution for 30 min.
5. After that the sections were rinsed in two changes of distilled water.
6. Again, they were stained in the metanil yellow solution for 30 – 60 sec.
7. Then sections were rinsed quickly in distilled water.
8. Soon after, the sections were dehydrated and cleared in xylene.
9. Then they were mounted with D.P.X.

Results:

Acidic mucins were stained deep rose to red.

Nuclei were stained black.

Other tissue elements were stained light yellow.

10. Hyaluronidase enzyme labile technique:[96]

Solutions preparation:

Hyaluronidase solution:

Bovine testicular hyaluronidase (BTH)	- 50 mgs
Phosphate buffer solution	- 100 ml

***Phosphate buffer solution*:**

Sodium chloride	- 8 gm
Sodium phosphate, monobasic	- 2 gm
Sodium phosphate, dibasic	- 0.3 gm
Distilled water	- 1000 ml

Method:

1. The sections were dewaxed and brought into distilled water for 5 min.
2. Then, the sections were incubated with the hyaluronidase solution for three hours at 37 ºC.
3. After that the sections were washed in running tap water for 5 minutes.
4. Soon after that, the sections were stained with alcian blue 2.5 for 5 minutes.
5. After that they were washed in running tap water for 2 – 3 minutes.
6. Soon after they were dehydrated, cleared and mounted with DPX.

Results:

If hyaluronic acid present, the sections were not stained blue.

If hyaluronic acid absent, the sections were stained blue.

3. DATA ANALYSIS PLAN

3.1 Statistical Analysis

Descriptive statistics were used to generate histochemical scores and distributions to summarize negative, weak, mild, moderate, and severe acid mucins in meniscal osteoarthritis. Further chi-square test was used to see the association between different histological staining intensities and sides of osteoarthritis menisci. One-way ANOVA (F-test) was applied to test various parts of different stains, staining intensities in medial and lateral OA menisci of both left and right legs. Further, a nonparametric method (Chi-square test) was used to see the association between the sides and OA. Significance was seen at 5% level. Analyses were performed using MS Excel and SPSS version 22.

1.1 Definitions and measurement methods of study variables:

Table 3.1 Coding used for Socio-demographic variables

Variables	Attribute	Coding
Socio-demographic variables		
Age	50 – 84 years	-
Gender	Female	1
	Male	2
Religion	Hindus	1
	Muslims	2
	Christians	3
Exercise	No	0
	Medium	1
	Light	2
	Active	3
	Moderate	4
Diet	Non-veg	0
	Veg	1
Blood group	A +ve	1
	B +ve	2
	O +ve	3
	AB +ve	4
	O –ve	5
Family history	No	0
	Yes	1

Table 3.2 Coding used for sides of legs and menisci variables

Variables	Attribute	Coding
Main study variables (osteoarthritis menisci of the knee joint)		
Sides of legs	Left	1
	Right	2
Sides of menisci	Medial	-
	Lateral	-

Table 3.3 Coding used for parts of menisci on severity of (OA) variables

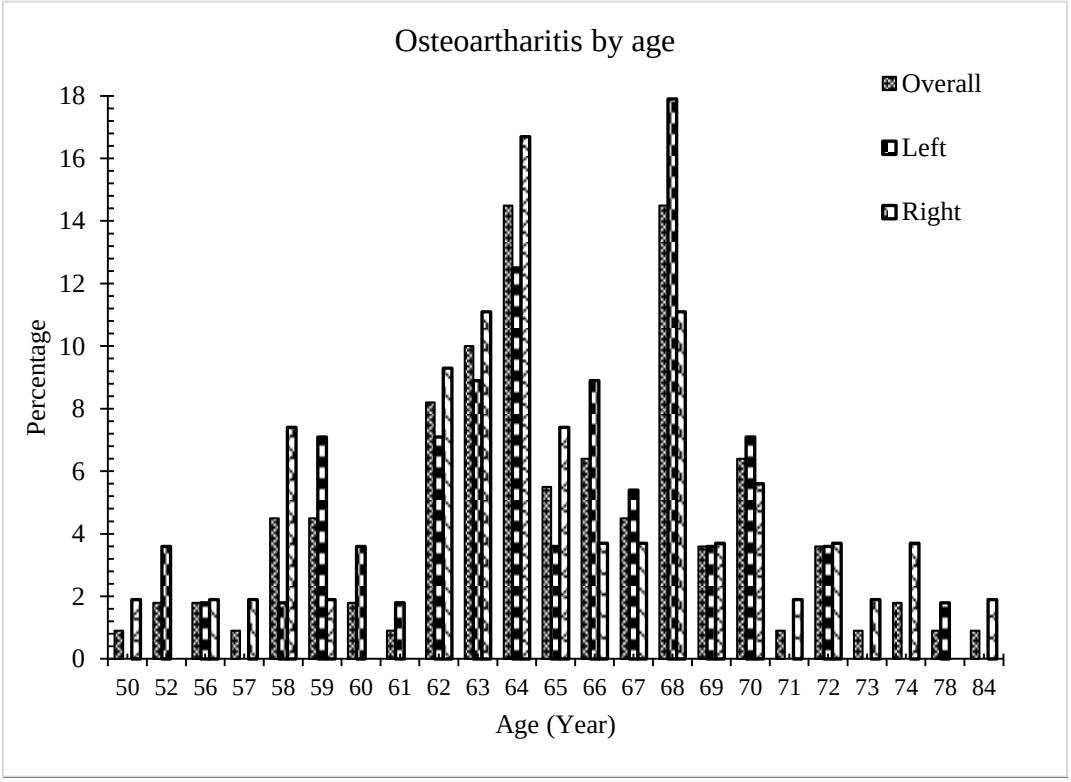
Variables	Attribute	Coding
Parts of (OA)menisci		
Medial anterior	Mild	1
Medial middle	Moderate	2
Medial posterior	Severe	3
Lateral anterior	Mild	1
Lateral middle	Moderate	2
Lateral posterior	Severe	3

Table 3.3 Coding used for grading of OA menisci and color intensity of different stains

Variables	Attribute	Coding
Grading score of OA menisci of different stains		
H & E stain grading		
Grade 1	0 -3 (normal)	1
Grade 2	4 – 6 (mild)	2
Grade 3	7 – 9 (moderate)	3
Grade 4	10 – 12 (severe)	4
Other special stain color intensity grading scores		
AB - 1		
AB – 2.5	- Negative or no stain (0%)	0
PAS	± Weak or variable stain (<25%)	1
PAS+AB-2.5	+ Mild or slight stain (26-50%)	2
AF	+ + Moderate stain (51 – 75%)	3
AF+AB-2.5	+ + + Strong stain (76 – 100%)	4
Orcein+AB-2.5		
Mucin carmine		
Hyaluronidase enzyme stain (treated with hyaluronidase)		
Negative	- No stain	1
Weak present	± Weak or variable stain	2
Mild	+ Mild or slight stain	3

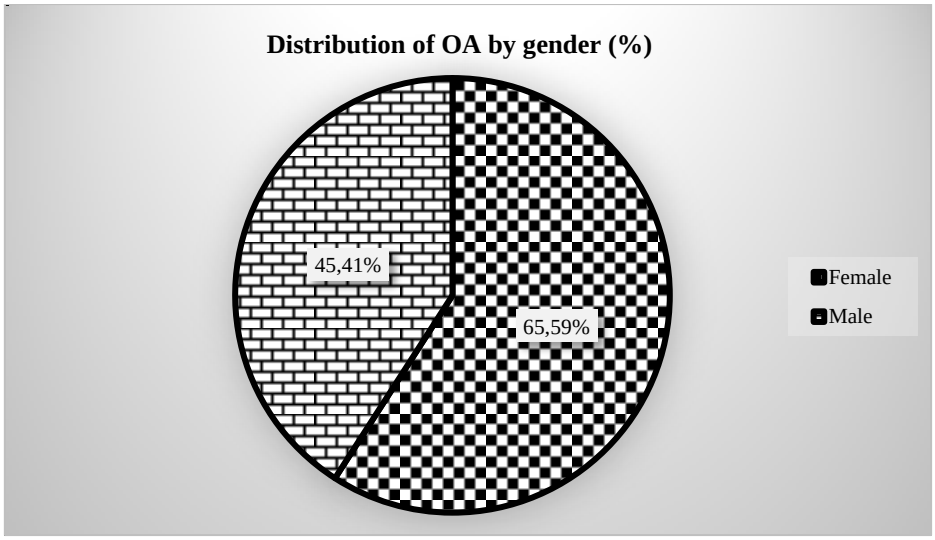
4. RESULTS AND OBSERVATIONS

4.1 Socio-demographic variations:



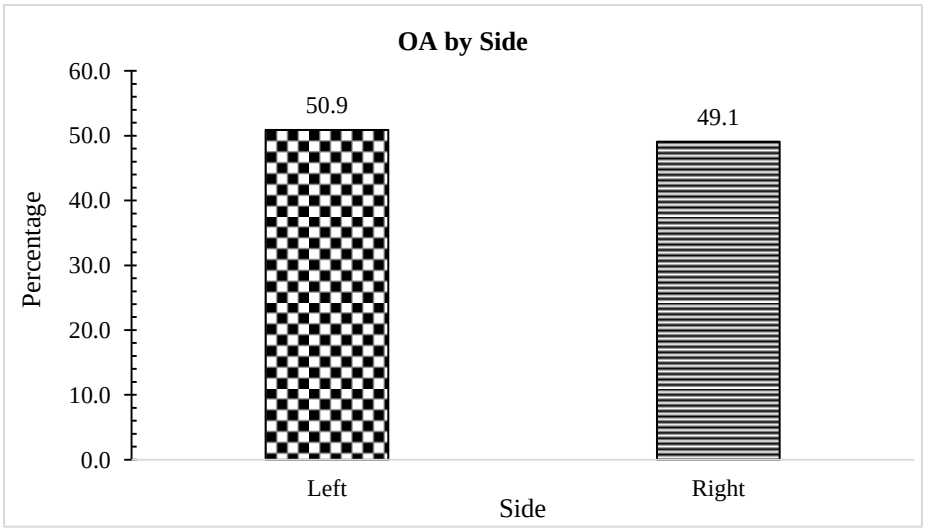
Graph 1: OA menisci by age and side of legs. OA: Osteoarthritis.

Osteoarthritis menisci vary by age and side, as shown in Graph 1. Among 110 OA patients, the minimum number of cases of osteoarthritis was found in 50 – 59 and 70 + years. In 110 patients, OA cases were higher in the age group 60 - 69 years in both legs of the knee joint.



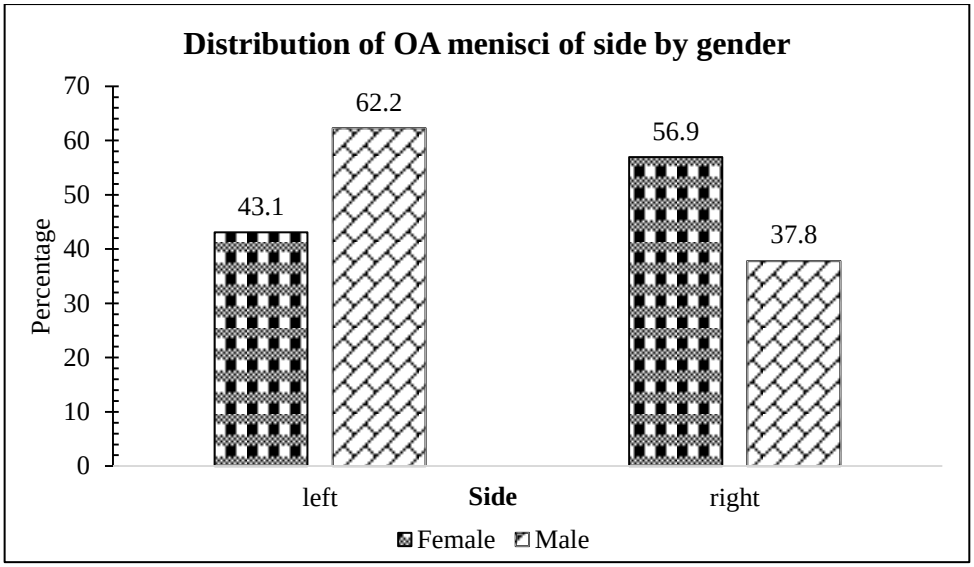
Graph 2: Distribution of OA menisci by gender.

Among 110 OA patients, about 59% and 41% of OA problems are in females and males respectively.



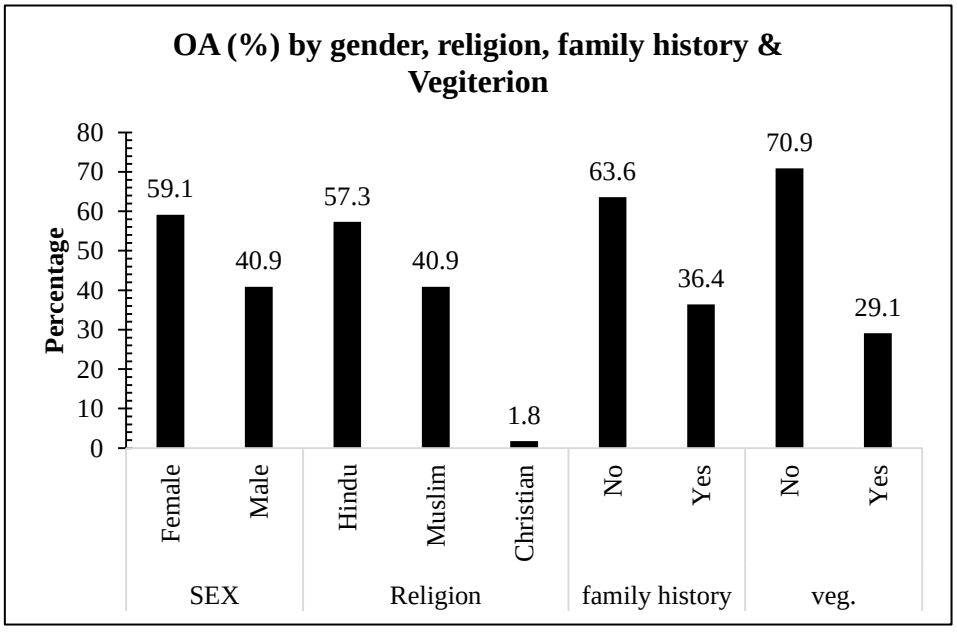
Graph 3: Osteoarthritis (OA) menisci by side of legs.

Graph 3: shows the distribution of Osteoarthritis (OA) menisci percentage on the side of the legs. Among 110 OA cases, approximately 51 % of people have left leg OA and 49 % have it in the right legs.



Graph 4: Distribution of OA menisci of side by gender

Graph 4: shows the distribution of OA menisci of side by gender. In 110 OA cases, 43% of females have left leg OA whereas 57 % have right leg OA. On the other hand, 62 % and 38 % of males have left and right leg OA respectively.

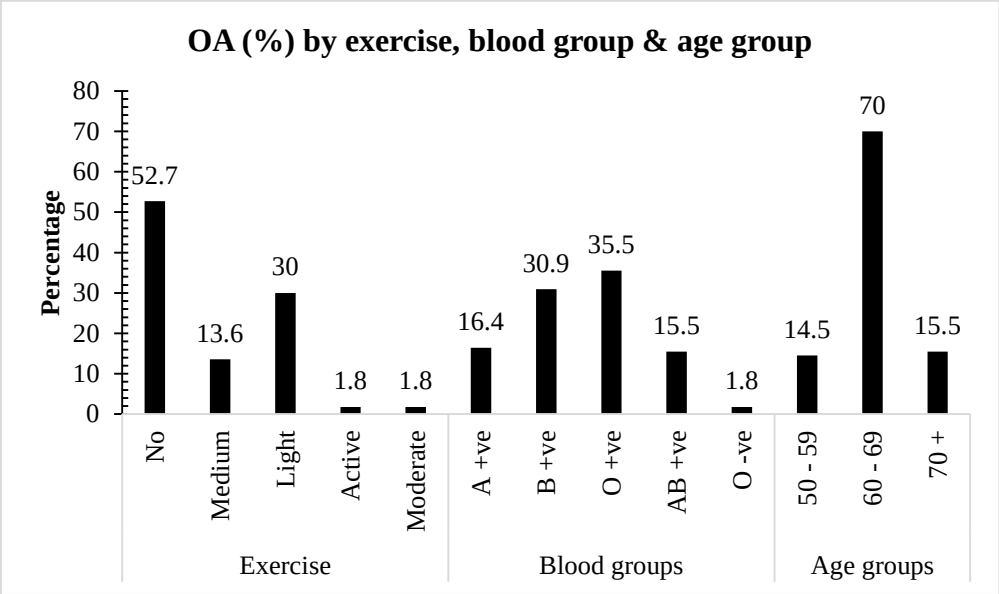


Graph 5: OA menisci by gender, religion, family history, and vegetarian food.

OA: Osteoarthritis

Gender, religions, family history, and vegetarian food:

The distribution of osteoarthritis (OA) in this series is shown in Graph 5. In the gender group, among 110 OA cases, women have a higher incidence of osteoarthritis than men, i.e. (59% of women and 41% of men). Among religions, in 110 OA cases hindus had more percentage of OA, than other religions (Muslims and Christians), (57% Hindu, 41% Muslim, and 2% Christian). Of these, in 110 OA cases 64% had no family history of OA, while 36% had a family history of OA. Whereas for dietary habits, in 110 OA cases non-vegetarians (71%) were more susceptible to OA than vegetarians (29%).



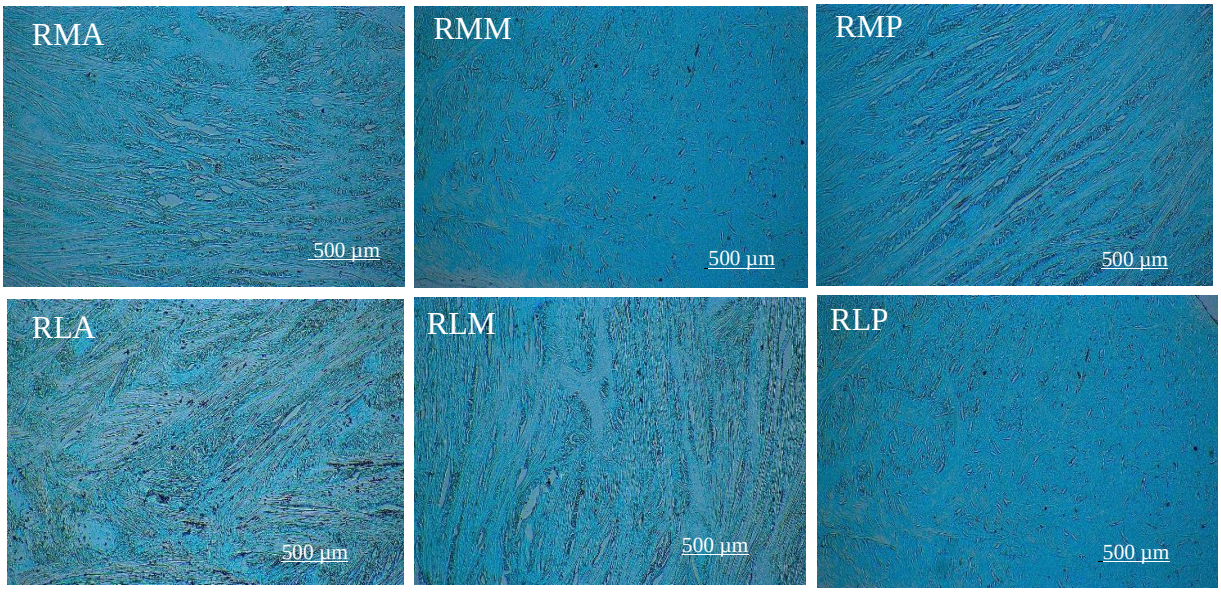
Graph 6: Osteoarthritis (OA) menisci by Exercise, Blood group & Age group.

Exercise, blood group, and age group:

Among different types of physical activity, i. e. (moderate, active, light, medium, and inactive), in 110 OA cases inactive people were more affected by OA (52.7%). In the blood group distribution, in 110 OA cases people with B+ve (30.9%)

and O+ve (35.5%) blood types had more OA than other blood types. In terms of age group distribution, in 110 OA cases, people aged 60 – 69 years were more susceptible to OA than other age groups (Graph 6).

Histochemical of mucopolysaccharides in osteoarthritic menisci of a human knee joint Alcian blue pH 1.0 (AB-1.0)



RMA – Right leg medial menisci anterior part**, RMM** – Right leg Medial menisci middle part, **RMP** –Right leg medial menisci posterior part
RLA –Right leg lateral menisci anterior part, R**LM** – Right leg lateral menisci middle part, **RLP** – Right leg lateral menisci posterior part

Figure 4.1.1a: The intensity of Sulfated acid mucins in the control group: magnification 100x, 500µm – Scale bar

Fig 4.1.1a shows an assessment of the histological staining intensity of alcian blue pH 1.0, in the extracellular matrix of sheep meniscus used as control group at 3 regions (anterior, middle, posterior) of medial and lateral menisci of right leg: RMA, RMM, RMP, RLA, RLM, and RLP with moderate staining and a score (+ +).

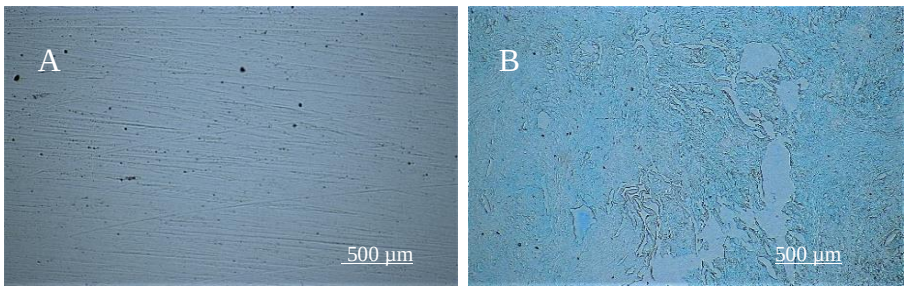


Figure 4.1.1b: The intensity of sulfated acid mucins in the test group: magnification 100x.

Fig. 4.1.1b shows an assessment of the histological staining intensity of alcian blue pH 1.0 in the extracellular matrix of OA human meniscus used as a test group at 3 regions (anterior, middle, posterior) of medial and lateral menisci of both legs: figure 'A' score: negative (-), figure 'B' score: weak or variable stain (±).

Table 4.1.1a: Alcian blue pH.1 intensity of sulfated mucins in medial menisci of both legs

Alcian blue pH.1 staining intensity of sulfated mucins		Left		Right		Overall	
		n1	%	n2	%	n	%
Control (normal menisci)	Moderate (+ +)	1	100	1	100	2	100
Test (OA menisci)							
Medial anterior	*Negative (-)	55	98.2	52	96.3	107	97.3
	Weak (±)	1	1.8	2	3.7	3	2.7
Medial middle	*Negative (-)	53	94.6	51	94.4	104	94.5
	Weak (±)	3	5.4	3	5.6	6	5.5
Medial posterior	*Negative (-)	54	96.4	50	92.6	104	94.5
	Weak (±)	2	3.6	4	7.4	6	5.5

Note: * F- test = 66.99 (Negative -), $P < 0.001$, n1+n2 = n.

Table 4.1.1b: Alcian blue pH.1 intensity of sulfated mucins in lateral menisci of both legs

Alcian blue pH.1 staining intensity of sulfated mucins		Left		Right		Overall	
		n1	%	n2	%	n	%
Control (normal menisci)	Moderate (+ +)	1	100	1	100	2	100
Test (OA menisci)							
Lateral anterior	*Negative (-)	53	94.6	53	98.1	106	96.4
	Weak (±)	3	5.4	1	1.9	4	3.6
Lateral middle	*Negative (-)	54	96.4	52	96.3	106	96.4
	Weak (±)	2	3.6	2	3.7	4	3.6
Lateral posterior	*Negative (-)	52	92.9	50	92.6	102	92.7
	Weak (±)	4	7.1	4	7.4	8	7.3

Note: * F- test = 107.98 (Negative -), $P < 0.001$, n1+n2 = n.

Table 4.1.1a, reveals a histochemical assessment of alcian blue pH 1 staining intensity level in the extracellular matrix of medial menisci of the left and right legs. The study showed the moderate staining intensity of alcian blue pH 1.0 in the control and its score of two plus (+ +) staining intensity of sulfated acid mucins. It was present in the medial and lateral menisci of in both legs of sheep. The test observation showed in the left leg medial meniscus anterior part (LMA) was 98.2 % negative and 1.8 % weak staining intensity of AB-1.0 while, it was 94.6 % negative and 5.4 % weak in the left leg medial meniscus middle part (LMM). Similarly, the staining intensity in the left leg medial meniscus posterior part (LMP) was 96.4 %

negative and 3.6 % weak. Right leg medial meniscus anterior part (RMA) was 96.3 % negative and 3.7 % weak staining intensity while, it was 94.4 % negative and 5.6 % weak staining intensity in right leg medial menisci middle part (RMM). Similarly, the staining intensity in right leg medial menisci posterior part (RMP) was 92.6 % negative and 7.4 % weak.

Similarly, the result in the left leg lateral meniscus anterior part (LLA) had 94.6 % negative and 5.4 % weak staining intensity of AB-1.0 while, it was 96.4 % negative and 3.6 % weak in the left leg lateral meniscus middle part (LLM). On similar lines, staining intensity in the left leg lateral meniscus posterior part (LLP) was 92.9 % negative and 7.1% weak. The right leg lateral meniscus anterior part (RLA) showed 98.1% negative and 1.9% weak staining intensity. While it was negative at 96.3 % and weak at 3.7% staining intensity in RLM. Similarly, the staining intensity in RLP was 92.6 % negative and 7.4 % weak (Table 4.1.1b).

Table 4.1.1c: Results of test statistics used in AB-1.0 intensity of sulphated mucins – different parts of medial and lateral menisci on sides of the legs

Source of variation	d.f.	s.s	M.S.S.	F-test	p-Value
Medial menisci anterior/middle/posterior (weak intensity)					
between	2	18.816	9.408	0.108409	>0.05
within	12	1041.39	86.7825		
total	14				
Medial menisci anterior/middle/posterior (negative intensity)					
between	2	553.9271	276.9636	66.99902	<0.001
within	312	1289.76	4.133845		
total	314				
Lateral menisci anterior/middle/posterior (weak intensity)					
between	2	54.76	27.38	0.319894	>0.05
within	13	1112.681	85.59087		
total	15				
Lateral menisci anterior/middle/posterior (negative intensity)					
between	2	942.7789	471.3894	107.9856	<0.001
within	311	1357.608	4.365298		
total	313				

S.S- Sum of Square, M.S.S.-Mean Sum of Square, d.f.-Degree of Freedom.

Table 4.1.1c reveals results of test statistics used in the different parts (anterior, middle, and posterior) of medial and lateral OA meniscus of AB-1.0. Negative intensity of sulphated mucins on both sides of the legs showing significant varying by F-test (p- <0.001).

Table 4.1.1d: Association of AB-1.0 intensity of sulfated mucins – medial and lateral menisci on side of the legs.

(AB-1.0) Sulfated acid mucins		Side				Chi-square (df), p-value
		Left		Right		
		n	%	n	%	
Medial	Negative (-)	55	98.2	53	98.1	0.001 (1), 0.979
	Weak (±)	1	1.8	1	1.9	
Lateral	Negative (-)	54	96.4	53	98.1	0.306 (1), 0.580
	Weak (±)	2	3.6	1	1.9	

Table 4.1.1d represents the association in the medial and lateral OA meniscus of AB-1.0 intensity of sulfated mucins on both sides of the legs. Medial menisci of the left and right leg have 98.2% and 98.1% respectively negative intensity of sulfated mucins. However, lateral menisci of the left and right leg have respectively 96.4% and 98.1% negative intensity of sulfated mucins. Moreover, the sides of the legs do not show a significant association between the medial and lateral meniscus.

Alcian blue at pH 2.5 (AB-2.5)

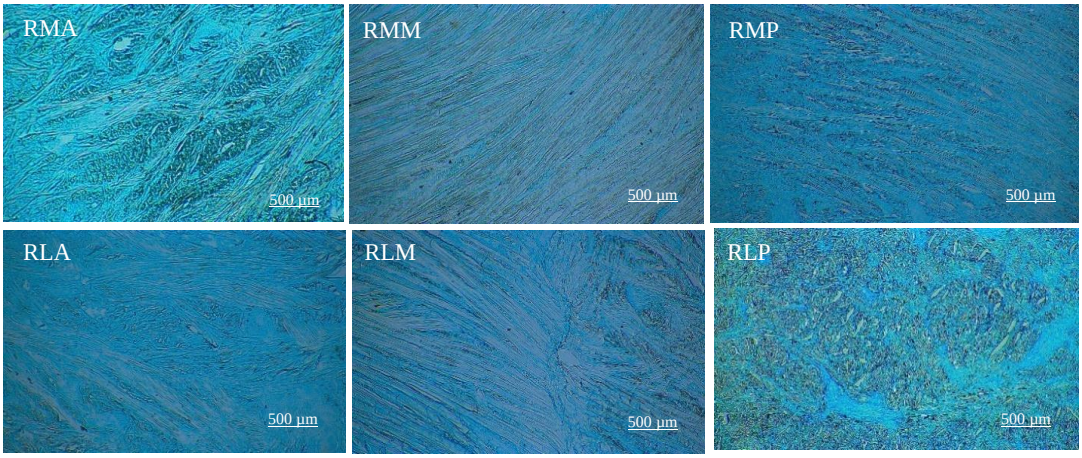


Figure 4.1.2a: The Intensity of acid mucins in control group: magnification 100x.

Fig. 4.1.2a shows assessment of histological staining intensity of alcian blue pH 2.5 in the extracellular matrix of sheep meniscus used as control group at 3 regions (anterior, middle, posterior) of medial and lateral menisci of right leg: RMA, RMM, RMP, RLA, RLM and RLP with strong staining and a score (+ + +).

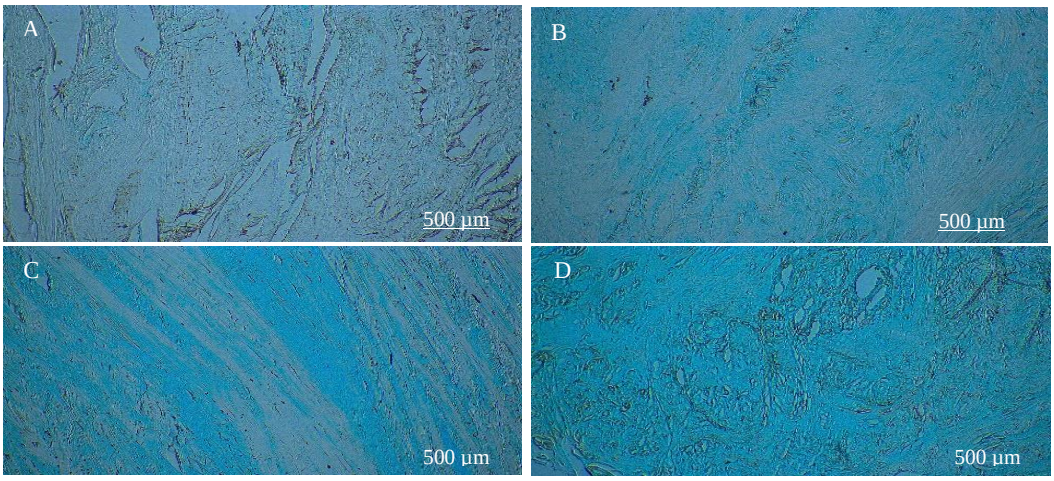


Figure 4.1.2b: The intensity of acid mucins in the test group: magnification 100x.

Fig. 4.1.2b: shows an assessment of the histological staining intensity of alcian blue pH 2.5 in the extracellular matrix of OA human meniscus used as a test group at 3 regions (anterior, middle, posterior) of medial and lateral menisci of both legs: figure 'A' score: negative (-), figure 'B' score: weak or variable stain (±), figure 'C' score: slight or mild stain (+) and figure 'D' score: moderate stain (+ +).

Table 4.1.2a: Alcian blue pH 2.5 staining intensity of acid mucins in medial meniscus of both legs.

Alcian Blue 2.5 staining intensity of acid mucins		Left leg		Right leg		Overall	
		n1	%	n2	%	n	%
Control (normal menisci)	Strong (+ + +)	1	100	1	100	2	100
Test (OA menisci)							
Medial anterior	Negative (-)	1	1.8	2	3.7	3	2.7
	*Weak (±)	29	51.8	27	50	56	50.9
	#Mild (+)	26	46.4	25	46.3	51	46.4
Medial middle	Negative (-)	1	1.8	0	0	1	0.9
	*Weak (±)	26	46.4	25	46.3	51	46.4
	#Mild (+)	29	51.8	29	53.7	58	52.7
Medial posterior	Negative (-)	1	1.8	1	1.9	2	1.8
	*Weak (±)	27	48.2	33	61.1	60	54.5
	#Mild (+)	25	44.6	20	37	45	40.9
	Moderate (++)	3	5.4	0	0	3	2.7

Note: * F = 20.35 (Weak), P < 0.05, # F = 37.44 (Mild), P < 0.05, n1+n2 = n

Table 4.1.2b: Alcian blue pH 2.5 staining intensity of acid mucins in lateral meniscus of both legs.

Alcian Blue 2.5 staining intensity of acid mucins		Left leg		Right leg		Overall	
		n1	%	n2	%	n	%
Control (normal menisci)	Strong (+ + +)	1	100	1	100	2	100
Test (OA menisci)							
Lateral anterior	Negative (-)	0	0	2	3.7	2	1.8
	*Weak (±)	36	64.3	28	51.9	64	58.2
	#Mild (+)	20	35.7	24	44.4	44	40
Lateral middle	Negative (-)	3	5.4	2	3.7	5	4.5
	*Weak (±)	30	53.6	36	66.7	66	60
	#Mild (+)	23	41.1	16	29.6	39	35.5
Lateral posterior	Negative (-)	1	1.8	1	1.9	2	1.8
	*Weak (±)	37	66.1	30	55.6	67	60.9
	#Mild (+)	18	32.1	23	42.6	41	37.3

Note: * F = 3.38 (Weak), P < 0.05, # F = 3.80 (Mild), P < 0.05, n1+n2 = n

Table 4.1.2a reveals a histochemical assessment of alcian blue pH 2.5 staining intensity level in the extracellular matrix of medial menisci of the left and right legs. The study showed a strong staining intensity of alcian blue pH 2.5 in the control and its score of three plus (+ + +) staining intensity of acid mucins. It is present in both legs of the medial and lateral menisci of sheep. The observation made in the left leg

medial meniscus anterior part (LMA) was 1.8% negative, 51.8% weak, and 46.4% mild staining intensity of AB-2.5, while it was 1.8% negative, 46.4% weak, and 51.8% mild in the left leg medial meniscus middle part (LMM). Similarly, the staining intensity in the left leg medial meniscus posterior part (LMP) was 1.8 % negative, 48.2 % weak, 44.6 % mild, and 5.4 % moderate. Right leg medial meniscus anterior part (RMA) was 3.7 % negative, 50% weak, and 46.3% mild staining intensity while, it was 46.3% weak, and 53.7% mild staining intensity in RMM. Similarly, the staining intensity in RMP was 1.9% negative, 61.1% weak, and 37% mild. Staining intensity in the medial OA menisci is significantly varying by parts ($p < 0.05$).

Table 4.1.2b, the result made in the left leg lateral meniscus anterior part (LLA) had 64.3% weak, and 35.7% mild staining intensity of AB-2.5 while, it was 5.4% negative, 53.6% weak, and 41.1% mild in the left leg lateral meniscus middle part (LLM). Similarly, staining intensity in the left leg lateral meniscus posterior part (LLP) was 1.8% negative, 66.1% weak, and 32.1% mild. The right leg lateral meniscus anterior part (RLA) showed 3.7% negative, 51.9% weak, and 44.4% mild staining intensity. While, it was negative (3.7%), weak (66.7%), and mild (29.6%) staining intensity in RLM. Similarly, the staining intensity in RLP was 1.9% negative, 55.6% weak, and 42.6% mild. Staining Intensity in lateral OA menisci significantly ($p < 0.05$) varied by parts.

Table 4.1.2c: Results of test statistics used in AB-2.5 intensity of acid mucins – different parts of medial and lateral menisci on sides of the legs.

Source of variation	d.f.	S.S	M.S.S.	F-test	p-value
Medial menisci anterior/middle/posterior (weak intensity)					
Between	2	1809.259	904.6293	20.35992	<0.05
Within	165	7331.257	44.43186		
Total	167				
Medial menisci anterior/middle/posterior (mild intensity)					
Between	2	3573.008	1786.504	37.44732	<0.05
Within	152	7251.481	47.70711		
Total	154				
Lateral menisci anterior/middle/posterior (weak intensity)					
Between	2	246.3305	123.1652	3.380786	< 0.05
Within	195	7104.034	36.43095		
Total	197				
Lateral menisci anterior/middle/posterior (mild intensity)					
Between	2	428.0719	214.036	3.807491	< 0.05
Within	122	6858.161	56.21444		
Total	124				

S.S- Sum of Square, M.S.S.-Mean Sum of Square, d.f.-Degree of Freedom

Table 4.1.2c states the results of test statistics used in different parts (anterior, middle, and posterior) of medial and lateral OA meniscus of AB-2.5 intensity of acid mucins on both sides of the legs showing significant Weak (±) and Mild (+) varying by F-test ($p < 0.05$).

Table 4.1.2d: Association of AB-2.5 intensity of acid mucins – medial and lateral menisci on side of the legs.

AB2.5 (acid) Mucins		Side				Chi-square (df), p-value
		Left		Right		
		n	%	n	%	
Medial	Weak	27	48.2	26	48.1	3.058 (2), 0.217
	Mild	26	46.4	28	51.9	
	Moderate	3	5.4	0	0	
Lateral	Weak	36	64.3	33	61.1	0.119 (1), 0.731
	Mild	20	35.7	21	38.9	

Table 4.1.2d states the association in the medial and lateral OA meniscus of AB-2.5 intensity of acid mucins on both sides of the legs. Medial menisci of the left and right leg have 46.4% and 51.9% respectively mild intensity of acid mucins. However, lateral menisci of the left and right leg have respectively 35.7% and 38.9% mild intensity of acid mucins. Moreover, the sides of the legs do not show a significant association between the medial and lateral meniscus.

PAS (*Periodic Acid-Schiff)*

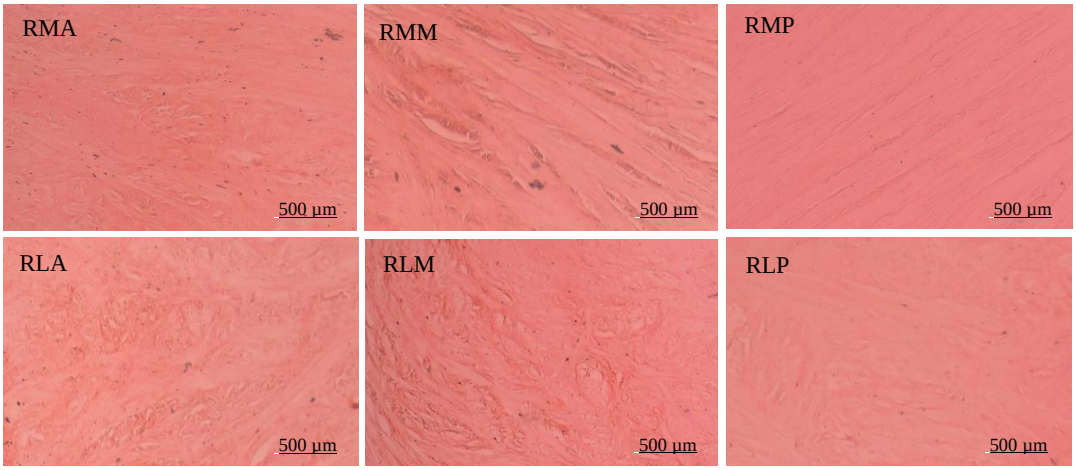


Figure 4.1.3a - The Intensity of neutral mucins (glycogen carbohydrate) in the control group: magnification 100x.

Fig 4.1.3a. shows an assessment of the histological staining intensity of PAS in the extracellular matrix of sheep meniscus used as control group at 3 regions (anterior, middle, posterior) of medial and lateral menisci of right leg: RMA, RMM, RMP, RLA, RLM, and RLP with moderate staining and a score (+ +) staining intensity of neutral mucins (glycogen carbohydrate) .

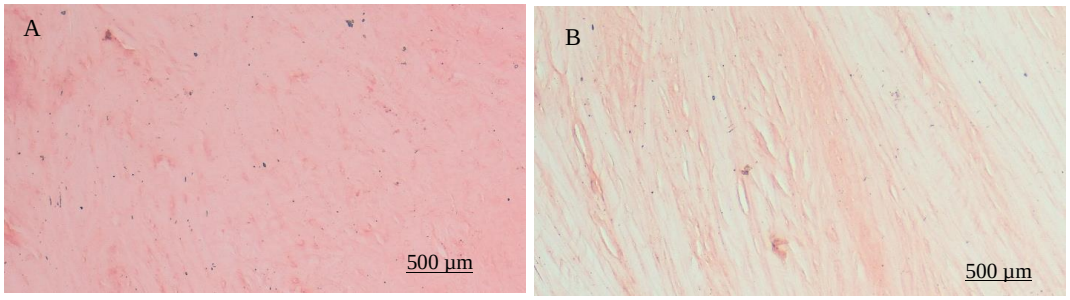


Figure 4.1.3b - The intensity of neutral mucins (glycogen carbohydrate) in the test group: magnification 100x.

Fig. 4.1.3b shows an assessment of the histological staining intensity of PAS in the extracellular matrix of OA human meniscus used as a test group at 3 regions (anterior, middle, posterior) of medial and lateral menisci of both legs: figure 'A' score: mild PAS positive (+), figure 'B' score weak PAS positive (±) staining intensity of neutral mucins (glycogen carbohydrate).

Table 4.1.3a: PAS staining color intensity level in medial menisci of both legs.

PAS staining intensity of (glycogen carbohydrate)		Left		Right		overall	
		n1	%	n2	%	n	%
Control (normal menisci)	Moderate (++)	1	100	1	100	2	100
Test (OA menisci)							
Medial anterior	*Mild (+)	52	92.9	47	87.0	99	90.0
	Weak (±)	4	7.1	7	13.0	11	10.0
Medial middle	*Mild (+)	49	87.5	46	85.2	95	86.4
	Weak (±)	7	12.5	8	14.8	15	13.6
Medial posterior	*Mild (+)	50	89.3	49	90.7	99	90.0
	Weak (±)	6	10.7	5	9.3	11	10.0

Note: * F- test = 40.97 (Mild), $P < 0.05$, n1+n2 = n

Table 4.1.3b: PAS staining color intensity level in lateral menisci of both legs.

PAS staining intensity of (glycogen carbohydrate)		Left		Right		overall	
		n1	%	n2	%	n	%
Control (normal menisci)	moderate (++)	1	100	1	100	2	100
Test (OA menisci)							
Lateral anterior	*Mild (+)	45	80.4	47	87.0	92	83.6
	Weak (±)	11	19.6	7	13.0	18	16.4
Lateral middle	*Mild (+)	51	91.1	46	85.2	97	88.2
	Weak (±)	5	8.9	8	14.8	13	11.8
Lateral posterior	*Mild (+)	48	85.7	51	94.4	99	90.0
	Weak (±)	8	14.3	3	5.6	11	10.0

Note: * F- test = 89.53 (Mild), $P < 0.05$, n1+n2 = n

Table 4.1.3a, reveals a histochemical assessment of PAS+ve staining intensity level in the extracellular matrix of medial menisci of the left and right legs. The study showed the moderate staining intensity of PAS+ve in the control and its score of three plus (+ +) neutral mucins (glycogen carbohydrate). It was present in both legs of the medial and lateral menisci of sheep. The test observation made in the left leg medial meniscus anterior part (LMA) was 92.9 % mild and 7.1 % weak staining intensity of PAS+ve while, it was 87.5 % mild and 12.5 % weak in the left leg medial meniscus middle part (LMM). Similarly, the staining intensity in the left leg medial meniscus posterior part (LMP) was 89.3 % mild and 10.7 % weak. Right

leg medial meniscus anterior part (RMA) was 87.0 % mild and 13.0 % weak staining intensity, while it was 85.2 % mild and 14.8 % weak staining intensity in RMM. Similarly, the staining intensity in RMP was 90.7 % mild and 9.3 % weak.

Again, the result observed in the left leg lateral meniscus anterior part (LLA) had 80.4 % mild and 19.6 % weak staining intensity of PAS+ve, while it was 91.1 % mild and 8.9 % weak in the left leg lateral meniscus middle part (LLM). Similarly, staining intensity in the left leg lateral meniscus posterior part (LLP) was 85.7 % mild and 14.3 % weak. The right leg lateral meniscus anterior part (RLA) showed 87.0 % mild and 13.0 % weak staining intensity. While it was mild at 85.2 % and weak at 14.8 % staining intensity in RLM. Similarly, the staining intensity in RLP was 94.4 % mild and 5.6 % weak (Table 4.1.3b).

Table 4.1.3c: Results of test statistics used in PAS intensity of neutral mucins – different parts of medial and lateral menisci on sides of the legs.

PAS staining intensity of neutral mucins (glycogen carbohydrate)					
Source of variation	d.f.	s.s	M.S.S.	F-test	p-Value
Medial menisci anterior/middle/posterior (mild +)					
between	2	832.0055	416.0027	40.97172	<0.05
within	290	2944.489	10.15341		
total	292				
Medial menisci anterior/middle/posterior (weak ±)					
between	2	115.5892	57.79459	0.718978	>0.05
within	34	2733.068	80.38434		
total	36				
Lateral menisci anterior/middle/posterior (mild +)					
between	2	2059.06	1029.53	89.5359	<0.05
within	285	3277.077	11.49852		
total	287				
Lateral menisci anterior/middle/posterior (weak ±)					
between	2	322.02	161.01	2.042906	>0.05
within	39	3073.754	78.81422		
total	41				

S.S- Sum of Square, M.S.S.-Mean Sum of Square, d.f.-Degree of Freedom.

Table 4.1.3c, states the results of test statistics used in the different parts (anterior, middle, and posterior) of medial and lateral OA meniscus of PAS staining intensity of neutral mucins on both sides of the legs showing significant on mild (+) varying by F-test (p- <0.05).

Table 4.1.3d: Association of PAS staining intensity of neutral mucins – medial and lateral menisci on sides of the legs.

PAS: neutral mucins (glycogen carbohydrate)	Side					Chi-square (df), p-value
	Left			Right		
	n		%	n	%	
Medial	Mild (+)	49	87.5	46	85.2	0.125 (1),0.724
	Weak (±)	7	12.5	8	14.8	
Lateral	Mild (+)	45	80.4	45	83.3	0.164 (1),0.686
	Weak (±)	11	19.6	9	16.7	

Table 4.1.3d, reveals the association in the medial and lateral OA meniscus of PAS color intensity of neutral mucins on both sides of the legs. Medial menisci of the left and right leg have 87.5% and 85.2% mild (+), and 12.5% and 14.8% weak (±) neutral mucins intensity respectively. However, lateral menisci of the left and right leg have respectively 80.4% and 83.3% mild (+), and 19.6%, and 16.7% weak (±) neutral mucins intensity. Moreover, the sides of the legs do not show a significant association between the medial and lateral meniscus.

Aldehyde fuchsin (AF)

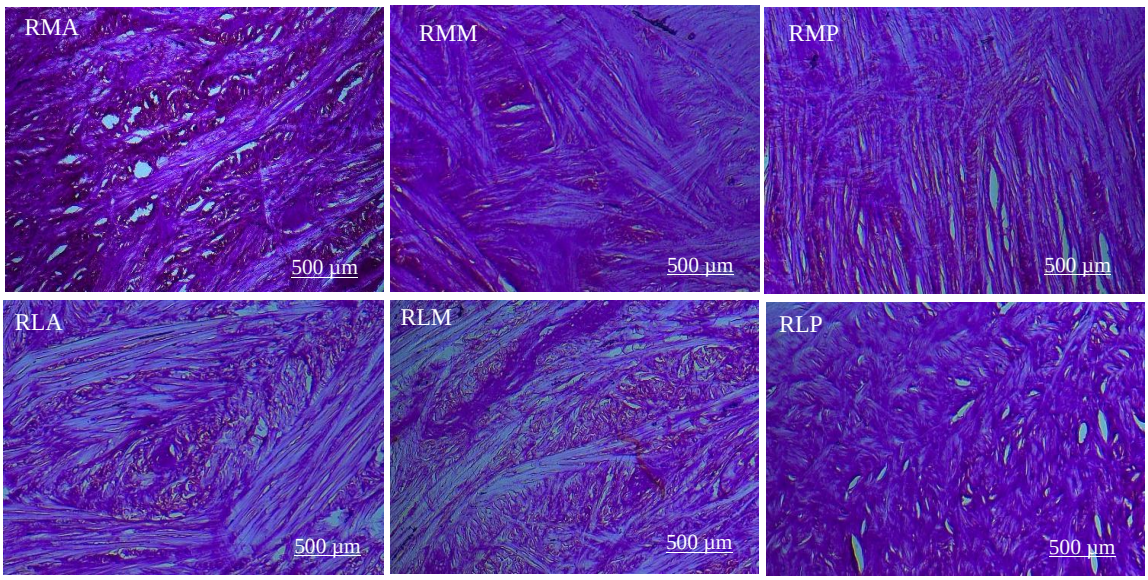


Figure 4.1.4a, the intensity of sulphomucins in the control group: magnification 100x.

Fig. 4.1.4a, shows assessment of histological staining intensity of aldehyde fuchsin (AF) in the extracellular matrix of sheep meniscus used as control group at 3 regions (anterior, middle, posterior) of medial and lateral menisci of right leg: RMA, RMM, RMP, RLA, RLM and RLP with strong staining and a score (+ + +).

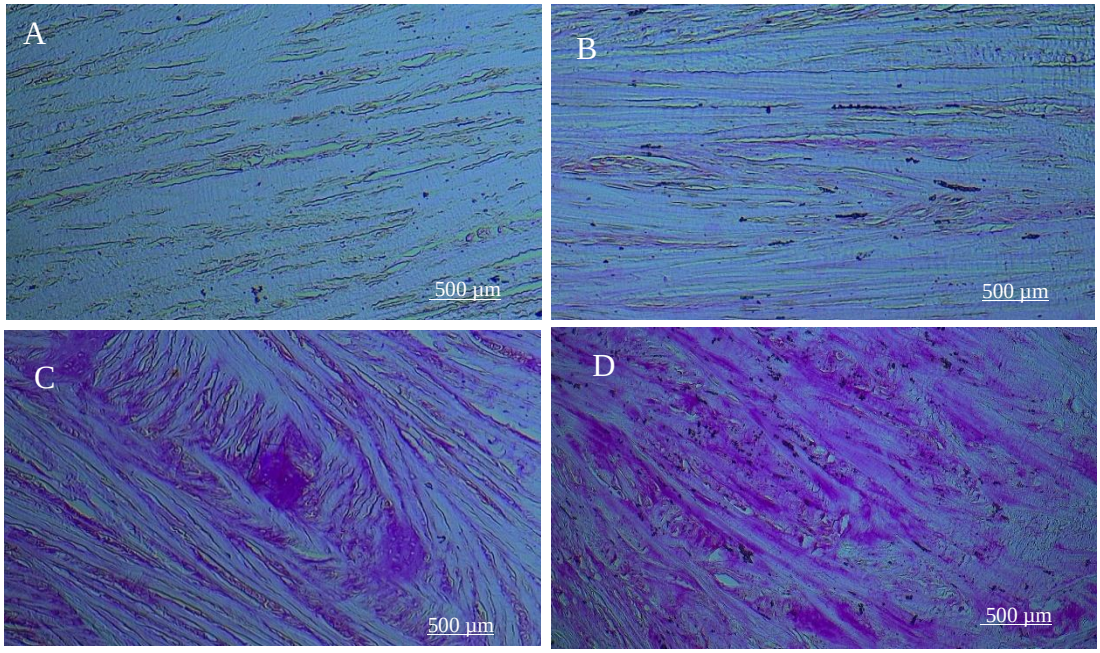


Figure 4.1.4b, the intensity of sulphomucins in the test group: magnification 100x.

Fig. 4.1.4b, shows an assessment of histological staining intensity of aldehyde fuchsin (AF) in the extracellular matrix of OA human meniscus used as a test group at 3 regions (anterior, middle, posterior) of medial and lateral menisci of both legs: figure 'A' score: negative color intensity of AF (-), figure 'B' score: weak or variable color intensity of AF (±), figure 'C' score: mild color intensity of AF (+) and figure 'D' score: moderate color intensity of AF (+ +).

Table 4.1.4a: AF staining intensity of sulphomucins in medial meniscus of both legs.

Aldehyde fuchsin staining intensity of sulphomucins		Left		Right		Overall	
		n1	%	n2	%	n	%
Control (normal)	Strong (+++)	1	100	1	100	2	100
Test (OA menisci)							
Medial anterior	Negative (-)	2	3.6	1	1.9	3	2.7
	*Weak (±)	38	67.9	37	68.5	75	68.2
	#Mild (+)	14	25	12	22.2	26	23.6
	Moderate (++)	2	3.6	4	7.4	6	5.5
Medial middle	Negative (-)	0	0	2	3.7	2	1.8
	*Weak (±)	40	71.4	43	79.6	83	75.5
	#Mild (+)	12	21.4	9	16.7	21	19.1
	Moderate (++)	4	7.1	0	0	4	3.6
Medial posterior	Negative (-)	0	0	2	3.7	2	1.8
	*Weak (±)	31	55.4	41	75.9	72	65.5
	#Mild (+)	23	41.1	10	18.5	33	30
	Moderate (++)	2	3.6	1	1.9	3	2.7

Note: * F- test = 77.15 (Weak), $P < 0.05$, # F- test = 11.80 (Mild), $P < 0.05$, n1+n2 = n

Table 4.1.4b: AF staining intensity of sulphomucins in lateral meniscus of both legs.

Aldehyde fuchsin staining intensity of sulphomucins		Left		Right		Overall	
		n1	%	n2	%	n	%
Control (normal)	Strong (+++)	1	100	1	100	2	100
Test (OA menisci)							
Lateral anterior	Negative (-)	1	1.8	0	0	1	0.9
	*Weak (±)	41	73.2	41	75.9	82	74.5
	#Mild (+)	14	25	12	22.2	26	23.6
	Moderate (++)	0	0	1	1.9	1	0.9
Lateral middle	Negative (-)	1	1.8	2	3.7	3	2.7
	*Weak (±)	40	71.4	41	75.9	81	73.6
	#Mild (+)	11	19.6	10	18.5	21	19.1
	Moderate (++)	4	7.1	1	1.9	5	4.5
Lateral posterior	Negative (-)	1	1.8	2	3.7	3	2.7
	*Weak (±)	35	62.5	39	72.2	74	67.3
	#Mild (+)	18	32.1	12	22.2	30	27.3
	Moderate (++)	2	3.6	1	1.9	3	2.7

Note: * F- test = 46.15 (Weak), $P < 0.05$, # F- test = 6.01 (Mild), $P < 0.05$, n1+n2 = n

Table 4.1.4a, reveals a histochemical assessment of aldehyde fuchsin staining intensity level in the extracellular matrix of medial menisci of the left and right legs. The study showed a strong staining intensity of aldehyde fuchsin in the control and its score of three plus (+ + +) sulphomucins. It is present in both legs of the medial and lateral menisci of sheep. The test observed in the left leg medial meniscus anterior part (LMA) was 3.6 % negative, 67.9 % weak, 25 % mild and 3.6 % moderate staining intensity of aldehyde fuchsin, while it was 71.4% weak, 21.4 % mild and 7.1 % moderate in the left leg medial meniscus middle part (LMM). Similarly, the staining intensity in the left leg medial meniscus posterior part (LMP) was 55.4 % weak, 41.1 % mild, and 3.6 % moderate. Right leg medial meniscus anterior part (RMA) was 1.9 % negative, 68.5 % weak, 22.2 % mild and 7.4 % moderate staining intensity, while it was 3.7 % negative, 79.6 % weak, and 16.7 % mild staining intensity in RMM. Similarly, the staining intensity in RMP was 3.7 % negative, 75.9 % weak, 18.5 % mild and 1.9 % moderate.

The result in left leg lateral meniscus anterior part (LLA) had 1.8 % negative, 73.2 % weak, and 25 % mild staining intensity of aldehyde fuchsin, while it was 1.8 % negative, 71.4 % weak, 19.6 % mild and 7.1 % moderate in the left leg lateral meniscus middle part (LLM). Similarly, staining intensity in the left leg lateral meniscus posterior part (LLP) was 1.8% negative, 62.5% weak, 32.1% mild and 3.6% moderate. The right leg lateral meniscus anterior part (RLA) showed 75.9% weak, 22.2% mild and 1.9% moderate staining intensity. While, it was negative 3.7%, weak 75.9%, mild 18.5% and moderate1.9% staining intensity in RLM. Similarly, the staining intensity in RLP was 3.7 % negative, 72.2 % weak, 22.2% mild and 1.9% moderate (Table 4.1.4b).

Table 4.1.4c: Results of test statistics used in aldehyde fuchsin intensity of sulphomucins – different parts of medial and lateral menisci on sides of the legs.

Aldehyde fuchsin staining intensity of sulphomucins					
Source of variation	d.f.	s.s	M.S.S.	F-test	p-Value
Medial menisci anterior/middle/posterior (negative)					
between	2	1.388571	0.694286	0.007892	>0.05
within	4	351.9	87.975		
total	6				
Medial menisci anterior/middle/posterior (weak)					
between	2	4211.723	2105.861	77.15556	<0.05
within	227	6195.672	27.29371		
total	229				
Medial menisci anterior/middle/posterior (mild)					
between	2	1606.694	803.3469	11.80116	<0.05
within	77	5241.665	68.07358		
total	79				
Medial menisci anterior/middle/posterior (moderate)					
between	2	18.26769	9.133846	0.105163	>0.05
within	10	868.545	86.8545		
total	12				

Lateral menisci anterior/middle/posterior (negative)					
between	2	2.777143	1.388571	0.015857	>0.05
within	4	350.28	87.57		
total	6				
Lateral menisci anterior/middle/posterior (weak)					
between	2	2353.785	1176.892	46.1557	<0.05
within	234	5966.605	25.49831		
total	236				
Lateral menisci anterior/middle/posterior (mild)					
between	2	832.4143	416.2071	6.010968	<0.05
within	74	5123.855	69.24128		
total	76				
Lateral menisci anterior/middle/posterior (moderate)					
between	2	13.68	6.84	0.079084	>0.05
within	6	518.94	86.49		
total	8				

S.S- Sum of Square, M.S.S.-Mean Sum of Square, d.f.-Degree of Freedom

Table 4.1.4c states the results of test statistics used in the different parts (anterior, middle, and posterior) of medial and lateral OA meniscus of AF. Staining intensity of sulphomucins on both sides of the legs showed significant mild (+) and weak (±) varying by F-test (p- <0.05).

Table 4.1.4d: Association of aldehyde fuchsin color intensity of sulphomucins – medial and lateral menisci on side of the legs.

Aldehyde fuchsin - sulphomucins		Side				Chi-square (df), p-value
		Left		Right		
		n	%	n	%	
Medial	Negative	0	0	2	3.7	6.238 (3), 0.101
	Weak	15	26.8	7	13	
	Mild	33	58.9	40	74.1	
	Moderate	8	14.3	5	9.3	
Lateral	Negative	1	1.8	2	3.7	0.864 (3), 0.834
	Weak	8	14.3	7	13	
	Mild	42	75	42	77.8	
	Moderate	5	8.9	3	5.6	

Table 4.1.4d states the association in the medial and lateral OA meniscus of AF color intensity of sulphomucins on both sides of the legs. Medial menisci of the left and right leg had 26.8% and 13 % weak, 58.9 % and 74.1% mild sulphomucin, intensity respectively. However, lateral menisci of the left and right leg had respectively 14.3% and 13% weak, 75%, and 77.8% mild sulphomucin intensity. Moreover, the sides of the legs do not show a significant association between the medial and lateral meniscus.

Mucicarmine stain (MC)

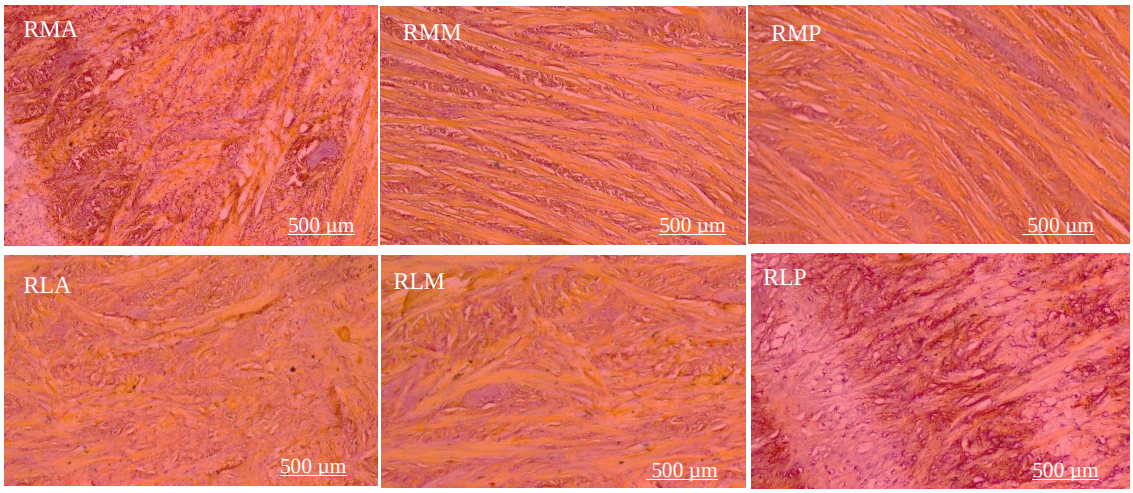


Figure 4.1.5a - The intensity of acid mucopolysaccharides in the control group: magnification 100x.

Fig 4.1.5a, shows an assessment of the histological staining intensity of Mucicarmine in the extracellular matrix of sheep meniscus used as control group at 3 regions (anterior, middle, posterior) of medial and lateral menisci of right leg: RMA, RMM, RMP, RLA, RLM, and RLP with moderate staining and a score (+ +).

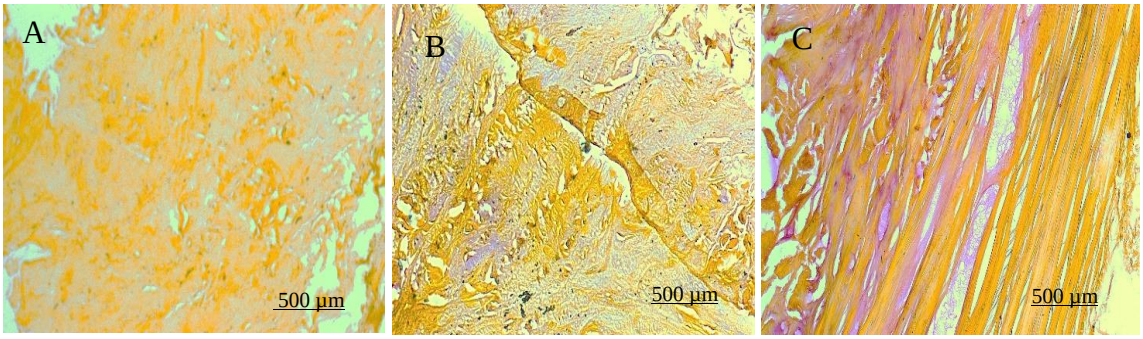


Figure 4.1.5b - The intensity of acid mucopolysaccharides in test group: magnification 100x.

Fig. 4.1.5b shows an assessment of histological staining intensity of Mucicarmine in the extracellular matrix of OA human meniscus used as a test group at 3 regions (anterior, middle, posterior) of medial and lateral menisci of both legs: figure 'A' score: negative (-), figure 'B' score: weak or variable stain (±), and figure 'C' score: Slight or mild stain (+) staining intensity of acid mucopolysaccharides.

Table 4.1.5a: Mucicarmine staining intensity level in medial menisci of both legs.

Mucicarmine: Acid mucopolysaccharides		Left		Right		Overall	
		n1	%	n2	%	n	%
Control (normal)	Moderate (++)	1	100	1	100	2	100
Test (OA) menisci							
Medial anterior	*Negative (-)	46	82.1	43	79.6	89	80.9
	#Weak (±)	10	17.9	11	20.4	21	19.1
Medial middle	*Negative (-)	54	96.4	46	85.2	100	90.9
	#Weak (±)	2	3.6	8	14.8	10	9.1
Medial posterior	*Negative (-)	52	92.9	47	87	99	90
	#Weak (±)	4	7.1	7	13	11	10

Note: * F = 248.7 (Negative), P < 0.05, # F = 6.15 (Weak), P < 0.05, n1+n2 = n

Table 4.1.5b: Mucicarmine staining intensity level in lateral menisci of both legs.

Mucicarmine: Acid mucopolysaccharides		Left		Right		Overall	
		n1	%	n2	%	n	%
Control (normal)	Moderate (++)	1	100	1	100	2	100
Test (OA) menisci							
Lateral anterior	*Negative (-)	52	92.9	50	92.6	102	92.7
	#Weak (±)	4	7.1	4	7.4	8	7.3
Lateral middle	*Negative (-)	47	83.9	44	81.5	91	82.7
	#Weak (±)	9	16.1	10	18.5	19	17.3
Lateral posterior	*Negative (-)	56	100	51	94.4	107	97.3
	#Weak (±)	0	0	3	5.6	3	2.7

Note: * F = 682.7 (Negative), P < 0.05, # F = 5.90 (Weak), P < 0.05, n1+n2 = n

Table 4.1.5a, reveals a histochemical assessment of mucicarmine staining intensity level in the extracellular matrix of medial menisci of the left and right legs. The study showed the moderate staining intensity of mucicarmine in the control and its score of two plus (+ +). It is present in both legs of the medial and lateral menisci of sheep. The test observation made in the left leg medial meniscus anterior part (LMA) was 17.9% weak and 82.1% negative (acid mucopolysaccharides) staining intensity of mucicarmine while it was 3.6% weak and 96.4% negative (acid mucopolysaccharides) in the left leg medial meniscus middle part (LMM). Similarly, the staining intensity in the left leg medial meniscus posterior part (LMP) was 7.1% weak and 92.9% negative (acid mucopolysaccharides). Right leg medial meniscus

anterior part (RMA) was 20.4% weak and 79.6% negative (acid mucopolysaccharides) staining intensity while it was 14.8% weak and 85.2% negative (acid mucopolysaccharides) staining intensity in RMM. Similarly, the staining intensity in RMP was 13% weak and 87% negative (acid mucopolysaccharides).

Similarly, (Table 4.1.5b) the result made in the left leg lateral meniscus anterior part (LLA) had 7.1% weak and 92.9% negative (acid mucopolysaccharides) staining intensity of Mucicarmine while it was 16.1% weak and 83.9% negative (acid mucopolysaccharides) in the left leg lateral meniscus middle part (LLM). On similar lines, staining intensity in the left leg lateral meniscus posterior part (LLP) was 100% negative (acid mucopolysaccharides). The right leg lateral meniscus anterior part (RLA) showed 7.4% weak and 92.6% negative (acid mucopolysaccharides) staining intensity. While it was 18.5% weak and 81.5% negative (acid mucopolysaccharides) staining intensity in RLM. Similarly, the staining intensity in RLP was 5.6% weak and 94.4% negative (acid mucopolysaccharides).

Table 4.1.5c: Results of test statistics used in mucicarmine intensity of acid mucopolysaccharides – different parts of medial and lateral menisci on sides of the legs.

Mucicarmine: Acid mucopolysaccharides					
Source of variation	d.f.	s.s	M.S.S.	F-test	p-Value
Medial menisci anterior/middle/posterior (negative)					
between	2	5651.59	2825.795	248.7453	<0.05
within	285	3237.656	11.36019		
total	287				
Medial menisci anterior/middle/posterior (weak)					
between	2	957.5764	478.7882	6.153964	<0.05
within	39	3034.262	77.8016		
total	41				
Lateral menisci anterior/middle/posterior (negative)					
between	2	10782.26	5391.132	682.7074	<0.05
within	297	2345.318	7.896695		
total	299				
Lateral menisci anterior/middle/posterior (weak)					
between	2	928.5987	464.2993	5.905806	<0.05
within	27	2122.671	78.61743		
total	29				

S.S- Sum of Square, M.S.S.-Mean Sum of Square, d.f.-Degree of Freedom

Table 4.1.5c, states the results of test statistics used in the different parts (anterior, middle, and posterior) of medial and lateral OA meniscus of mucicarmine. Intensity of acid mucopolysaccharides on both sides of the legs showed significant negative (-) and weak (±) varying by F-test (p- <0.05).

Table 4.1.5d: Association of mucicarmine staining intensity of acid mucopolysaccharides – medial and lateral menisci on side of the legs.

Mucicarmine: Acid mucopolysaccharides		Side				Chi-square (df), p-value
		Left		Right		
		n	%	n	%	
Medial	Negative	42	75	34	63	1.86(1), 0.172
	Weak	14	25	20	37	
Lateral	Negative	45	80.4	42	77.8	0.11(1), 0.739
	Weak	11	19.6	12	22.2	

Table 4.1.5d, states the association in the medial and lateral OA meniscus of mucicarmine staining intensity of acid mucopolysaccharides on both sides of the legs. Medial menisci of the left and right leg have 75% and 63% negative, 25% and 37% weak color intensity of acid mucopolysaccharides respectively. However, lateral menisci of the left and right legs have respectively 80.4% and 77.8% negative, 19.6% and 22.2% weak staining intensity of acid mucopolysaccharides. Moreover, the sides of the legs do not show a significant association between the medial and lateral meniscus.

Hyaluronidase enzyme labile technique

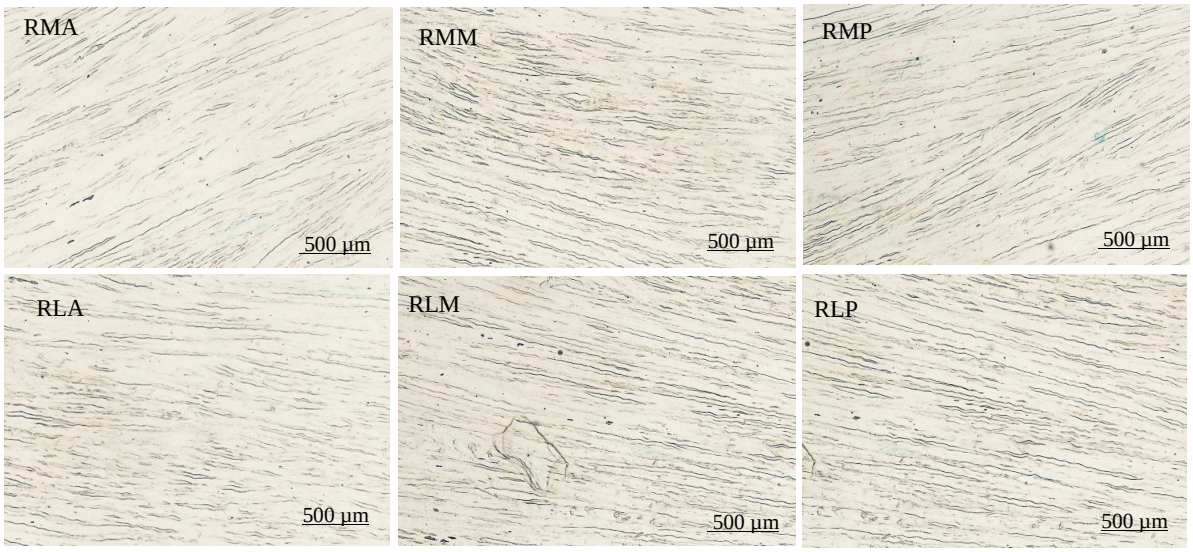


Figure 4.1.6a: The intensity of hyaluronic acid in the control group of sheep menisci: magnification 100x.

Fig. 4.1.6a. shows an assessment of the histological staining intensity of hyaluronic acid in the extracellular matrix of sheep meniscus used as control group and sections treated with bovine testicular hyaluronidase at 3 regions (anterior, middle, posterior) of medial and lateral menisci of right leg: RMA, RMM, RMP, RLA, RLM, and RLP showed negative staining intensity with score (-). Note: While treated with bovine testicular hyaluronidase, negative (-) intensity indicate that there is presence of hyaluronic acid.

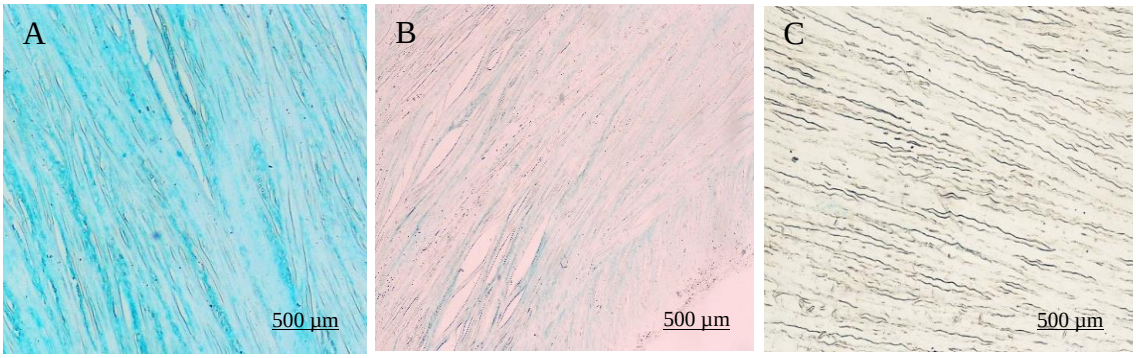


Figure 4.1.6b. The intensity of hyaluronic acid in the test group of OA menisci: magnification 100x.

Fig. 4.1.6b: shows an assessment of the histological staining intensity of hyaluronic acid in the extracellular matrix of OA human meniscus used as a test group and sections treated with bovine testicular hyaluronidase at 3 regions (anterior, middle, posterior) of medial and lateral menisci of both legs: figure 'A' score: Mild (+), figure 'B' score: Weak or variable stain (±), and figure 'C' score: Negative intensity (-). Note: When treated with bovine testicular hyaluronidase, negative (-) intensity indicated that there is presence of hyaluronic acid. Weak or variable (±) blue color intensity indicated the weak presence of hyaluronic acid and mild (+) blue color intensity indicated a total absence of hyaluronic acid.

Table 4.1.6a: Hyaluronidase enzyme labile stain level in medial menisci of both legs.

Hyaluronidase enzyme : (Hyaluronic acid)		Left		Right		Overall	
		n1	%	n2	%	n	%
Control (normal)	Negative (-)	1	100	1	100	2	100
Test (OA menisci)							
Medial anterior	*Negative (-)	8	14.3	6	11.1	14	12.7
	Weak (±)	20	35.7	18	33.3	38	34.5
	#Mild (+)	28	50.0	30	55.6	58	52.7
Medial middle	*Negative (-)	10	17.9	8	14.8	18	16.4
	Weak (±)	17	30.4	22	40.7	39	35.5
	#Mild (+)	29	51.8	24	44.4	53	48.2
Medial posterior	*Negative (-)	13	23.2	10	18.5	23	20.9
	weak (±)	20	35.7	18	33.3	38	34.5
	#Mild (+)	23	41.1	26	48.1	49	44.5

Note: * F- test = 4.05 (Negative), $P < 0.05$, # F- test = 19.36 (Mild), $P < 0.05$, n1+n2 = n

Table 4.1.6b: Hyaluronidase enzyme labile stain level in lateral menisci of both legs.

Hyaluronidase enzyme : (Hyaluronic acid)		Left		Right		Overall	
		n1	%	n2	%	n	%
Control (normal)	Negative (-)	1	100	1	100	2	100
Test (OA menisci)							
Lateral anterior	Negative (-)	10	17.9	9	16.7	19	17.3
	*Weak (±)	22	39.3	25	46.3	47	42.7
	#Mild (+)	24	42.9	20	37.0	44	40.0
Lateral middle	Negative (-)	11	19.6	8	14.8	19	17.3
	*Weak (±)	20	35.7	23	42.6	43	39.1
	#Mild (+)	25	44.6	23	42.6	48	43.6
Lateral posterior	Negative (-)	10	17.9	6	11.1	16	14.5
	*Weak (±)	22	39.3	20	37.0	42	38.2
	#Mild (+)	24	42.9	28	51.9	52	47.3

Note: * F- test =4.70 (Weak), $P < 0.05$, # F- test = 12.47 (Mild), $P < 0.05$, n1+n2 = n

Table 4.1.6a, reveals a histochemical assessment of hyaluronidase enzyme labile staining intensity level in the extracellular matrix of medial menisci of the left and right legs. The study showed the negative (-) staining intensity of hyaluronidase enzyme labile in the control and its score of (-) present of hyaluronic acid. It is present in both legs of the medial and lateral menisci of sheep. The test observation

made in the left leg medial meniscus anterior part (LMA) was 14.3% negative, 35.7% weak and 50% mild staining intensity of hyaluronidase enzyme labile technique. While it was 17.9% negative, 30.4% weak and 51.8% mild in the left leg medial meniscus middle part (LMM). Similarly, the staining intensity in the left leg medial meniscus posterior part (LMP) was 23.2% negative, 35.7% weak and 41.1% mild. Right leg medial meniscus anterior part (RMA) was 11.1% negative, 33.3% weak and 55.6% mild staining intensity, while it was 14.8 % negative, 40.7% weak and 44.5% mild staining intensity in RMM. Similarly, the staining intensity in RMP was 18.5% negative, 33.3% weak and 48.2% mild.

Similarly, in the left leg lateral meniscus anterior part (LLA) had 17.0% negative, 39.3% weak and 42.9% mild staining intensity of hyaluronidase enzyme labile technique, while it was 19.6% negative, 35.7% weak and 44.6% mild in the left leg lateral meniscus middle part (LLM). Again, staining intensity in the left leg lateral meniscus posterior part (LLP) was 17.9% negative, 39.3% weak and 42.9% mild. The right leg lateral meniscus anterior part (RLA) showed 16.7% negative, 46.3% weak and 37.0% staining intensity. It was negative at 14.8%, weak at 42.6%, and 42.6% mild staining intensity in RLM. Similarly, the staining intensity in RLP was 11.1% negative, 37.0% weak, and 51.9% mild (Table 4.1.6b).

Table 4.1.6c: Results of test statistics used in hyaluronidase enzyme intensity for hyaluronic acid – different parts of medial and lateral menisci on sides of the legs.

Hyaluronidase enzyme : (Hyaluronic acid)					
Source of variation	d.f.	s.s	M.S.S.	F-test	p-Value
Medial menisci anterior/middle/posterior (negative)					
between	2	608.812	304.406	4.052824	<0.05
within	52	3905.7	75.10961		
total	54				
Medial menisci anterior/middle/posterior (weak)					
between	2	25.77391	12.88696	0.217646	>0.05
within	112	6631.604	59.21075		
total	114				
Medial menisci anterior/middle/posterior (mild)					
between	2	1805.609	902.8047	19.36679	<0.05
within	157	7318.731	46.61612		
total	159				
Lateral menisci anterior/middle/posterior (negative)					
between	2	88.27259	44.1363	0.581178	>0.05
within	51	3873.085	75.94283		
total	53				

Lateral menisci anterior/middle/posterior (weak)					
between	2	512.3373	256.1686	4.70401	<0.05
within	129	7025.017	54.4575		
total	131				
Lateral menisci anterior/middle/posterior (mild)					
between	2	1274.092	637.0461	12.47889	<0.05
within	141	7198.038	51.04991		
total	143				

S.S- Sum of Square, M.S.S.-Mean Sum of Square, d.f.-Degree of Freedom

Table 4.1.6c, states the results of test statistics used in the different parts (anterior, middle, and posterior) of medial and lateral OA meniscus of hyaluronidase enzyme intensity for hyaluronic acid on both sides of the legs showing significant on negative (-) and mild (+) for medial meniscus and weak (±) and mild (+) for lateral meniscus varying by F-test (p- <0.05).

Table 4.1.6d: Association of hyaluronidase enzyme labile staining intensity of hyaluronic acid – medial and lateral menisci on sides of the legs.

Hyaluronidase enzyme: (Hyaluronic acid)		Side				chi square (df), p-value
		Left		Right		
		n	%	n	%	
Medial	Negative (-)	9	16.1	8	14.8	0.906 (2), 0.636
	Weak (±)	16	28.6	20	37.0	
	Mild (+)	31	55.4	26	48.1	
Lateral	Negative (-)	12	21.4	5	9.3	3.152 (2), 0.207
	Weak (±)	18	32.1	21	38.9	
	Mild (+)	26	46.4	28	51.9	

Table 4.1.6d: states the association in the medial and lateral OA meniscus of hyaluronidase enzyme labile staining intensity of hyaluronic acid on both sides of the legs. Medial menisci of the left and right leg have 55.4% and 48.1% mild (+), 28.6% and 37.0% weak (±), and 16.1% and 14.8% negative (-) of hyaluronic acid respectively. However, lateral menisci of the left and right leg have respectively 46.4% and 51.9% mild (+), 32.1 and 38.9% weak (±), and 21.4% and 9.3% negative (-) of hyaluronic acid. Moreover, the sides of the legs do not show a significant association between the medial and lateral meniscus.

Combine PAS + AB-2.5

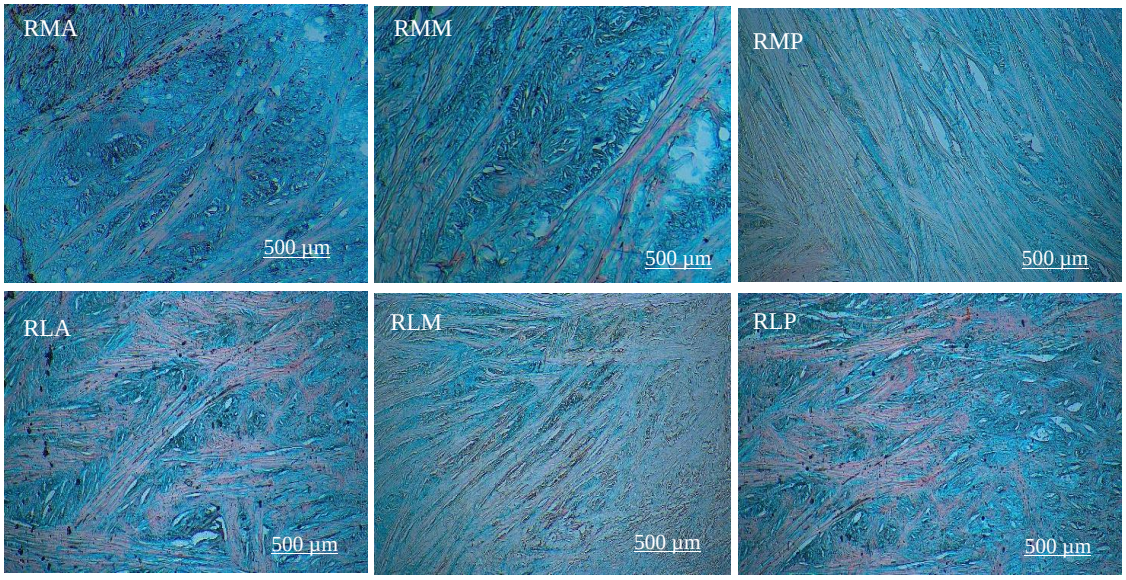


Figure 4.1.7a: - The Intensity of neutral and acidic mucins in the control group: magnification 100x.

Fig. 4.1.7a: shows an assessment of the histological staining intensity of PAS + AB -2.5 in the extracellular matrix of sheep meniscus used as control group at 3 regions (anterior, middle, posterior) of medial and lateral menisci of right leg: RMA, RMM, RMP, RLA, RLM and RLP showed strong staining with score (+ + +) of both stains.

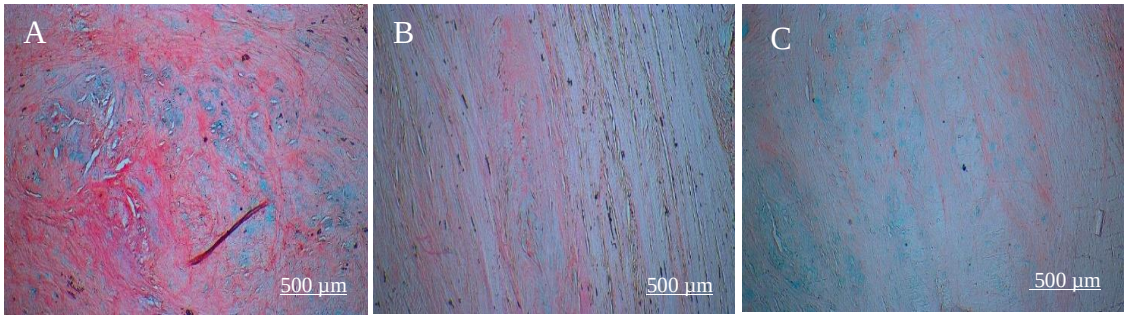


Figure 4.1.7b: - The intensity of neutral and acidic mucins in the test group: magnification 100x.

Fig. 4.1.7b: shows an assessment of the histological staining intensity of PAS+AB-2.5 in the extracellular matrix of OA human meniscus used as a test group at 3 regions (anterior, middle, posterior) of medial and lateral menisci of both legs: figure ‘A’ score: moderate PAS (+ +) and mild AB-2.5 (+) , figure ‘B’ score mild PAS (+) and weak AB-2.5 (±). , figure ‘C’ score weak PAS (±) and weak AB-2.5 (±) staining intensity.

Table 4.1.7a: PAS + AB -2.5 staining intensity of neutral and acidic mucins in medial menisci of both legs.

PAS + AB- 2.5 for neutral and acidic mucins.		Left		Right		Overall	
		n1	%	n2	%	n	%
Control (normal)	Strong (+++ P/AB)	1	100	1	100	2	100
Test (OA menisci)							
Medial anterior	*Mild +P	45	51.7	42	48.3	87	79.1
	Moderate ++P	2	28.6	5	71.4	7	6.4
	Weak ± P	9	56.3	7	43.8	16	14.5
	Mild +AB	2	28.6	5	71.4	7	6.4
	#Weak ± AB	54	52.4	49	47.6	103	93.6
Medial middle	*Mild +P	48	54.5	40	45.5	88	80.0
	Moderate ++P	3	27.3	8	72.7	11	10.0
	Weak ± P	5	45.5	6	54.5	11	10.0
	Mild +AB	3	27.3	8	72.7	11	10.0
	#Weak ± AB	53	53.5	46	46.5	99	90.0
Medial posterior	*Mild +P	41	48.8	43	51.2	84	76.4
	Moderate ++P	5	55.6	4	44.4	9	8.2
	Weak ± P	10	58.8	7	41.2	17	15.5
	Mild +AB	5	55.6	4	44.4	9	8.2
	#Weak ± AB	51	50.5	50	49.5	101	91.8

Note: * F- test = 15.65 (Mild +P), $P < 0.05$, # F- test = 45.01 (Weak ±A), $P < 0.05$, n1+n2 = n, P/AB: PAS/Alcian blue-2.5

Table 4.1.7b: PAS + AB -2.5 staining intensity of neutral and acidic mucins in lateral menisci of both legs.

PAS + AB- 2.5 for neutral and acidic mucins.		Left		Right		Overall	
		n1	%	n2	%	n	%
Control (normal)	Strong (+++ P/AB)	1	100	1	100	2	100
Test (OA menisci)							
Lateral anterior	*Mild +P	48	51.6	45	48.4	93	84.5
	Moderate ++P	5	62.5	3	37.5	8	7.3
	Weak ± P	3	33.3	6	66.7	9	8.2
	Mild +AB	5	62.5	3	37.5	8	7.3
	#Weak ± AB	51	50.0	51	50.0	102	92.7
Lateral middle	*Mild +P	51	51.0	49	49.0	100	90.9
	Moderate ++P	2	66.7	1	33.3	3	2.7
	Weak ± P	3	42.9	4	57.1	7	6.4
	Mild +AB	2	66.7	1	33.3	3	2.7
	#Weak ± AB	54	50.5	53	49.5	107	97.3
Lateral posterior	*Mild +P	50	52.1	46	47.9	96	87.3
	Moderate ++P	5	50.0	5	50.0	10	9.1
	Weak ± P	1	25.0	3	75.0	4	3.6
	Mild +AB	5	50.0	5	50.0	10	9.1
	#Weak ± AB	51	51.0	49	49.0	100	90.9

Note: * F- test = 87.85 (Mild +P), $P < 0.05$, # F- test = 196.08 (Weak ±A), $P < 0.05$

n1+n2 = n, P/AB: PAS/Alcian blue-2.5

Table 4.1.7a, reveals a histochemical assessment of PAS + AB-2.5 staining intensity level in the extracellular matrix of medial menisci of the left and right legs. The study showed a strong staining intensity of PAS + AB-2.5 in the control and its score of three plus (+ + +). It is present in both legs of the medial and lateral menisci of sheep. The test observation made in the left leg medial meniscus anterior part (LMA) was 51.7% mild, 28.6% moderate and 56.3% weak PAS +ve (neutral mucin), while 26.8% mild and 52.4% weak (acidic mucins) staining intensity of PAS + AB-2.5. It was 54.5% mild, 27.3% moderate and 45.5% weak PAS +ve (neutral mucin), while 27.3% mild and 53.5% weak (acidic mucins) in the left leg medial meniscus middle part (LMM). Similarly, the staining intensity in the left leg medial meniscus posterior part (LMP) was 48.8% mild, 55.6% moderate and 58.8% weak PAS +ve (neutral mucin), while 55.6% mild and 50.5% (acidic mucins). Right leg medial meniscus anterior part (RMA) was 48.3% mild, 71.4% moderate and 43.8% weak PAS +ve (neutral mucin), while 71.4% mild and 47.6% weak (acidic mucins) staining intensity, whereas it was 45.5% mild, 72.7% moderate and 54.5% weak PAS +ve (neutral mucin), while 72.7% mild and 46.5% weak (acidic mucins) staining intensity in RMM. Similarly, the staining intensity in RMP was 51.2% mild, 44.4% moderate and 41.2% weak PAS +ve (neutral mucin), while 44.4 % mild and 49.5 % weak (acidic mucins).

Similarly, table 4.1.7b, shows the results of the left leg lateral meniscus anterior part (LLA) had 51.6% mild, 62.5% moderate and 33.3% weak PAS +ve (neutral mucin), while 62.5% mild and 50.0% weak (acidic mucins) staining intensity of PAS + AB-2.5, while it was 51.0% mild, 66.7% moderate and 42.9% weak PAS +ve (neutral mucin), while 66.7% mild and 50.5% weak (acidic mucins) in the left leg lateral meniscus middle part (LLM). Also, staining intensity in the left leg lateral

meniscus posterior part (LLP) was 52.1% mild, 50.0% moderate and 25.0% weak PAS +ve (neutral mucin), while 50.0% mild and 51.0% weak (acidic mucins). The right leg lateral meniscus anterior part (RLA) showed 48.4% mild, 37.5% moderate and 66.7% weak PAS +ve (neutral mucin), while 37.5% mild and 50.0% weak (acidic mucins) staining intensity. It was 49.0 % mild, 33.3% moderate and 57.1% weak PAS +ve (neutral mucin), while 33.3% mild and 49.5% weak (acidic mucins) staining intensity in RLM. Similarly, the staining intensity in RLP was 47.9% mild, 50.0% moderate and 75.0% weak PAS +ve (neutral mucin), while 50.0% mild and 49.0% weak (acidic mucins).

Table 4.1.7c: Results of test statistics used in PAS + AB-2.5 color intensity of neutral and acidic mucins – different parts of medial menisci on sides of the legs.

PAS + AB-2.5 (Neutral and acidic mucins)					
Source of variation	d.f.	s.s	M.S.S.	F-test	p-Value
Medial menisci anterior/middle/posterior (mild +P)					
between	2	611.6979	305.8489	15.65947	<0.05
within	256	5000	19.53125		
total	258				
Medial menisci anterior/middle/posterior (moderate + +P)					
between	2	57.54515	28.77257	0.345843	>0.05
within	24	1996.694	83.19559		
total	26				
Medial menisci anterior/middle/posterior (weak ± P)					
between	2	214.1998	107.0999	1.366571	>0.05
within	41	3213.223	78.3713		
total	43				
Medial menisci anterior/middle/posterior (mild +AB)					
between	2	57.54515	28.77257	0.345843	>0.05
within	24	1996.694	83.19559		
total	26				
Medial menisci anterior/middle/posterior (weak ± AB)					
between	2	667.594	333.797	45.01052	<0.05
within	300	2224.793	7.415978		
total	302				

S.S- Sum of Square, M.S.S.-Mean Sum of Square, d.f.-Degree of Freedom.

Table 4.1.7c: states the results of test statistics used in different parts (anterior, middle, and posterior) of medial OA meniscus of PAS + AB-2.5. Staining intensity of neutral and acidic mucins on both sides of the legs showing significant on mild (+) PAS and weak (±) AB varying by F-test (p- <0.05).

Table 4.1.7d: Results of test statistics used in PAS + AB-2.5 color intensity of neutral and acidic mucins – different parts of lateral menisci on sides of the legs.

PAS + AB-2.5 (neutral and acidic mucins)					
Source of variation	d.f.	s.s	M.S.S.	F-test	p-Value
Lateral menisci anterior/middle/posterior (mild +P)					
between	2	1972.181	986.0905	87.85955	<0.05
within	286	3209.917	11.22349		
total	288				
Lateral menisci anterior/middle/posterior (moderate ++P)					
between	2	94.05746	47.02873	0.560331	>0.05
within	18	1510.744	83.93021		
total	20				
Lateral menisci anterior/middle/posterior (weak ± P)					
between	2	58.01653	29.00826	0.342144	>0.05
within	17	1441.322	84.78367		
total	19				
Lateral menisci anterior/middle/posterior (mild +AB)					
between	2	94.05746	47.02873	0.560331	>0.05
within	18	1510.744	83.93021		
total	20				
Lateral menisci anterior/middle/posterior (weak ± AB)					
between	2	2241.167	1120.584	196.0809	<0.05
within	306	1748.76	5.714903		
total	308				

S.S- Sum of Square, M.S.S.-Mean Sum of Square, d.f.-Degree of Freedom.

Table 4.1.7d: states the results of test statistics used in the different parts (anterior, middle, and posterior) of lateral OA meniscus of PAS + AB-2.5. Staining intensity of neutral and acidic mucins on both sides of the legs showed significant mild (+) PAS and weak (±) AB varying by F-test (p- <0.05).

Table 4.1.7e: Association of PAS + AB-2.5 color intensity of neutral and acidic mucins – medial and lateral menisci on sides of the legs.

PAS + AB-2.5 (neutral and acidic) mucins		Side				Chi-square (df), p-value
		Left		Right		
		n	%	n	%	
Medial	Mild (+P)	40	69.0	37	67.3	3.649 (3), 0.456
	Moderate (+ +P)	9	15.5	11	20.0	
	Mild (+AB)	0	0.0	3	5.7	
	Weak (± AB)	54	100.0	50	94.3	
	Weak (± P)	9	15.5	7	12.7	
Lateral	Mild (+P)	47	79.7	45	78.9	0.213 (4), 0.995
	Moderate (+ +P)	9	15.3	8	14.0	
	Mild (+AB)	3	5.7	3	5.9	
	Weak (± AB)	50	94.3	48	94.1	
	Weak (± P)	3	5.1	4	7.0	

Table 4.1.7e: states the association in the medial and lateral OA meniscus of PAS + AB-2.5 color intensity of neutral and acidic mucins on both sides of the legs. Medial menisci of the left and right leg have 69.0% and 67.3% mild (+P), 15.5% and 20.0% moderate (+ +P), and 15.5% and 12.7% weak (± P) PAS +ve (neutral) mucin intensity respectively. However, acidic mucins have 100% and 94.3% weak (± AB) and 5.7% mild (+AB) on the right side of the leg. While lateral menisci of the left and right leg have respectively 79.7% and 78.9% mild (+P), 15.3%, and 14.0% moderate (+ +P), and 5.1% and 7.0% weak (± P) PAS +ve (neutral) mucin intensity. Whereas, staining intensity of acidic mucins have 94.3% and 94.1% weak (± AB), and 5.7% and 5.9% mild (+AB) respectively. However, the sides of the legs do not show a significant association between the medial and lateral meniscus.

AF + AB-2.5 (combine technique)

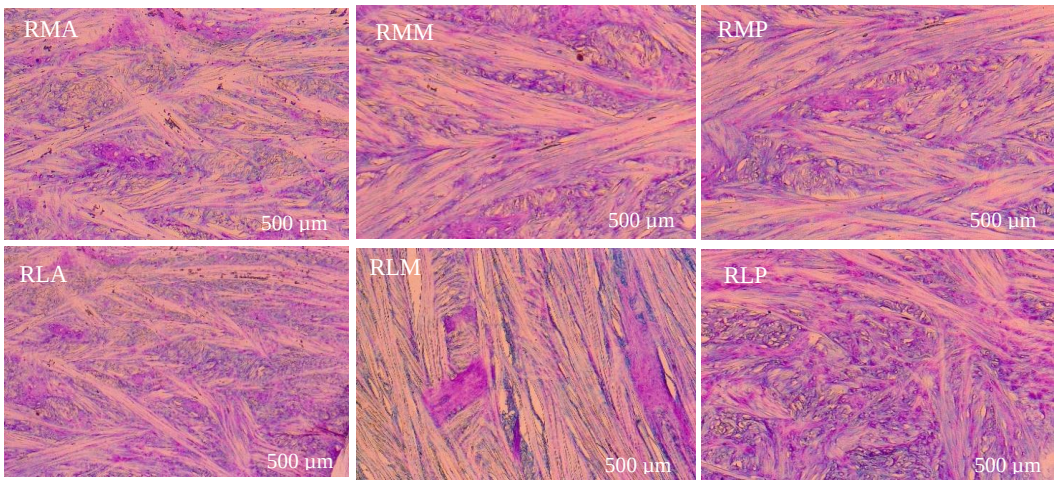


Figure 4.1.8a - The Intensity of AF + AB 2.5 (sulfated + carboxylated) mucins in the control group: magnification 100x.

Fig 4.1.8a: shows an assessment of the histological staining intensity of AF + AB 2.5 in the extracellular matrix of sheep meniscus used as control group at 3 regions (anterior, middle, posterior) of medial and lateral menisci of right leg: RMA, RMM, RMP, RLA, RLM, and RLP showed moderate staining with score (+ +).

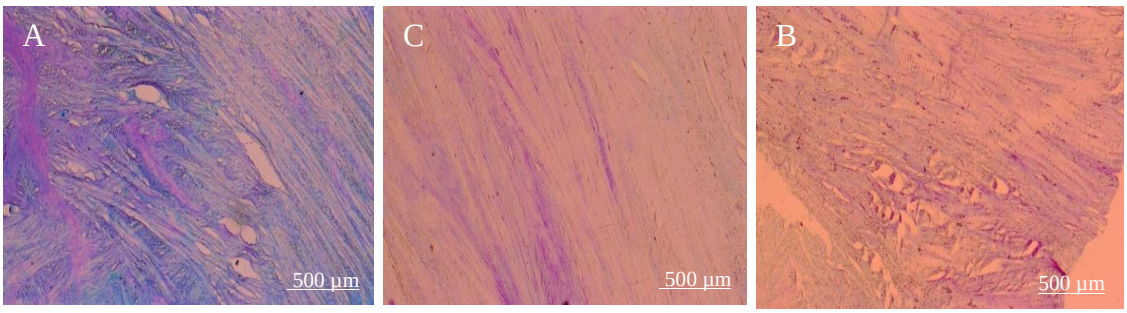


Figure 4.1.8b. The intensity of AF + AB 2.5 (sulfated + carboxylated) mucins in the test group: magnification 100x.

Fig. 4.1.8b shows an assessment of the histological staining intensity of AF + AB 2.5 in the extracellular matrix of OA human meniscus used as a test group at 3 regions (anterior, middle, posterior) of medial and lateral menisci of both legs. Figure 'A' score: mild color intensity of AB (+) and weak AF (±), figure 'B' score: mild

color intensity of AF (+) and weak staining intensity of AB (±), and figure 'C' score: weak staining intensity of AB (±) and AF (±).

Table 4.1.8a: AF + AB 2.5 staining intensity of sulfated + carboxylated mucins in medial menisci of both legs.

AF + AB 2.5 (sulfated + carboxylated) mucins		Left		Right		Overall	
		n1	%	n2	%	n	%
Control (normal)	Moderate (++) AF & AB	1	100	1	100	2	100
Test (OA) menisci							
Medial anterior	Mild (+) AB	3	50.0	3	50.0	6	5.5
	*Weak (±) AB	53	51.0	51	49.0	104	94.5
	#Mild (+) AF	28	49.1	29	50.9	57	51.8
	$Weak (±) AF	28	52.8	25	47.2	53	48.2
Medial middle	Mild (+) AB	4	57.1	3	42.9	7	6.4
	*Weak (±) AB	52	50.5	51	49.5	103	93.6
	#Mild (+) AF	27	52.9	24	47.1	51	46.4
	$Weak (±) AF	29	49.2	30	50.8	59	53.6
Medial posterior	Mild (+) AB	1	25.0	3	75.0	4	3.6
	*Weak (±) AB	55	51.9	51	48.1	106	96.4
	#Mild (+) AF	19	47.5	21	52.5	40	36.4
	$Weak(±) AF	37	52.9	33	47.1	70	63.6

Note: * F- test = 43.26 (Weak ±AB), P<0.05, # F- test = 57.23 (Mild +AF), P < 0.05, $F-test = 95.41(Weak ±AF), P<0.05, n1+n2 = n, AB: Alcian blue 2.5, AF: Aldehyde fuschion

Table 4.1.8b: AF + AB 2.5 staining intensity of sulfated + carboxylated mucins in lateral menisci of left and right legs.

AF + AB 2.5 (sulfated + carboxylated) mucins		Left		Right		Overall	
		n1	%	n2	%	n	%
Control (normal)	Moderate (++) AF & AB	1	100	1	100	2	100
Test (OA) menisci							
Lateral anterior	Mild (+) AB	0	0.0	2	100.0	2	1.8
	*Weak (±) AB	56	51.9	52	48.1	108	98.2
	#Mild (+) AF	23	46.0	27	54.0	50	45.5
	$Weak (±) AF	33	55.0	27	45.0	60	54.5
Lateral middle	Mild (+) AB	2	50.0	2	50.0	4	3.6
	*Weak (±) AB	54	50.9	52	49.1	106	96.4
	#Mild (+) AF	25	48.1	27	51.9	52	47.3
	$Weak (±) AF	31	53.4	27	46.6	58	52.7
Lateral posterior	Mild (+) AB	0	0.0	1	100.0	1	0.9
	*Weak (±) AB	56	51.4	53	48.6	109	99.1
	#Mild (+) AF	23	48.9	24	51.1	47	42.7
	$Weak (±) AF	33	52.4	30	47.6	63	57.3

Note: * F- test = 107.8 (Weak ±AB), P<0.05, # F- test = 5.16 (Mild +AF), P < 0.05, $F-test = 7.75(Weak ±AF), P<0.05, n1+n2 = n, AB: Alcian blue 2.5, AF: Aldehyde fuschion

Table 4.1.8a: reveals a histochemical assessment of AF + AB 2.5 staining intensity level in the extracellular matrix of medial menisci of the left and right legs. The study shows the moderate staining intensity of AF + AB 2.5 in the control and its score of two plus (+ +) sulfated + carboxylated mucins. It is present in both legs

of the medial and lateral menisci of sheep. However, the test observation made in the left leg medial meniscus anterior part (LMA) was 49.1% mild, 52.8 % weak (sulfated mucin), and 50.0 % mild and 51.0% weak (carboxylated mucin) staining intensity of AF + AB 2.5 while, it was 52.9% mild, 49.2% weak (sulfated mucin) and 57.1% mild, 50.5% weak (carboxylated mucin) in the left leg medial meniscus middle part (LMM). Similarly, the staining intensity in the left leg medial meniscus posterior part (LMP) was 47.5% mild, 52.9% weak (sulfated mucin) and 25.0 % mild, 51.9% weak (carboxylated mucin). Right leg medial meniscus anterior part (RMA) was 50.9 % mild, 47.2% weak (sulfated mucin) and 50.0 % mild, 49.0% weak (carboxylated mucin) staining intensity, while it was 47.1% mild, 50.8 % weak (sulfated mucin) and 42.9 % mild, 49.5% weak (carboxylated mucin)staining intensity in RMM. Similarly, the staining intensity in RMP was 52.5% mild, 47.1 % weak (sulfated mucin) and 75.0% mild, 48.1% weak (carboxylated mucin).

Similarly, table 4.1.8b, shows the result in the left leg lateral meniscus anterior part (LLA) had 46.0% mild, 55.0% weak (sulfated mucin), and 51.9% weak (carboxylated mucin) staining intensity of AF + AB 2.5, while it was 48.1% mild, 53.4% weak (sulfated mucin) and 50.0 % mild, 50.9% weak (carboxylated mucin) in the left leg lateral meniscus middle part (LLM). Similarly, staining intensity in the left leg lateral meniscus posterior part (LLP) was 48.9 % mild, 52.4% weak (sulfated mucin), and 51.4% weak (carboxylated mucin). The right leg lateral meniscus anterior part (RLA) showed 54.0 % mild, 45.0% weak (sulfated mucin), and 100 % mild, 48.1% weak (carboxylated mucin) staining intensity. While, it was 46.6% mild, 51.9% weak (sulfated mucin) and 50.0 % mild, 49.1% weak (carboxylated mucin) staining intensity in RLM. Similarly, the staining intensity in RLP was 51.1 % mild, 47.6% weak (sulfated mucin), and 100 % mild, 48.6% weak (carboxylated mucin).

Table 4.1.8c: Results of test statistics used in AF + AB 2.5 staining intensity of sulfated + carboxylated mucins – different parts of medial menisci on sides of the legs.

AF + AB 2.5 (sulfated + carboxylated) mucins					
Source of variation	d.f.	s.s	M.S.S.	F-test	p-Value
Medial menisci anterior/middle/posterior (weak ± AB)					
between	2	404.1666	202.0833	43.26566	<0.05
within	310	1447.934	4.670754		
total	312				
Medial menisci anterior/middle/posterior (moderate + AB)					
between	2	18.95965	9.479825	0.110294	>0.05
within	14	1203.306	85.95041		
total	16				
Medial menisci anterior/middle/posterior (weak ± AF)					
between	2	7649.133	3824.566	95.41152	<0.05
within	179	7175.207	40.08495		
total	181				
Medial menisci anterior/middle/posterior (mild + AF)					
between	2	5642.238	2821.119	57.23466	<0.05
within	145	7147.107	49.2904		
total	147				

S.S- Sum of Square, M.S.S.-Mean Sum of Square, d.f.-Degree of Freedom.

Table 4.18c: states the results of test statistics used in the different parts (anterior, middle, and posterior) of medial OA meniscus of AF + AB 2.5. Staining intensity of sulfated + carboxylated mucins on both sides of the legs showing significant weak (±) AB, weak (±) AF and mild (+) AF varying by F-test (p- <0.05).

Table 4.1.8d: Results of test statistics used in AF + AB 2.5 staining intensity of sulfated + carboxylated mucins – different parts of medial menisci on sides of the legs.

AF + AB 2.5 (sulfated + carboxylated) mucins					
Source of variation	d.f.	s.s	M.S.S.	F-test	p-Value
Lateral menisci anterior/middle/posterior (weak ± AB)					
between	2	413.3511	206.6755	107.8501	<0.05
within	320	613.2231	1.916322		
total	322				
Lateral menisci anterior/middle/posterior (Moderate + AB)					
between	2	8.264463	4.132231	0.046948	>0.05
within	4	352.0661	88.01653		
total	6				
Lateral menisci anterior/middle/posterior (weak ± AF)					
between	2	635.9984	317.9992	7.758344	<0.05
within	178	7295.868	40.98802		
total	180				
Lateral menisci anterior/middle/posterior (mild + AF)					
between	2	513.8943	256.9471	5.16056	<0.05
within	146	7269.421	49.79056		
total	148				

S.S- Sum of Square, M.S.S.-Mean Sum of Square, d.f.-Degree of Freedom.

Table 4.1.8d: states the results of test statistics used in different parts (anterior, middle, and posterior) of lateral OA meniscus of AF + AB 2.5. Staining intensity of sulfated + carboxylated mucins on both sides of the legs showing significant weak (±) AB, weak (±) AF and mild (+) AF varying by F-test (p- <0.05).

Table 4.1.8e: Association of AF + AB-2.5 color intensity of sulfated & carboxylated mucins – medial and lateral menisci on sides of the legs.

AF + AB-2.5 (sulfated and carboxylated) mucins		Side				Chi-square (df), p-value
		Left		Right		
		n	%	n	%	
Medial	Weak (±) AB	52	98.1	50	96.2	0.593 (3), 0.898
	Mild (+) AB	1	1.9	2	3.8	
	Weak (±) AF	31	52.5	27	48.2	
	Mild (+) AF	28	47.5	29	51.8	
Lateral	Weak (±) AB	51	98.1	52	96.3	0.871 (3), 0.833
	Mild (+) AB	1	1.9	2	3.7	
	Weak (±) AF	33	55.0	27	50.0	
	Mild (+) AF	27	45.0	27	50.0	

Table 4.1.8e: stated the association in the medial and lateral OA meniscus of AF + AB-2.5 color intensity of sulfated & carboxylated mucins on both sides of the legs. Medial menisci of the left and right leg have 98.1% and 96.2% weak and 1.9% and 3.8% mild carboxylated mucins, whereas 52.5% and 48.2% weak, and 47.5% and 51.8% mild sulfated mucins intensity respectively. However, lateral menisci of the left and right leg have respectively 98.1% and 96.3% weak, and 1.9% and 3.7% mild intensity of carboxylated mucins. While 55.0% and 50% weak, and 45% and 50% mild intensity of sulfated mucins. Moreover, the sides of the legs do not show a significant association between the medial and lateral meniscus.

Orcein + AB -2.5

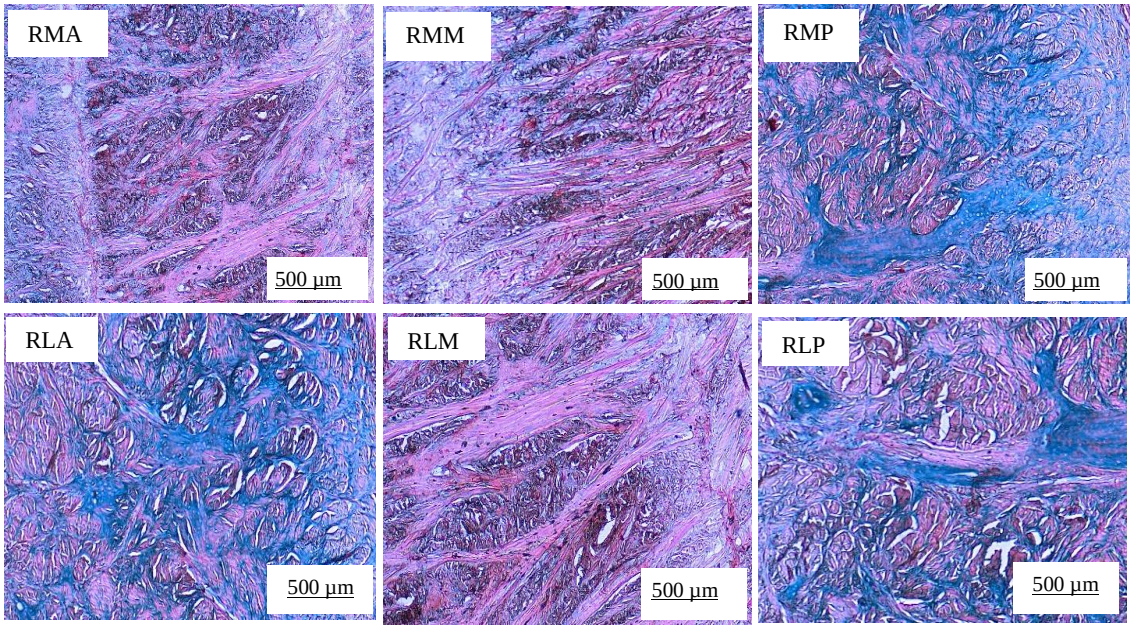


Figure 4.1.9a - The intensity of sulpho and sialo mucins in the control group: magnification 100x.

Fig. 4.1.9a. shows an assessment of the histological staining intensity of Orcein + AB -2.5 in the extracellular matrix of sheep meniscus used as control group at 3 regions (anterior, middle, posterior) of medial and lateral menisci of right leg: RMA, RMM, RMP, RLA, RLM, and RLP with moderate staining and a score (+ +).

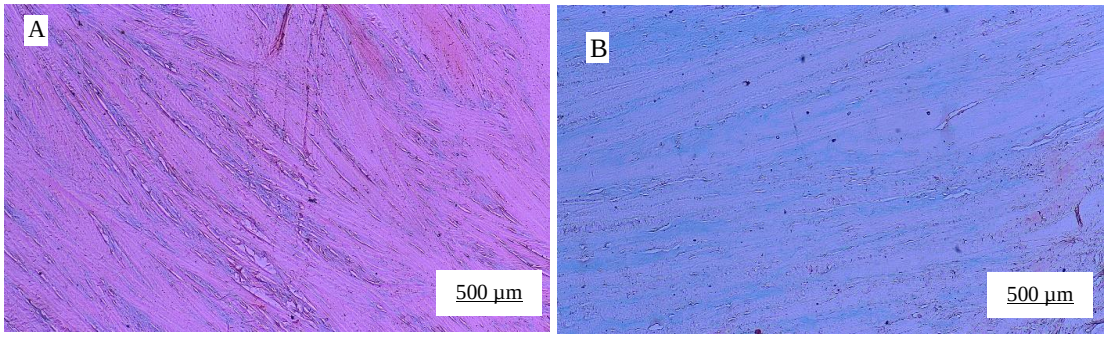


Figure4.1.9b. The intensity of sulpho and sialo mucins in the test group: magnification 100x.

Fig. 4.1.9b shows an assessment of the histological staining intensity of Orcein + AB-2.5 in the extracellular matrix of OA human meniscus used as a test group at 3 regions (anterior, middle, posterior) of medial and lateral menisci of both legs: figure 'A' score: weak 'AB-2.5' (±) and mild 'O' (+), figure 'B' score: weak 'O'(±) and mild 'AB-2.5' (+) staining intensity.

Table 4.1.9a: Orcein + AB -2.5 staining intensity of sulpho and sialo mucins in medial menisci of both legs.

Orcein + AB 2.5 (sulpho and sialo) mucin		Left		Right		Overall	
		n1	%	n2	%	n	%
Control (normal)	Moderate (++) O/AB	1	100	1	100	2	100
Test (OA) menisci							
Medial anterior	*Mild (+) O	45	54.9	37	45.1	82	74.5
	#Weak (±) O	11	39.3	17	60.7	28	25.5
	$Mild (+) AB	11	39.3	17	60.7	28	25.5
	@Weak (±) AB	45	54.9	37	45.1	82	74.5
Medial middle	*Mild (+) O	50	55.6	40	44.4	90	81.8
	#Weak (±) O	6	30.0	14	70.0	20	18.2
	$Mild (+) AB	6	30.0	14	70.0	20	18.2
	@Weak (±) AB	50	55.6	40	44.4	90	81.8
Medial posterior	*Mild (+) O	47	52.2	43	47.8	90	81.8
	#Weak (±) O	9	45.0	11	55.0	20	18.2
	$Mild (+) AB	9	45.0	11	55.0	20	18.2
	@Weak (±) AB	47	52.2	43	47.8	90	81.8

Note: * F- test = 80.11 (Mild +O), P<0.05, # F- test = 6.08 (Weak ± O), P < 0.05, $F-test = 6.08(Mild +AB), P<0.05, @F-test = 80.11 (Weak ±AF), n1+n2 = n, O/AB: Orcein / Alcian blue-2.5.

Table 4.1.9b: Orcein + AB -2.5 staining intensity of sulpho and sialo mucins in lateral menisci of both legs.

Orcein + AB 2.5 (sulpho and sialo) mucins		Left		Right		Overall	
		n1	%	n2	%	n	%
Control (normal)	Moderate (++) O/AB	1	100	1	100	2	100
Test (OA) menisci							
Lateral anterior	*Mild (+) O	43	50	43	50.0	86	78.2
	Weak (±) O	13	54	11	45.8	24	21.8
	Mild (+) AB	13	54	11	45.8	24	21.8
	#Weak (±) AB	43	50	43	50.0	86	78.2
Lateral middle	*Mild (+) O	44	53	39	47.0	83	75.5
	Weak (±) O	12	44	15	55.6	27	24.5
	Mild (+) AB	12	44	15	55.6	27	24.5
	#Weak (±) AB	44	53	39	47.0	83	75.5
Lateral posterior	*Mild (+) O	48	57	36	42.9	84	76.4
	Weak (±) O	8	31	18	69.2	26	23.6
	Mild (+) AB	8	31	18	69.2	26	23.6
	#Weak (±) AB	48	57	36	42.9	84	76.4

Note: * F- test = 7.7 (Mild +O), P<0.05, # F- test = 7.7 (Weak ± AB), P < 0.05, n1+n2 = n, O/AB: Orcein / Alcian blue-2.5.

Table 4.1.9a, reveals a histochemical assessment of Orcein + AB-2.5 staining intensity level in the extracellular matrix of medial menisci of the left and right legs. The study shows the moderate staining intensity of Orcein + AB 2.5 in the control and its score of two plus (+ +). It is present in both legs of the medial and lateral menisci

of sheep. The test observation in the left leg medial meniscus anterior part (LMA) was 54.9% mild, 39.3% weak (sulphomucin), and 39.3% mild, 54.9% weak (sialomucin) staining intensity of Orcein + AB 2.5, while it was 55.6% mild, 30% weak (sulphomucin) and 30% mild, 55.6% weak (sialomucin) in the left leg medial meniscus middle part (LMM). Similarly, the staining intensity in the left leg medial meniscus posterior part (LMP) was 52.2% mild, 45.0% weak (sulphomucin) and 45.0% mild, 52.2% weak (sialomucin). Right leg medial meniscus anterior part (RMA) was 45.1% mild, 60.7% weak (sulphomucin) and 60.7% mild, 45.1% weak (sialomucin) staining intensity, while it was 44.4% mild, 70.0% weak (sulphomucin) and 70.0% mild, 44.4% weak (sialomucin) staining intensity in RMM. Similarly, the staining intensity in RMP was 90.0% mild, 20% weak (sulphomucin) and 20% mild, 90% weak (sialomucin).

Similarly, in table 4.1.9b, the result in the left leg lateral meniscus anterior part (LLA) showed 50% mild, 54% weak (sulphomucin), and 54% mild, 50% weak (sialomucin) staining intensity of Orcein + AB 2.5, while it was 53.0% mild, 44.0% weak (sulphomucin) and 44.0% mild, 53.0% weak (sialomucin) in the left leg lateral meniscus middle part (LLM). Similarly, staining intensity in the left leg lateral meniscus posterior part (LLP) was 57% mild, 31% weak (sulphomucin), and 31% mild, 57.0% weak (sialomucin). The right leg lateral meniscus anterior part (RLA) showed 50.0% mild, 45.8% weak (sulphomucin), and 45.8% mild, 50.0% weak (sialomucin) staining intensity, while it was 47.0% mild, 55.6% weak (sulphomucin), and 55.6% mild, 47% weak (sialomucin) staining intensity in RLM. Similarly, the staining intensity in RLP was 42.9% mild, 69.2% weak (sulphomucin), and 69.2% mild, 42.9% weak (sialomucin).

Table 4.1.9c: Association of Orcein + AB -2.5 staining intensity of sulpho and sialo mucins – different parts of medial menisci on sides of the legs.

Orcein + AB 2.5 (sulpho and sialo) mucins					
Source of variation	d.f.	s.s	M.S.S.	F-test	p-Value
Medial menisci anterior/middle/posterior (mild +O)					
between	2	2979.749	1489.874	80.11526	<0.05
within	259	4816.529	18.59664		
total	261				
Medial menisci anterior/middle/posterior (weak ±O)					
between	2	871.1716	435.5858	6.080728	<0.05
within	65	4656.198	71.63382		
total	67				
Medial menisci anterior/middle/posterior (mild +AB)					
between	2	871.1716	435.5858	6.080728	<0.05
within	65	4656.198	71.63382		
total	67				
Medial menisci anterior/middle/posterior (weak ±AB)					
between	2	2979.749	1489.874	80.11526	<0.05
within	259	4816.529	18.59664		
total	261				

S.S- Sum of Square, M.S.S.-Mean Sum of Square, d.f.-Degree of Freedom.

Table 4.1.9c: states the association in the different parts (anterior, middle, and posterior) of medial OA meniscus of Orcein + AB -2.5 staining intensity of sulpho and sialo mucins on both sides of the legs showing significant mild + O, weak ± O, mild + AB and weak ± AB varying by F-test (p- <0.05).

Table 4.1.9d: Association of Orcein + AB -2.5 staining intensity of sulpho and sialo mucins – different parts of lateral menisci on sides of the legs.

Orcein + AB 2.5 (sulpho and sialo) mucins					
Source of variation	d.f.	s.s	M.S.S.	F-test	p-Value
Lateral menisci anterior/middle/posterior (mild +O)					
between	2	327.0179	163.509	7.71389	<0.05
within	250	5299.174	21.19669		
total	252				
Lateral menisci anterior/middle/posterior (weak ±O)					
between	2	96.91961	48.4598	0.695813	>0.05
within	74	5153.719	69.64485		
total	76				
Lateral menisci anterior/middle/posterior (mild +AB)					
between	2	96.91961	48.4598	0.695813	>0.05
within	74	5153.719	69.64485		
total	76				
Lateral menisci anterior/middle/posterior (weak ±AB)					
between	2	327.0179	163.509	7.71389	<0.05
within	250	5299.174	21.19669		
total	252				

S.S- Sum of Square, M.S.S.-Mean Sum of Square, d.f.-Degree of Freedom.

Table 4.1.9d: states the association in different parts (anterior, middle, and posterior) of medial OA meniscus of Orcein + AB -2.5 staining intensity of sulpho and sialo mucins on both sides of the legs showing significant mild + O and weak ± AB varying by F-test (p- <0.05).

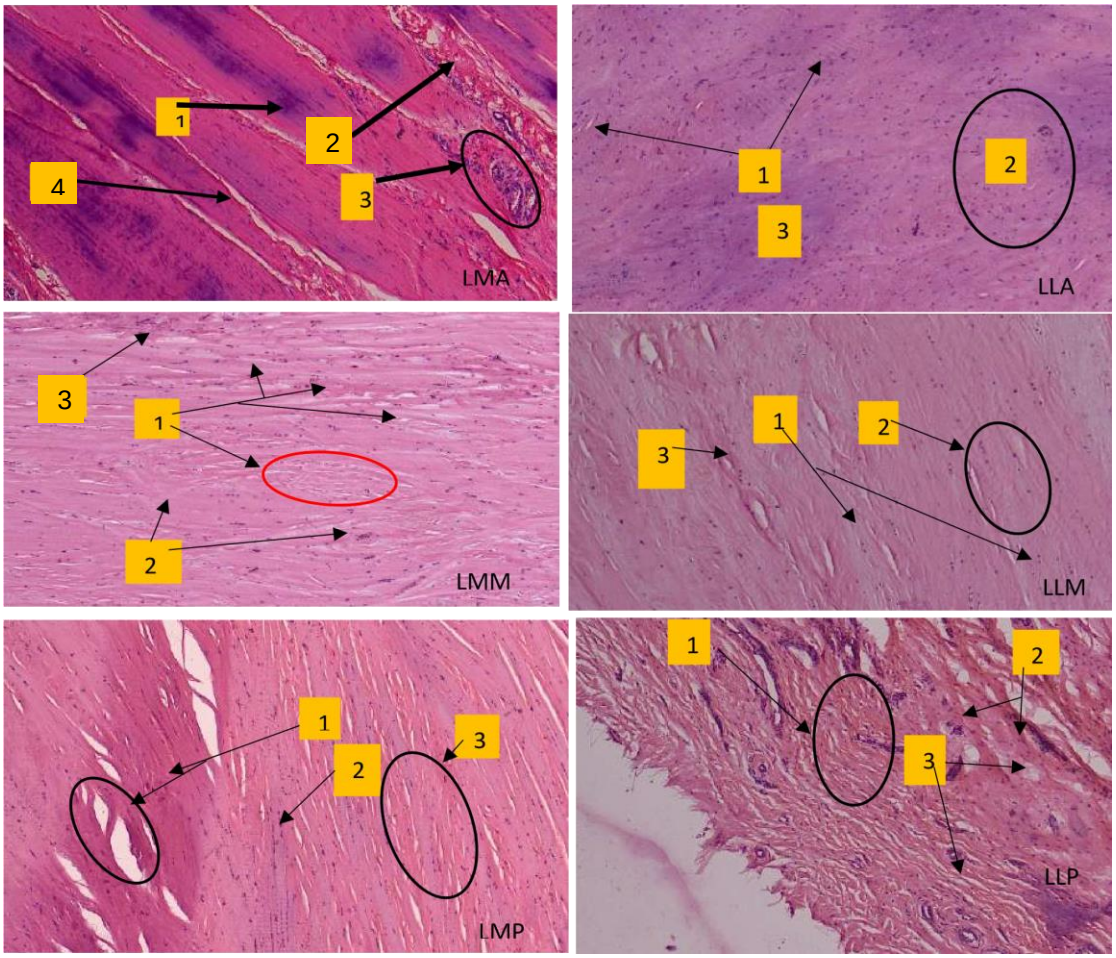
Table 4.1.9e: Association of Orcein + AB-2.5 color intensity of sulphomucins & sialomucins – medial and lateral menisci on sides of the legs.

Orcein + AB -2.5 (Sulpho and Sialo) mucins		Side				Chi-square (df), p-value
		Left		Right		
		n	%	n	%	
Medial	Mild (+) O	48	85.7	42	77.8	2.778 (3), 0.427
	Weak (±) O	8	14.3	12	22.2	
	Mild (+) AB	9	16.1	14	25.9	
	Weak (±) AB	47	83.9	40	74.1	
Lateral	Mild (+) O	47	83.9	43	78.2	2.196 (3), 0.533
	Weak (±) O	9	16.1	12	21.8	
	Mild (+) AB	12	21.4	17	32.1	
	Weak (±) AB	44	78.6	36	67.9	

Table 4.1.9e, states the association in the medial and lateral OA meniscus of Orcein + AB-2.5 color intensity of sulphomucins & sialomucins on both sides of the legs. Medial menisci of the left and right leg have 85.7% and 77.8% mild, and 14.3% and 22.2% weak intensity of sulphomucins. While 16.1% and 25.9% mild, and 83.9% and 74.1% weak intensity of sialomucin respectively. However, lateral menisci of the left and right leg have respectively 83.9% and 78.2% mild, and 16.1% and 21.8% weak intensity of sulphomucins, while 21.4%, and 32.1% mild, and 78.6% and 67.9% weak intensity of sialomucins. Moreover, the sides of the legs do not show a significant association between the medial and lateral OA meniscus.

Microscopic structure of menisci in osteoarthritic patients

Haematoxylin and Eosin (H & E)



LMA – Left leg medial menisci anterior part**, LMM** – Left leg Medial menisci middle part, **LMP** –Left leg medial menisci posterior part
LLA – Left leg lateral menisci anterior part, **LLM** – Left leg lateral menisci middle part, **LLP** – Left leg lateral menisci posterior part

Figure 4.2.1a -: Haematoxylin and Eosin (H & E) stain, magnification 100x (Olympus Microscope)

Histological assessment of meniscus in LMA

Histological evaluation of the left leg medial meniscus (LMA) of the anterior part is shown in Fig. 4.2.1a, where 1 indicates bands of degenerative changes, 2 degenerated extracellular matrix substance (mucoid) is observed, 3 diffuse hypercellularity with inflammation is observed, 4 separation of fibrocartilage with organized collagen fibers.

Histological assessment of meniscus in LMM

Histologic evaluation of the meniscus in the middle medial meniscus (LMM) of the left leg is shown in Fig. 4.2.1a, where 1 indicates moderate abrasion, fraying and waviness, few indentations, and few clefts, 2 the disorganized cellular pattern showed hypocellular to acellular areas and cell clusters and 3 indicates the presence of degenerated extracellular matrix; most of the collagen fibers appear unorganized.

Histological assessment of meniscus in LMP

Figure 4.2.1a, shows the histological evaluation of the meniscus in the left leg medial meniscus posterior part (LMP), where 1 indicates moderate to severe abrasion with a tear, 2 diffuse hypercellularity is seen. 3 fibrocartilage separation in the presence of disorganized collagen fibers.

Histological assessment of meniscus in LLA

Fig. 4.2.1a, discloses histological assessment of meniscus in left leg lateral meniscus anterior part (LLA), where 1 indicates slight abrasion and wrinkle, 2 indicates diffuse hypercellularity, and 3 indicates loose and degenerative changes.

Histological assessment of meniscus in LLM

Fig. 4.2.1a, reveals a histological assessment of meniscus in the left leg lateral meniscus middle part (LLM), where 1 represents a slightly worn and undulating surface, 2 represents a normal distribution of cells. The cells appear to be more arranged between fibers, and 3 degenerated extracellular matrix substance and collagen fibers are unorganized.

Histological assessment of meniscus in LLP

Fig. 4.2.1a, shows a histological assessment of the meniscus in the left leg lateral meniscus posterior part (LLP), where 1 indicates severe abrasion, tear, and disruption, 2 depicts diffuse hypercellularity with clusters of cells, and 3 fibrocartilage dissociation (edema, mucosal degeneration, cystic fissures, and disjointed collagen fibers.

Alcian blue at pH 2.5 (AB-2.5)

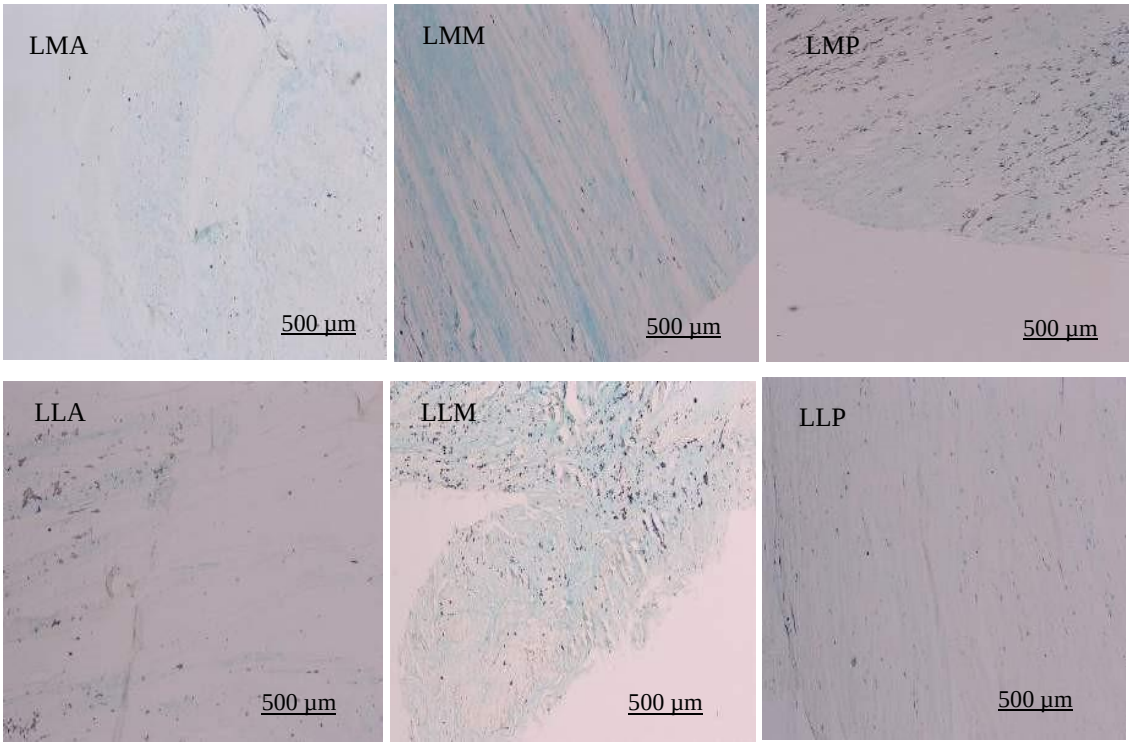


Figure 4.2.1b -: Mucoid degeneration: magnification 100x.

Alcian blue at pH 2.5 staining intensity: LMA, LMP, LLA, and LLP shows slight staining intensity to Alcian blue 2.5, with a score of 1, while LMM and LLM shows moderate staining intensity for Alcian blue 2.5 score as 2 (fig. 4.2.1b).

Analysis of osteoarthritic (OA) menisci.

Microscopic analysis of the menisci from Grade II, III, and IV osteoarthritic knee joints indicates fibrocartilaginous disruption. The deteriorated extracellular matrix exhibits fine fibrillation, a loss of structure, and the merging of the meniscus cells' and the matrix's gaps. There was a lot of variation in the cell distribution, including places that were hypercellular, hypocellular, and acellular as well as regions with big, plentiful cell groupings. The meniscus surface was discovered to include clusters of aberrant cells, which are often observed around worn areas (Fig. 4.2.1a). The pattern of mucoid degeneration's intensity, with its light and moderate staining regions, showed variation. (Fig. 4.2.1b).

Table 4.2.1a: Osteoarthritis (OA) menisci by side of legs and parts of medial menisci.

Osteoarthritis		Left		Right		Overall		Chi-square (df),p-value
		n	%	n	%	n	%	
Medial anterior	Mild	5	8.9	2	3.7	7	6.4	1.309 (2), 0.520
	Moderate	43	76.8	43	79.6	86	78.2	
	Severe	8	14.3	9	16.7	17	15.5	
Medial middle	Mild	1	1.8	1	1.9	2	1.8	0.196 (2), 0.907
	Moderate	52	92.9	49	90.7	101	91.8	
	Severe	3	5.4	4	7.4	7	6.4	
Medial posterior*	Mild	8	14.3	1	1.9	9	8.2	6.057 (2),0.048
	Moderate	42	75.0	44	81.5	86	78.2	
	Severe	6	10.7	9	16.7	15	13.6	

Note: * The Chi-square statistic is significant at the .05 level,

Table 4.2.1b: Osteoarthritis (OA) menisci by side of legs and parts of lateral menisci

Osteoarthritis		Left		Right		Overall		Chi-square (df),p-value
		n	%	n	%	n	%	
Lateral anterior*	Mild	5	8.9	0	0.0	5	4.5	6.213 (2), 0.045
	Moderate	48	85.7	53	98.1	101	91.8	
	Severe	3	5.4	1	1.9	4	3.6	
Lateral middle	Mild	7	12.5	4	7.4	11	10.0	0.794 (2), 0.672
	Moderate	43	76.8	44	81.5	87	79.1	
	Severe	6	10.7	6	11.1	12	10.9	
Lateral posterior*	Mild	7	12.5	1	1.9	8	7.3	6.466 (2), 0.039
	Moderate	47	83.9	47	87.0	94	85.5	
	Severe	2	3.6	6	11.1	8	7.3	

Note: * The Chi-square statistic is significant at the 0.05 level

Table 4.2.1a, reveals the histological grade distribution of the OA meniscus on the sides of the leg and parts of medial menisci. The study showed 8.9% mild, 76.8% moderate, and 14.3% severe OA in the left leg medial meniscus anterior part (LMA), whereas 1.8% mild, 92.9% moderate, and 5.4% severe OA in LMM. In LMP, mild, moderate, and severe OA are 14.3, 75 and 10.7 percent respectively.

Left leg lateral meniscus anterior part (LLA) had 8.9% mild, 85.7% moderate, and 5.4% severe OA. However, LLM had 12.5% mild, 76.8% moderate, and 10.7% severe OA, whereas LLP had 12.5% mild, 83.9% moderate, and 3.6% severe OA (Table 4.2.1b).

Right leg medial meniscus anterior part (RMA) had 3.7% mild, 79.6% moderate, and 16.7% severe osteoarthritis, whereas RMM had 1.9% mild, 90.7% moderate, and 7.4% severe osteoarthritis. Similarly, RMP had 1.9% mild, 81.5% moderate, and 16.7% severe osteoarthritis (Table 4.2.1a). The right leg lateral meniscus anterior part (RLA) showed 98.1% moderate and 1.9% severe osteoarthritis. Similarly, RLM had mild (7.4%), moderate (81.5%), and severe (11.1%) osteoarthritis. However, RLP was 1.9% mild, 87.0% moderate, and 11.1% severe OA (Table 4.2.1b). The posterior part of the medial menisci of both legs is significantly associated with osteoarthritis (Table 4.2.1a), whereas, the anterior and posterior parts of the lateral menisci have a significant association ($p<0.05$) (Table 4.2.1b).

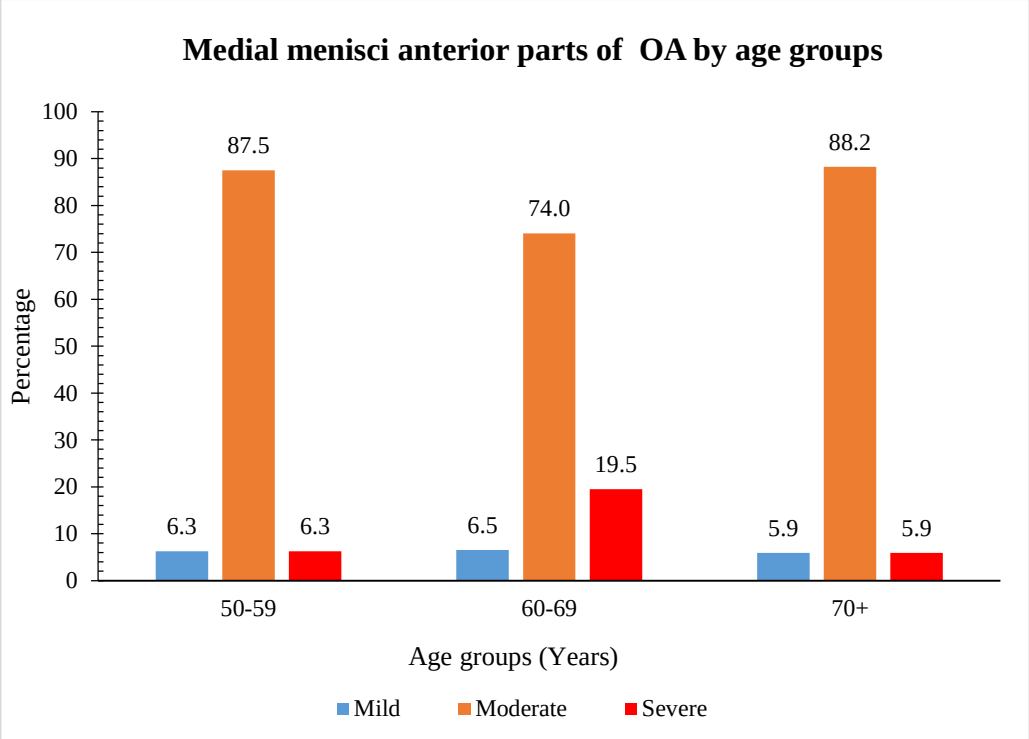
To compare the age-related changes in histological structure in osteoarthritic menisci

Table 4.3.1a: Medial menisci anterior parts of OA menisci by age groups

Age groups	Medial menisci anterior parts						n
	Mild		Moderate		Severe		
	n1	%	n2	%	n3	%	
50-59	1	6.3	14	87.5	1	6.3	16
60-69	5	6.5	57	74.0	15	19.5	77
70+	1	5.9	15	88.2	1	5.9	17

Note: n=n1+n2+n3

Table 4.3.1a reveals the medial menisci anterior parts of OA menisci by age groups, at 50 – 59 years age group. It was found that medial menisci anterior parts of OA menisci was 6.3% mild, 87.5% moderate and 6.3% severe, whereas in 60-69 years age group mild, moderate and severe medial menisci anterior parts of OA menisci were 6.5%, 74% and 19.5% respectively. While in 70+ years age group, medial menisci anterior parts of OA menisci were 5.9% mild, 88.2% moderate and 5.9% severe.



Graph 4.3.1a: Showing medial menisci anterior parts of OA menisci by age groups

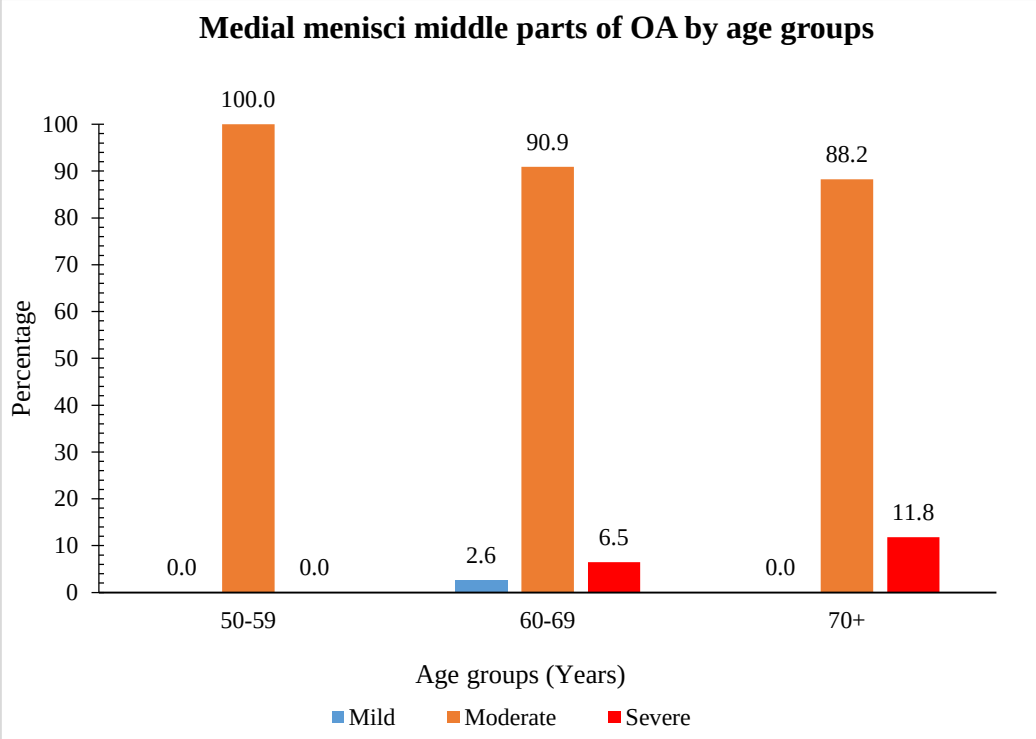
Graph 4.3.1a shows OA patients by age groups, it was higher in moderate (Grade 3) OA in the medial anterior parts of menisci i.e. 87.5%, 74.0%, and 88.2% in 50-59, 60-69, and 70 and above years respectively.

Table 4.3.1b: Medial menisci middle parts of OA menisci by age groups

Age groups	Medial menisci middle parts						n
	Mild		Moderate		Severe		
	n1	%	n2	%	n3	%	
50-59	0	0.0	16	100.0	0	0.0	16
60-69	2	2.6	70	90.9	5	6.5	77
70+	0	0.0	15	88.2	2	11.8	17

Note: n=n1+n2+n3

Table 4.3.1b reveals the medial menisci middle parts of OA menisci by age groups. At 50 – 59 years age group, it was found that medial menisci middle parts of OA menisci was 0% mild, 100% moderate and 0% severe, whereas 60-69 years age group was mild, moderate and severe in medial menisci middle parts of OA menisci were 2.6%, 90.9% and 6.5% respectively. While in 70+ years age group, medial menisci middle parts of OA menisci were 0% mild, 88.2% moderate and 11.8% severe.



Graph 4.3.1b: Showing medial menisci middle parts of OA menisci by age groups

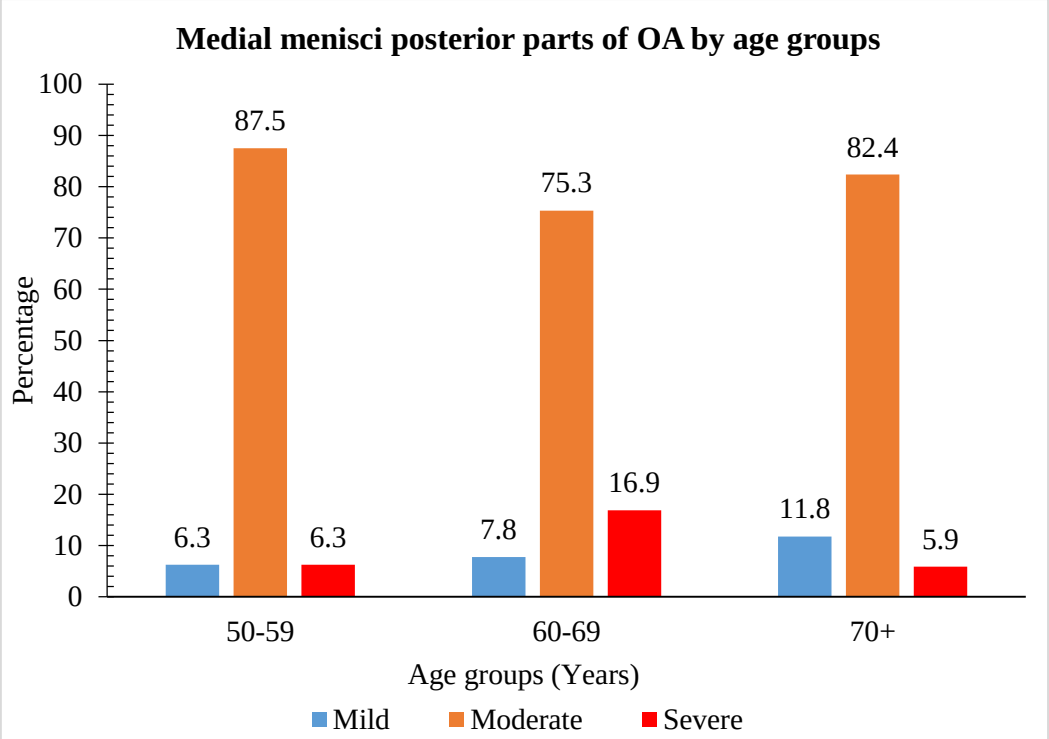
Graph 4.3.1b shows the OA patients by age groups. It was higher in moderate (Grade 3) OA in medial menisci middle parts of menisci. i.e. 100%, 90.9% and 88.2% in 50-59, 60-69 and 70 and above years respectively.

Table 4.3.1c: Medial menisci posterior parts of OA menisci by age groups

Age groups	Medial menisci posterior parts						n
	Mild		Moderate		Severe		
	n1	%	n2	%	n3	%	
50-59	1	6.3	14	87.5	1	6.3	16
60-69	6	7.8	58	75.3	13	16.9	77
70+	2	11.8	14	82.4	1	5.9	17

Note: n=n1+n2+n3

Table 4.3.1c reveals the medial menisci posterior parts of OA menisci by age groups, at 50 – 59 years age group. It was found that medial menisci posterior parts of OA menisci was 6.3% mild, 87.5% moderate and 6.3% severe, whereas in the 60-69 years age group, the mild, moderate and severe in medial menisci posterior parts of OA menisci were 7.8%, 75.3% and 16.9% respectively. While in 70+ years age group, medial menisci posterior parts of OA menisci were 11.8% mild, 82.4% moderate and 5.9% severe.



Graph 4.3.1c: Showing medial menisci posterior parts of OA menisci by age groups

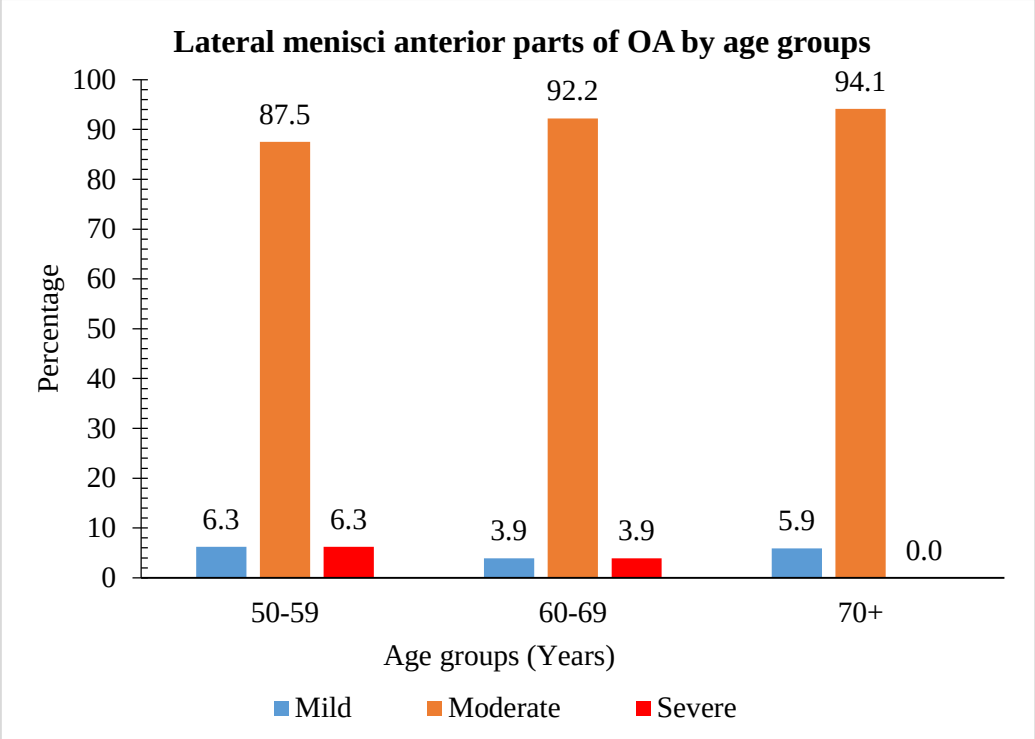
Graph 4.3.1c shows the OA patients by age groups. It was higher in moderate (Grade 3) OA in medial menisci posterior parts of menisci. i.e. 87.5%, 75.3% and 82.4% in 50-59, 60-69 and 70 and above years respectively.

Table 4.3.1d: Lateral menisci anterior parts of OA menisci by age groups

Age groups	Lateral menisci anterior parts						n
	Mild		Moderate		Severe		
	n1	%	n2	%	n3	%	
50-59	1	6.3	14	87.5	1	6.3	16
60-69	3	3.9	71	92.2	3	3.9	77
70+	1	5.9	16	94.1	0	0.0	17

Note: n=n1+n2+n3

Table 4.3.1d reveals the lateral menisci anterior parts of OA menisci by age groups. At 50 – 59 years age group, it was found that lateral menisci anterior parts of OA menisci were 6.3% mild, 87.5% moderate and 6.3% severe, whereas in the 60-69 years age group the mild, moderate and severe in lateral menisci anterior parts of OA menisci was 3.9%, 92.2% and 3.9% respectively. While in 70+ years age group, lateral menisci anterior parts of OA menisci were 5.9% mild, 94.1% moderate and 0% severe.



Graph 4.3.1d: Showing lateral menisci anterior parts of OA menisci by age groups

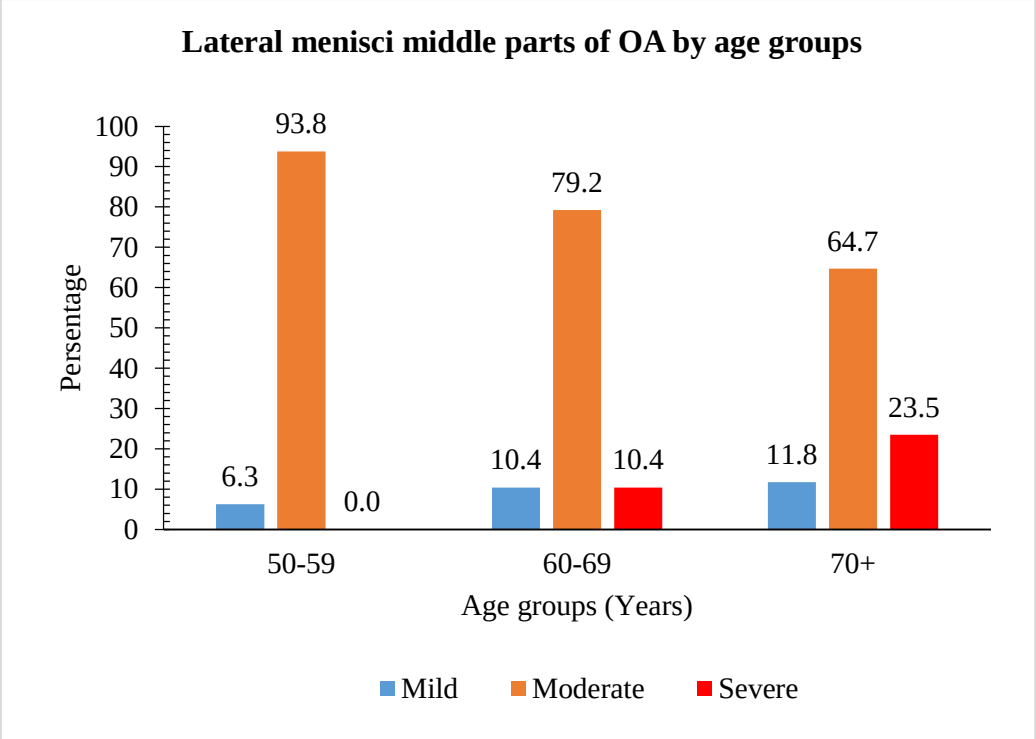
Graph 4.3.1d shows the OA patients by age groups. It was higher in moderate (Grade 3) OA in lateral menisci anterior parts of menisci. i.e. 87.5%, 92.2% and 94.1% in 50-59, 60-69 and 70 and above years respectively.

Table 4.3.1e: Lateral menisci middle parts of OA menisci by age groups

Age groups	Lateral menisci middle parts						n
	Mild		Moderate		Severe		
	n1	%	n2	%	n3	%	
50-59	1	6.3	15	93.8	0	0.0	16
60-69	8	10.4	61	79.2	8	10.4	77
70+	2	11.8	11	64.7	4	23.5	17

Note: n=n1+n2+n3

Table 4.3.1e reveals the lateral menisci middle parts of OA menisci by age groups, at 50 – 59 years. It was found that the lateral menisci middle parts of OA menisci was 6.3% mild, 93.8% moderate and 0% severe, whereas in the 60-69 years age group the mild, moderate and severe in lateral menisci middle parts of OA menisci was 10.4%, 79.2% and 10.4% respectively. While in 70+ years age group, the lateral menisci middle parts of OA menisci were 11.8% mild, 64.7% moderate and 23.5% severe.



Graph 4.3.1e: Showing lateral menisci middle parts of OA menisci by age groups

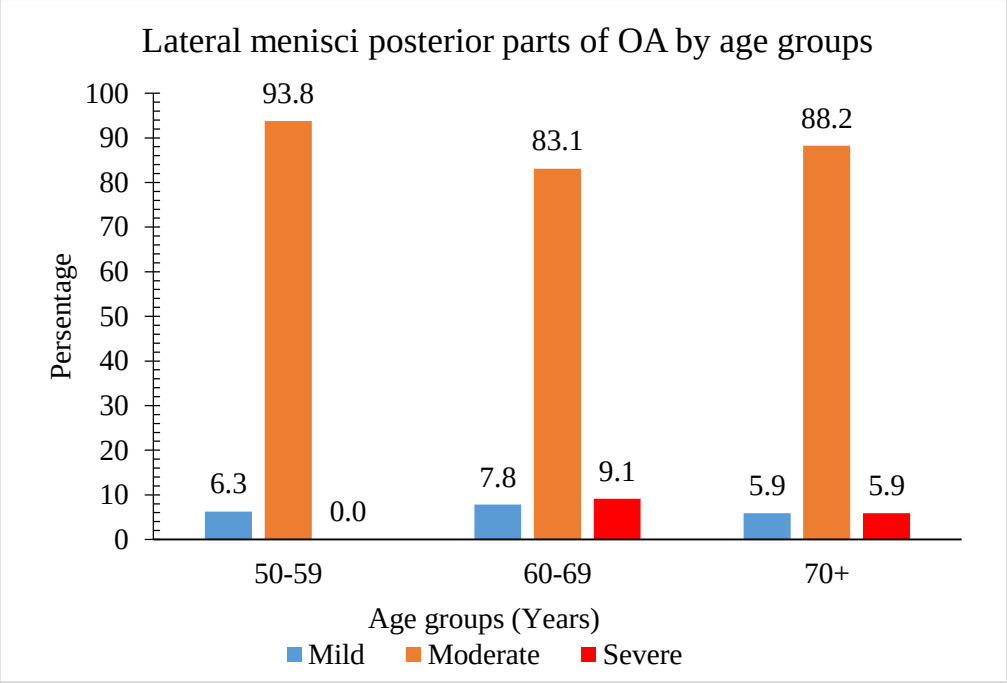
Graph 4.3.1e shows the OA patients by age groups. It was higher in moderate (Grade 3) OA in lateral menisci middle parts of menisci. i.e. 93.8%, 79.2% and 64.7% in 50-59, 60-69 and 70 and above years respectively.

Table 4.3.1f: Lateral menisci posterior parts of OA menisci by age groups

Age groups	Lateral menisci posterior parts						n
	Mild		Moderate		Severe		
	n1	%	n2	%	n3	%	
50-59	1	6.3	15	93.8	0	0.0	16
60-69	6	7.8	64	83.1	7	9.1	77
70+	1	5.9	15	88.2	1	5.9	17

Note: n=n1+n2+n3

Table 4.3.1f reveals the lateral menisci posterior parts of OA menisci by age groups, at 50 – 59 years. It was found that lateral menisci posterior parts of OA menisci was 6.3% mild, 93.8% moderate and 0% severe, whereas in the 60-69 years age group, the mild, moderate and severe in lateral menisci posterior parts of OA menisci was 7.8%, 83.1% and 9.1% respectively. While in 70+ years age group, the lateral menisci posterior parts of OA menisci were 5.9% mild, 88.2% moderate and 5.9% severe.



Graph 4.3.1f: Showing lateral menisci posterior parts of OA menisci by age groups

Graph 4.3.1f shows the OA patients by age groups. It was higher in moderate (Grade 3) OA in the lateral menisci posterior parts of the menisci. i.e. 93.8%, 83.1%, and 88.2% in 50-59, 60-69, and 70 and above years respectively.

5. DISCUSSION

Menisci are complex fibrocartilaginous tissues.[97] The structural indication of OA is multiple disruption and loss of articular fibrocartilage. Through load distribution and stress absorption in both tibiofemoral compartments, the menisci play a significant role. Microscopic examination demonstrated a significant link between OA and deteriorated meniscus.[98 - 100] The menisci in the knee are specialized tissues that are essential for the transfer of loads, shock absorption, and joint stability.[101 - 104] Meniscal injuries are frequent in both athletes and the general public, which is consistent with the role that menisci play in knee joint function. It is unclear how complicated a role meniscal tissue composition plays in the etiology of meniscal tears and the subsequent development of knee OA.[105 – 110]

Additionally, the mechanistic relationship between meniscus injury and knee OA is not fully understood. To investigate meniscal alterations during the development of OA, experimental animal models are employed.[111, 112] Degeneration of the human meniscus has also been described by several authors.[113 - 117] However, the majority of meniscus grading systems are based on MRI.[118 - 121] The present study, however, offers a more methodical evaluation of aging and OA meniscal alterations at the microscopic level and verifies additional information regarding the severity of OA through a grading system.

Further, sheep's normal meniscal tissue was taken as control of three different parts (anterior, middle, and posterior) of the medial and lateral menisci of both legs for comparison purposes. Because gait analyses in sheep model have shown a similar pattern of hind limb loading to humans, and post-surgical GRF (Ground reaction

forces) changes comparable to OA patients.[122, 123] Another researcher examined the viscoelastic characteristics of the human meniscus with those of the porcine, ovine, and bovine menisci. They found that the ovine (sheep) model had the most in common with the human meniscus. A few studies also used histology and scanning electron microscopy to compare the extracellular matrix collagen, vascularization pattern, and cell density of ovine and rabbit menisci with those of humans, finding that sheep (ovine) menisci are more structurally similar to the ultrastructure.[124 – 127]

Their cellular and chemical makeup, as well as, perhaps more crucially, the organization and interactions of their constituents, determine their capacity to carry out these mechanical duties. In order to maintain the extracellular meshwork's stability and maintain the menisci's structural integrity and mechanical qualities, mucopolysaccharides, proteoglycans, and other non-collagenous proteins are essential.[128, 129]

Microscopic degeneration is a nearly constant finding in older patients and middle-aged and is common in both the lateral and medial menisci. In elderly participants with uniform degeneration of the matrix and collagen, other factors may contribute to severe meniscal tears. Meniscal degeneration is a complication of OA. However, inaccessible meniscal injuries can also lead to OA.[130]

Socio-demographic variations:

This study demonstrates that OA is related to blood types, age groups, gender, religion, family history, dietary practices, and physical activity. However, in the majority of earlier investigations, OA did not link with blood types, eating habits, family history, religion, or activity.

In the current study, among 110 cases, left leg has more OA than the right leg. In 110 Osteoarthritis cases in both left and right legs were higher in the 60-69 years of age group. While another studies described, that the chance of developing osteoarthritis increases with age by previously injured joint overload, joint misalignment, and obesity. In fact, these factors can worsen the osteoarthritis. Age-related changes in the extracellular matrix and cells of joint tissue may make older people more susceptible to osteoarthritis. Aging may contribute to the imbalance between catabolic and anabolic activity in the joints that characterizes OA. Aging chondrocytes do not respond well to growth factor stimulation and are unable to keep the homeostasis of the articular cartilage.[106,131 -134]

The current study found that across gender groups, among 110 cases, women (59%) had more OA than men (41%), because risk factors for osteoarthritis in women, as in men, are many like anatomical differences, obesity, previous trauma, genetic disorder, and hormonal issues. Menopausal women often gain weight, and increased joint stress leads to increase osteoarthritis in women. Men's hips are narrower anatomically than those of women. The outside of the knee is subjected to more stress because of the angle created by the hip bone's broader width than the knee. Some women eventually develop osteoarthritis as a result of this "knock-kneed" position, no matter how minor it may be. Males have a narrower femur, a thinner patella, a higher quadriceps angle, and different-sized tibial condyles, among other male-to-female anatomical variations that may be relevant.[14, 15, 135 – 138]

The data observed for the present study shows, in 110 OA cases, Hindus (57%) had higher rates of OA than Muslim religion (41%), In contrast, another study discovered that Muslims of the same ethnicity but with distinct religious practices

have a lower frequency of OA than Hindus. It is possible to stretch the soft tissue around the knee and lessen stiffness and contact pressure on the articular cartilage by performing Muslim prayers with the knees in deep flexion since infancy.[16, 17, 18, 139 – 141]

In the category of family history, among 110 cases, 64% had no family history of OA. While other studies described that, people inherit an increased risk of developing osteoarthritis. This predisposition can be passed down from generation to generation in families, but the pattern of inheritance is unknown. In the first cross-sectional comparison, the author reported a higher prevalence and severity of cartilage defects in the offspring. The longitudinal study claim that knee cartilage loss, changes in cartilage defects, and decreased physical performance, in addition to genetics, all contribute to the development of knee osteoarthritis, this was likely polygenic but reflects a shared environment. The authors concluded that offspring with a family history of knee osteoarthritis have an increased risk of knee pain independent of structural factors, suggested that genetic factors may be involved in the OA.[19 - 22]

In this study among 110 OA cases, Non-vegetarians (71%) were more susceptible to OA than vegetarians. According to Chang Xu et.al (2020), western unhealthy dietary patterns that include in longitudinal cohorts of men and women, consumption of foods like French fries, red and processed meats, poultry, refined grains, sugar-sweetened beverages, and pizza was linked to faster radiographic and symptomatic knee OA progression. In contrast, adherence to promoting a healthy, sensible diet that includes fruits, vegetables, beans, fish, and whole grains was linked to slower radiographic and symptomatic knee OA progression.[23 – 25, 142,143]

In this study also showed that among 110 OA cases, people who do not active in physical activity were more likely to be affected by OA (52.7%). Other studies came to the conclusion that physical activity increased the risk of osteoarthritis if it involved sports that subjected normal joints to high levels of impact or torsional loading on a regular basis, as well as if it involved sports that could injure the ligaments, tendons, and menisci that support the joints. Mild to moderate exercise certainly reduces the development of knee OA and is useful in treating and managing the pain and functional decline associated with OA, according to evidence data. It also does not speed up or promote knee OA. Modern data suggests that mild to moderate physical exercise has both preventative and therapeutic benefits for those with knee OA.[26-28,144,145]

In this study among 110 OA cases, B+ve (30.9%) and O+ve (35.5%) blood group types were more susceptible to osteoarthritis than people with other blood groups. According to another study, blood type AB was the main risk factor for osteoarthritis of the knee joint, regardless of age or gender. The correlation between the AB blood type and primary knee osteoarthritis may be mediated by blood group-related LeY antigen. Despite rigorous methodology, the inherent limitations of retrospective studies were mandatory. The outcomes of their research was confirmed by prospective cohort studies.[29-32, 146,147]

Mucopolysaccharides /Proteoglycans / Glycosaminoglycans

In this study, the anterior, middle, and posterior regions of the medial and lateral menisci in both legs showed less acid mucins (proteoglycan) staining intensity than the control menisci. However, in other investigations, normal meniscal tissue revealed a preponderance of acid mucins (80%) and scarce neutral mucins (20%),

suggested that the menisci were rich in acid mucins and had a significant role in viscosity. Sulfated GAGs made up around 80% of the meniscus's overall GAG content. Normal human meniscal proteoglycans comprise around 40% chondroitin 6-sulfate, 10%–20% chondroitin 4-sulfate, 20–30% dermatan sulfate, and 15% keratan sulfate.[63, 148]

According to research by Videman et al. (1979) on proteoglycan content, rabbits with OA brought on by limb immobilization had more proteoglycan in their menisci.[60] After osteoarthritis (OA) was brought on by limb immobilization, Djurasovic et al. (1998) observed a reduction in proteoglycan content in the menisci of mature beagle dogs.[61] According to Adams et al. (1983), proteoglycan concentration in the menisci reduced throughout the first trimester but steadily rose in the months that followed after OA was induced.[62]

In patients with meniscal injuries, Peters and Smillie (1972) observed higher proteoglycan levels in the area of the meniscus with degenerative tears.[149] Human meniscus samples from OA patients were used in research by Ghosh et al. (1975), who discovered that the degenerative area of the OA meniscus had more proteoglycan than normal control menisci.[76] According to Lopez-Franco M. et al (2016), who revealed that proteoglycan alterations in human menisci found that decreased acidic mucin (proteoglycan) was associated with a propensity for meniscus degeneration.[150]

According to Herwig, Egner, and Buddecke's (1984) research, collagen, and glycosaminoglycan contents declined as chondroitin 6-sulfate levels rose, but water content increased with increasing degeneration.[4]

According to Dahl et al. (1985), the hyaluronate molecular weight and concentration in synovial fluid can be decreased in osteoarthritis following anterior cruciate ligament transection (ACLT) because of the buildup of liquid from inflamed synovial vessels in the joint cavity. As a result, the fluid loses viscoelasticity and cartilage is more susceptible to deterioration.[151]

According to Teeple et al. (2011), pure human lubricin (LUB) or LUB+HA (hyaluronic acid) treatment significantly reduced the radiographic and histologic scores of cartilage injury (P =.039 and P =.015, respectively).[152] According to D.Warnecke et al. (2020), there was a considerable trend for the proteoglycan content to decrease as the degeneration progresses.[153] According to Pauli C. et al. (2011), described a significant increased in the proteoglycan content as meniscus degeneration advances.[52]

Microscopic structure of OA menisci:

In this study medial meniscus of the posterior part of both legs, revealed moderate to severe abrasion, torn surfaces, dispersed cellularity, collagen fibers disorganization, and seldom mucoid degeneration. The lateral menisci of both legs had minor surface folds and abrasions, scattered cellularity, and loose fibers, while the anterior part had degenerative changes. While extensive surface abrasion, tearing, and destruction are seen in posterior part of lateral meniscus, along with generalized hypercellularity, fibrocartilage dissociation, and broken collagen fibers.

In another study, histological analysis (H&E staining) of articular cartilage in a human knee with osteoarthritis found that the surface loses its ability to repair and turns dull and uneven. In moderate OA cartilage, structural alterations were visible,

such as a decrease in the thickness of the surface and medial cartilage. The collagen network structure was damaged, resulting in reduced cartilage thickness. Chondrocytes and cartilage tissue no longer maintain their repair activity. In severe OA cartilage fissures were deep on the surface, cells in the peripheral zone disappeared, and intermediate, and circular zones lacked of cells and were not arranged in the parallel fashion. The surface of the cartilage had slight modifications; it was no longer smooth, and the subchondral bone showed fibrillation. [35, 53, 58]

In other studies on animals, in the rabbit OA model, cell density in the meniscus increased or decreased depending on the area, and clusters of cells were frequently found in degenerated areas.[53, 154 – 157]

Various areas of the meniscus in both legs were examined in this study. The medial menisci had mild (14.3% on left and 1.9% on right), moderate (75.0% on left and 81.5% on right), and severe (10.7 on left and 16.7% on right) osteoarthritis in the posterior part, and indicates a significant at 5% level. While, the anterior and posterior part of lateral menisci were significant at the 5% level. In other studies of human OA menisci, histological changes in the anterior part of the OA meniscus were moderate. [130, 158 – 160]

Furthermore, in other research on human menisci, there was substantial matrix loss in one or more meniscal areas in cases of mild to severe OA. Biomechanically, during knee flexion, the femoral condyles roll back onto the tibial plateau and additional stress is transmitted to the rear section of the meniscus, often subluxing the posterior portion of the lateral meniscus during deep flexion.[60, 149, 161 - 163]

However, the anterior portion is seldom as damaged. This might mean that the anterior section is more resistant to deterioration or that it experiences less harmful biomechanical stress. On macroscopic and histological inspection, the medial and lateral menisci's inner margins tend to deteriorate.[161, 164, 165]

6. CONCLUSION

The prevalence of OA is higher in the left leg's knee menisci than in the right legs. The age group of 60-69 years old had greater incidences of OA in both legs. In comparison to males and people of other religions, women and Hindus had greater incidences of OA. Non-vegetarians and inactive people are more likely to get OA. Additionally, B +ve and O +ve blood types exhibit much greater variance in their susceptibility to OA than other blood groups ($p < 0.05$).

The results of this study may be useful to doctors, clinicians, and policymakers in developing programs to address dietary practices and physical activity levels in order to slow the progression of OA.

In OA cartilage, there was a significant loss of collagen and proteoglycan, indicating that these two substances are more actively involved in the degenerative process and the emergence of OA. Strong mucopolysaccharide staining intensities was seen in the control group. However, the meniscus in the test group had different degrees of decreased (moderate to negative) staining intensity levels. According to test statistics used for various histochemical stains, the color intensity of various medial and lateral menisci parts (anterior, middle, and posterior) with a side of the legs showed significant variation by F-test ($p < 0.05$). Additionally, the medial and lateral meniscus were not significantly associated with each other in the chi-square test correlation of distinct histochemical stain intensities with a side of the legs.

Meniscal degeneration starts deeper in the tissue than on the surface. At the inner border of the meniscus, tissue fibrillation and rips are first noticed. Over time, they proceed to the articular surface of the meniscus and eventually result in the entire

destruction or loss of meniscus tissue. OA menisci in both legs were significantly associated with posterior part of the medial menisci ($P < 0.05$), while the anterior and posterior parts of lateral menisci were significantly associated with OA menisci.

The alterations of the proteoglycans /Glycosaminoglycans in human OA menisci give information on the scientific evidence of the progressive nature of OA. Thus, this research will aid medical practitioners in the creation of medications with altered structural properties for the treatment of OA.

Implication of study

This work may contribute to understanding how carbohydrate moieties and their alteration and age-related changes may help prevent and design a futuristic plan to reverse the process of degenerative changes. It may help to classify morpho chemically and to understand the phases of disease.

This research will contribute to a better understanding of the disease process and be crucial for the creation of innovative, structure-modifying medications for the treatment of osteoarthritis.

The findings of this study will also help to know the microscopic arrangement of cells, collagen fibers, and glycosaminoglycan changes in normal and osteoarthritic menisci.

Limitations and recommendations

Regarding the histochemical study of mucopolysaccharides in osteoarthritic menisci of the human knee joint, there is a lack of literature for comparison of the

results of the different types of proteoglycans. So similar studies may be required to confirm results of this study.

In this study, it was observed that mucopolysaccharides level was decreased in OA menisci of the human knee joint when compared with normal menisci of sheep as a control. So further study is required to compare the mucopolysaccharides in OA menisci and normal menisci of human knee joints.

A study on recent advances in glycobiology and classification of mucopolysaccharides is important to understand pathophysiological changes in normal and elderly aged and OA.

Pathophysiological changes in OA and its comparison with a normal human being is important to know in a multi-step process of OA.

In addition, enzymes are also responsible for the breakdown of meniscal cartilage for the growth and advancement of knee OA. Hence, there is a need for further study of collagen and proteoglycan-degrading enzymes which gives a better understanding of how osteoarthritis (OA) develops in the knee joint.

7. SUMMARY

Menisci are intricate fibrocartilaginous tissues that are crucial for the knee joint's stability, shock absorption, load distribution, and articular cartilage protection. Each knee joint has two menisci i) Medial meniscus and ii) Lateral meniscus.

A pathogenic mismatch between the repair and regenerative mechanisms leads to osteoarthritis, which is one of the main factors in chronic disability. The disease primarily affects middle-aged and older persons and athletes, while it can sometimes afflict younger people due to trauma or overuse. Individuals who have this illness experience discomfort and functional loss. Many things, such as diet, trauma, strain, and genetic anomalies, might contribute to OA. Unfortunately, our knowledge of the molecular processes behind the disease's genesis and development is still limited. The exact mechanism underlying the sickness is unknown despite extensive research on it. OA's etiology is still a mystery.

Mucopolysaccharides or glycosaminoglycans are long unbranched polysaccharides made up of repeated disaccharide units. An amino sugar and a uronic sugar or galactose make up the repeating unit, with the exception of keratan. Due to their strong polarity, mucopolysaccharides draw water. As a result, they serve as a lubricant or a shock absorber for the body.

There are several categories for mucopolysaccharides:

i) **Neutral mucopolysaccharides** lack a free acidic group and are made up of hexosamine and hexose units.

ii) **Acidic mucopolysaccharides** consists of hexosamine associated with glucuronic acid, sialic acid, or sulfate radical. Acid mucins are further classified into Sialomucin and Sulphomucin depending on the sialic acid or ester sulfate groups.

Meniscal injuries are known to cause severe musculoskeletal morbidity. Due to their distinctive and intricate anatomy, treatment and repair of menisci are difficult for patients, surgeons, and physical therapists. The majority of people often have knee OA. It is difficult to define symptoms and radiographic changes for research purposes since they are not well connected. There is currently no cure for OA. Nonpharmacological, pharmacological, and surgical treatments can all be used to control OA. Surgery is normally only utilized after all other medical options have failed and functional impairment negatively affects the patient's quality of life.

However, as OA progresses, the collagen matrix becomes more disordered, and the content of mucopolysaccharides in the cartilage changes. Regarding the classification of mucopolysaccharides in health and disease, very little information is available. The present research was therefore useful for understanding OA and its relationship to molecular changes in histochemistry. This work may contribute to understanding how carbohydrate moieties and their alteration and age-related changes may help prevent and design a futuristic plan to reverse the process of degenerative changes. Understanding the stages of the disease and morpho-chemically categorizing it may be helpful. The results of this study will be essential for the creation of novel, structure-modifying drugs for the treatment of osteoarthritis and for a deeper comprehension of the disease process.

The present study was carried out in the Department of Anatomy J.N. Medical College, Belagavi. Specimens were collected from consecutive OA patients who underwent total knee joint replacement surgery, and who have undergone lower limb amputation surgery from the Orthopedics department unit of KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi, Karnataka, India. The sample size was 110. Medial and lateral osteoarthritic menisci were collected from 110 human knee joints of both sexes. Patients were excluded in case of any malignancy in the menisci or torn menisci and injuries to the menisci.

Normal meniscal tissue from an 8-month-old male domesticated ruminant sheep of average weight of 13 kg was used in this study. Sheep meniscal tissue was used as a control. Samples were put in 10% formalin for fixation. For each meniscus, three separate parts (anterior, middle, and posterior) were processed. Tissue samples were brought in for routine tissue processing and studied for different routine and special histological stains with color intensity.

Histological staining techniques were used to stain the following parts of the menisci of both legs: - LMA, LMM, LMP, and LLA, LLM, LLP for left leg medial, and lateral menisci of anterior, middle, and posterior parts. Whereas for the right leg medial and lateral menisci of the anterior, middle, and posterior parts were RMA, RMM, RMP and RLA, RLM, and RLP. For the histological evaluation of meniscus specimens, degeneration grade was assessed by the C. Pauli (2011) microscopic grading system. All images were captured with an Olympus BX-41 microscope equipped with Graphix software elements. (U-TV1X-2) T7 Tokyo, Japan. The histochemical grading of the meniscal OA was done by a Pathologist of KAHER's, J.N. Medical College, Belagavi. Descriptive statistics, nonparametric method

(Chi-square test), One-way ANOVA (F-test), and significance were seen at a 5% level. Analyses were performed using MS Excel and SPSS version 22.

In 110 OA cases, left leg had more osteoarthritis than the right. Women are more prone than males to acquire OA. In both the left and right legs, OA instances were greater in the age ranges from 60 to 69 years, and they were more prevalent in women on the right leg and males on the left. In this study, the OA cases are more in Hindus than in other religions. Nonvegetarians and physically inactive are more likely to be affected by OA. In addition, B +ve and O +ve blood group types were more susceptible to OA than other blood groups.

In this study, analysis of mucopolysaccharides in osteoarthritic menisci of a human knee joint used ten (10) different types of special histological stains and measure the color intensity as a negative stain (- : 0%), weak (±: <25%), mild stain (+: 26-50%), moderate stain (+ +: 51-75%), and strong stain (+ + +: 76-100%).

The stain alcian blue pH 1.0 was used to see the sulfated acid mucins. The control group used in this stain showed moderate (+ +) staining intensity, while the test group showed different color intensity negative (-) and weak stain (±) at 3 regions (anterior, middle, posterior) of the medial and lateral menisci of both legs. While comparing the control group and test group it was observed that there was decreased color intensity of sulfated acid mucins in osteoarthritis menisci. The results of test statistics used for intensity of sulphated acid mucins in different parts of medial and lateral menisci with a side of the legs, and found negative (-) intensity of sulphated mucins on both sides of the legs, showing significant variation ($p < 0.001$).

The stain alcian blue pH 2.5 was used to observe the acid mucins. The control group used in this stain showed strong (+ + +) staining intensity, whereas the test group showed different color intensities negative (-), weak (±), mild (+), and moderate (+ +) stains at 3 regions (anterior, middle, posterior) of medial and lateral menisci of both legs. While comparing the control group and test group, it was observed that there were decreased color intensity of acid mucins in osteoarthritis menisci. The results of test statistics used for the intensity of acid mucins in different parts of medial and lateral menisci with a side of the legs, and found Weak (±) and Mild (+) intensity of acid mucins on both sides of the legs showed significant variation ($p < 0.05$).

The PAS and aldehyde fuchsin stains were used to observe the neutral mucins (glycogen carbohydrate) and sulphomucins respectively. The control group used in these stains showed moderate (+ +) and strong (+ + +) staining intensity respectively, whereas the test group showed different color intensities mild (+) and weak (±) for PAS, while negative (-), weak (±), Mild (+), and moderate (+ +) for aldehyde fuchsin (AF) stains at 3 regions (anterior, middle, posterior) of medial and lateral menisci of both legs. While comparing the control group and test group, it was observed that there were a decreased color intensity of neutral mucins (glycogen carbohydrate) and sulphomucins in osteoarthritis menisci respectively. The results of test statistics used for the intensity of acid mucins and sulphomucins in different parts of medial and lateral menisci with a side of the legs, and found Mild (+) intensity of neutral mucins and Mild (+) and Weak (±) intensity of sulphomucins on both sides of the legs showed significant variation ($p < 0.05$).

The stain mucicarmine (MC) was used to observe the acid mucopolysaccharides. The control group used in this stain showed moderate (+ +) staining intensity, whereas the test group showed different color intensities negative (-), weak (±), and mild (+) stains at 3 regions (anterior, middle, posterior) of medial and lateral menisci of both legs. While comparing the control and test groups, it was observed that there were decreased color intensity of acid mucopolysaccharides in osteoarthritis menisci. The results of test statistics used for the intensity of acid mucopolysaccharides in different parts of medial and lateral menisci with a side of the legs, and found Negative (-) and Weak (±) intensity of acid mucopolysaccharides on both sides of the legs showed significant variation ($p < 0.05$).

Hyaluronidase enzyme labile technique was used to observe the hyaluronic acids. The control group used in this stain showed negative (-) staining intensity, whereas the test group showed different color intensities negative (-), weak (±), and mild (+) stains at 3 regions (anterior, middle, posterior) of medial and lateral menisci of both legs. While comparing the control and test groups, it was observed that there were an increase in the color intensity of hyaluronic acids in osteoarthritis menisci. The results of test statistics used for the intensity of hyaluronic acids in different parts of medial and lateral menisci with a side of the legs, were found negative (-) and mild (+) for medial meniscus and weak (±) and mild (+) for lateral meniscus of hyaluronic acids on both sides of the leg. This indicates a significant variation ($p < 0.05$) in both medial and lateral menisci. If tissues are treated with Bovine testicular hyaluronidase, negative (-) intensity indicates strong presence of the hyaluronic acid, weak (±) blue color intensity indicates average presence of hyaluronic acid and mild (+) blue color intensity indicates absence of hyaluronic acid.

The combined stain (PAS + AB -2.5) were used to observe the neutral and acidic mucins. The control group used in this stain showed strong (+ + +) staining intensity, whereas the test group showed different color intensities of moderate (+ +), mild (+), and weak (±) for PAS, and mild (+), weak (±) for AB - 2.5 stains at 3 regions (anterior, middle, posterior) of medial and lateral menisci of both legs. While comparing the control and test groups, it was observed that there were decreased color intensity of neutral and acidic mucins in osteoarthritis menisci. The results of test statistics used for the intensity of neutral and acidic mucins in different parts of medial and lateral menisci with a side of the legs, were found mild (+) PAS and weak (±) AB -2.5 intensity of neutral and acidic mucins on both sides of the legs. This showed significant variation ($p < 0.05$) of neutral and acidic mucin on both sides of legs.

The combined technique (AF + AB-2.5) and (Orcein + AB -2.5) stains were used to observe the (sulfated + carboxylated) and (sulpho and sialo) mucins respectively. The control group used in both combined stains showed moderate (+ +) staining intensity, whereas the test group showed different color intensities of mild (+) and weak (±) AF and mild (+) and weak (±) AB-2.5 stain and mild (+) and weak (±) Orcein and mild (+) and weak (±) AB – 2.5 stains at 3 regions (anterior, middle, posterior) of medial and lateral menisci of both legs. While comparing the control group and test group, it was observed that there were a decreased color intensity of (sulfated + carboxylated) and (sulpho and sialo) mucins respectively. The results of test statistics used for the intensity of (sulfated + carboxylated) mucins in different parts of medial and lateral menisci with a side of the legs, were found weak (±) AB, weak (±) AF and mild (+) AF intensity of sulfated + carboxylated mucins on both sides of the legs. This again showed significant variation ($p < 0.05$) of sulfated and

carboxylated mucin on both sides of the legs, whereas mild (+) O, weak (±) O, mild (+) AB – 2.5 and weak ± AB -2.5 intensity in medial menisci and mild + O and weak ± AB -2.5 in lateral menisci of sulpho and sialo mucins on both sides of the legs and showed significant variation ($p < 0.05$) of sulpho and sialo mucin on both sides of the legs.

Microscopic evaluation of menisci from osteoarthritic knee joints (Grade II, III, and IV) revealed fibrocartilaginous disruption. The degenerated extracellular matrix showed fine fibrillation and loss of structure. The fusion of spaces were occupied by cells of meniscus and matrix. Great variability in cell distribution was observed with hypercellular, hypocellular, and acellular areas, as well as areas containing large and abundant cell groups. Clusters of abnormal cells were found near the surface of the meniscus, typically associated with a worn area. Variability was observed in the intensity pattern of mucoid degeneration with light and moderate staining areas.

Degeneration of the meniscus begins in the substance of the tissue rather than the surface. Tissue fibrillation and tears are first observed at the inner border, spread over time to the articular surface of the meniscus, and progress to complete destruction or loss of meniscus tissue. In this study microscopic structures of OA menisci in both legs were significantly associated with medial menisci of the posterior part ($p < 0.05$), while lateral menisci of the anterior and posterior parts was significantly associated with OA menisci.

When age-related changes in microscopical structure in osteoarthritic menisci of the medial (anterior, middle, posterior) and lateral (anterior, middle, posterior) menisci of both legs, were recorded it was observed that moderate (Grade 3) OA was higher in all 50-59, 60-69, and 70 and above years of age groups.

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ANNEXURES

I) PROFORMA

QUESTIONNAIRE PROFORMA

JAWAHARLAL NEHRU MEDICAL COLLEGE, NEHRU NAGAR,

BELAGAVI – 590010

Project Title

"Histochemical Study of Mucopolysaccharides and Microscopic Structure of Osteoarthritic Menisci of the Human Knee Joint"

A. Demographic information

1. Identification no: From :
2. Name of participant :
3. Age in completed years:

   i) 20 – 29 ii) 30 – 39 iii) 40 – 49 iv) 50 – 59 v) 60 – 65
4. Sex: i) Male ii) Female
5. Marital status :

   i) Married ii) Unmarried iii) Divorced iv) Widow v) Single
6. Religion: i) Hindu ii) Muslims iii) Christian iv) Others
7. Education:

   i) Illiterate ii) Primary iii) Secondary iv) Higher Secondary

   v) Graduate vi) post graduate
8. Type of Family: i) Nuclear ii) Joint iii) Broken iv) 3rd generation

9. Annual Income: According to the modified BG Prasad classification Per capita monthly income in Rupees (it will be kept according to given scale and family member)

   i) Upper class ii) Upper Middle iii) Middle class

   iv) Lower middle v) Lower class

10. Duration of the Job / Business start in years:

   i) <1 ii) 1 – 5 iii) 5 – 10 iv) >10

B. Life style related

11. Have you ever smoked cigarette?

   i) Yes ii) No

12. If the answer is yes, tell us at what age did you start smoking?

   i) ---------- years ii) Forgot

13. On average how many cigarettes are you taking in a day? (Sticks)

   i) 1 – 9 ii) 10 – 19 iii) >20

14. Are you still smoker?

   i) Yes ii) No

15. If the answer is no , when did you quit smoking ?

   ----

16. Do you take chewing Tobacco?

   i) Yes ii) No iii) Previously, but not now

17. What type of the chewing tobacco do you consume?

   i) Gutkha ii) Pan parag iii) Jarda/ Khaini iv) Others

18. Are you taking Alcohol?

   i) Yes ii) No

19. If the answer is yes, Average Number of days taking alcohol / month?

i) 0 – 1 ii) 1 – 3 iii) 4 – 6 iv) 7 – 12 v) >12

20. What level of physical activities do you have while at work?

i) Light ii) Moderate iii) Active

21. What level of physical activities do you have while commuting to and from work?

i) Using motorized transportation, or no work (0 min of walking or cycling)

ii) Walking or cycling 1 – 29 min

iii) Walking or cycling >30 min.

22. Do you go for Yoga/ meditation?

i) Yes ii) No iii) Not regular

23. Do you make physical exercise?

i) Yes ii) No iii) Not regular

24. Are you vegetarian?

i) Yes ii) No

25. Do you have any close relative who is/was suffering from osteoarthritis?

i) Yes ii) No

if the answer is yes, who is the person?

i) Father ii) Mother iii) Sibling iv) Father's sibling v) Mother's sibling

vi) Child vii) Father's parents viii) Mother's parents ix) sibling's child

ಪ್ರಶ್ನಾವಳಿಗಳ ನಮೂನೆ
ಕೆ.ಎಲ್.ಇ. ವಿಶ್ವವಿದ್ಯಾಲಯ,
ಜವಾಹರಲಾಲ ನೆಹರು ಮೇಡಿಕಲ್ ಕಾಲೇಜು, ನೆಹರು ನಗರ, ಬೆಳಗಾವಿ.

ಸಂಶೋಧನ ಶೀರ್ಷಿಕೆ

ಮಾನವನ ಕೀಲು ಸಂಧಿಗಳ ನ್ಯೂನ್ಯತೆಗಳ ಉತಾರ ಒಂದು ಸೋಕ್ಷ್ಮತೆಯ ಅಧ್ಯಯನ

ಡೇಮೋಗ್ರಾಫಿಕ್ ಮಾಹಿತಿ.

1. ಗುರುತಿನ ಸಂಖ್ಯೆ : ದಿನಾಂಕ :
2. ಭಾಗವಹಿಸುವವರ ಹೆಸರು : ಇಂದ :
3. ವಯಸ್ಸು :

   1) 20 – 29 2) 30 – 39 3) 40 – 49 4) 50 – 59 5) 60 – 65
4. ಲಿಂಗ : (i) ಗಂಡು (ii) ಹೆಣ್ಣು
5. ವಿವಾಹಿತರೆ ?

   1) ಅವಿವಾಹಿತರೆ ? 2) ವಿಚ್ಛೇದಿತರೆ ? 3) ವಿಧವೆಯೇ ? 4) ಒಬ್ಬಂಟಿಯೇ ?
6. ಜಾತಿ : 1) ಹಿಂದು, 2) ಮುಸ್ಲಿಂ 3) ಕ್ರೈಸ್ತ್ 4) ಇತರೆ
7. ಶಿಕ್ಷಣ : 1) ಅಶಿಕ್ಷಿತ 2) ಪ್ರಾಥಮಿಕ 3) ಪ್ರೌಢಶಿಕ್ಷಣ 4) ಪ್ರೌಢಶಿಕ್ಷಣಕ್ಕಿಂತ ಮೇಲು
   5) ಪಧವಿದರ 6) ಸ್ನಾತಕೋತ್ತರ ಪಧವಿ.
8. ಕುಟುಂಬ ರೀತಿ : 1) ಒಂದೇ ಕುಟುಂಬ 2) ಕೂಡು ಕುಟುಂಬ 3) ಒಡೆದ ಕುಟುಂಬ 4) ಮೂರನೇ ತಲೆಮಾರು
9. ವಾರ್ಷಿಕ ಆದಾಯ : ಬಿ.ಜಿ. ಪ್ರಸಾದ ರ ಪ್ರಕಾರ ಮಾಸಿಕ ಆದಾಯ ರೂಪಾಯಿಗಳಲ್ಲಿ

   1) ಉನ್ನತ ವರ್ಗ 2) ಉನ್ನತ ಮದ್ಯಮ (3) ಮದ್ಯಮ ವರ್ಗ 4) ಕೆಳ ಮಧ್ಯಮ 5) ಕೆಳವರ್ಗ
10. ಯಾವ ವರ್ಷದಲ್ಲಿ ಪ್ರಾರಂಭ ನೌಕರಿ/ ವ್ಯಾಪಾರ

    1) < 1 2) 1–5 3) 5–10 4) >10

ಬಿ– ಜೀವನ ಕ್ರಮ

11. ನೀವು ಸಿಗರೇಟ್ ಸೇದುತ್ತಿರುವಿರಾ ? 1) ಹೌದು 2) ಇಲ್ಲ
12. ಒಂದು ವೇಳೆ ಸೇದುತ್ತಿರಿ– ಎನ್ನುವರಾಗಿ ಯಾವ ವರ್ಷದಿಂದ ಪ್ರಾರಂಭಿಸಿದ್ದೀರಿ.

    1) _____ವರ್ಷಗಳು 2) ಮರೆತುಹೋಗಿದೆ.
13. ಒಂದು ದಿನದಲ್ಲಿ ಸರಾಸರಿ ಎಷ್ಟು ಸಿಗರೇಟ್ ಸೇದುತ್ತೀರಾ ?

    1) 1 – 9 2) 10 – 19 3) > 20
14. ಇನ್ನೂ ನೀವು ಸೇದುತ್ತಿರುವಿರಾ ? 1) ಹೌದು 2) ಇಲ್ಲ

15. ಇಲ್ಲ ಎನ್ನುವದಾದರೆ ಯಾವಾಗ ಸೆದುವದನ್ನು ಬಿಟ್ಟಿದ್ದೀರಾ ? _____

16. **ತಂಬಾಕು ಸೇವೆನೆ ಮಾಡುತ್ತೀರಾ** ? 1) ಹೌದು 2) ಇಲ್ಲ 3) ಮುಂಚೆ ಸೇದುತ್ತಿದೆ ಈಗ ಇಲ್ಲ

17. **ಯಾವ ತರಹದ ತಂಬಾಕು ಸೇವನೆ ಮಾಡುತ್ತೀರಾ**

1) ಗುಟುಕಾ 2) ಪಾನ ಪರಾಗ 3) ಜರ್ದಾ/ಖೈನಿ 4) ಇತರೆ.

18. **ನೀವು ಮಧ್ಯಪಾನ ಸೇವಿಸುತ್ತೀರಾ** ? 1) ಹೌದು 2) ಇಲ್ಲ

19. **ಹೌದು ಇನ್ನುವದಾದರೆ ತಿಂಗಳಲ್ಲಿ ಸರಾಸರಿ ಎಷ್ಟು ದಿನ ಸೇವನೆ ಮಾಡುತ್ತೀರಾ** ?

1) 0–1 2) 1–3 3) 4–6 4) 7–12 5) > 12

20. **ನೀವು ಕೆಲಸ ಮಾಡುವ ವೇಳೆ ಯಾವ ಹಂತ ದೈಹಿಕ ಚಟುವಿಕೆಗಳನ್ನು ಮಾಡುತ್ತೀರಾ** ?

1) ಸಾಧಾಹರಣ 2) ಮಧ್ಯಮ 3) ಚುರುಕಾಗಿ

21. **ಯಾವ ಹಂತದ ದೈಹಿಕ ಶ್ರಮವನ್ನು ಕೆಲಸದಲ್ಲಿ ಮಾಡುತ್ತೀರಾ** ?

1) ಸ್ವಯಂಚಲಿಕ ವಾಹನ ವರ್ಗಾವಣೆ / ಮನೆಯಿಂದಲೇ ಮಾಡುವ ಕೆಲಸ

2) ನಡೆಯುವಿಕೆ ಅಥವಾ ಸೈಕಲ್ 1– 29 ನಿಮಿಷ

3) ನಡೆಯುವಿಕೆ ಅಥವಾ ಸೈಕಲ್ ? 30 ನಿಮಿಷ.

22. **ನೀವು ಯೋಗ ಮಾಡುತ್ತೀರಾ** ? 1) ಹೌದು 2) ಇಲ್ಲ 3) ಸತತ ಇಲ್ಲ

23. **ನೀವು ದೈಹಿಕ ವ್ಯಾಯಾಮ ಮಾಡುತ್ತೀರಾ** ? 1) ಹೌದು 2) ಇಲ್ಲ 3) ಸತತ ಇಲ್ಲ

24. **ನೀವು ಸಸ್ಯಹಾರಿಗಳೇ** ? 1) ಹೌದು 2) ಇಲ್ಲ

25. **ನಿಮ್ಮ ಹತ್ತಿರ ಸಂಬಂಧಿಕರು (ಕೀಲು ನೋವಿನಿಂದ) ಮೊಳೆ ಬೇನೆಯಿಂದ ಬಳಲುತ್ತಿದ್ದಾರೆಯೇ** ?

1) ಹೌದು 2) ಇಲ್ಲ

ಒಂದು ವೇಳೆ ಹೌದು ಎಂದಾದರೆ ಆ ವ್ಯಕ್ತಿ ಯಾರು ?

1) ತಂದೆ 2) ತಾಯಿ 3) ಸಂಬಂಧ 4) ತಂದೆಯ ಸಂಬಂಧ 5) ತಾಯಿ ಸಂಬಂಧ

6) ಮಗು 7) ತಂದೆಯ ಪೋಷಕರು 8) ತಾಯಿಯ ಪೋಷಕರು 9) ಸಂಬಂಧಿಗಳ ಮಗು.

****

प्रश्नाच्या नमूने

के. एल.इ. विश्वविद्यालय,

जवाहरलाल नेहरु मेडिकल कॉलेज, नेहरु नगर, बेळगावि.

शिर्षक : गुडध्याच्या चक्रतीमधील दोषांचा अभ्यास करणारे संशोधन

डेमोग्राफिक विवरणे

१. ओळख नंबर : दिनांक :

२. सहभागीचे नांव :

३. पूर्ण वय : 1) 20 – 29 2) 30 – 39 3) 40 – 49 4) 50 – 59 5) 60 – 65

४. लिंग : (i) पुरूष (ii) स्त्री

५. वैवाहीक जिवन ? 1) विवाहीत, 2) अविवाहीत 3) सोठचि ी 4) विधवा

६. धर्म : 1) हिन्दु 2) मुस्लिम 3) क्रिस्चैन 4) अन्य

७. शिक्षण : 1) अशिक्षीत 2) प्राइमरी 3)माध्यमिक 4)महाविद्यालय 5)पदवीधर
6) उच्चपदवीधर

८. कुटूब पध्दती प्रकार : 1) स्वतंत्र्य 2) एकत्रित 3) विच्छेदीत 4) तिसरी पिढी

९. वार्षिक उत्पन : 1) प्रथम श्रेणी 2) प्रथम मध्यम श्रेणी 3) मध्यम श्रेणी 4) खालची मध्यम श्रेणी
5)खालची श्रेणी

१०. नोकरीच्या किंवा व्यवसाच्या काहावधी(वर्ष मध्ये): 1) < 1 2) 1–5 3) 5–10 4) >10

जिवन क्रम प्रकार

११. धुम्रपान कधी केले आहे का : 1) हो. 2) नाही.

१२. धुम्रपान कोणत्या वर्षापासून वयाच्या करता आहात : 1) _ _ _ _ _वर्ष 2) यादनाही

१३. दिवसाल किती सिगरेट वापरता : 1) 1–9 2) 10–19 3)>20

१४. तूम्ही अजुन धुम्रपान करता का ? 1) हो. 2) नाही.

१५. जर तूमच उत्तर नाही असे आहे तर तुम्ही कधी धुम्रपान सोडले._____

१६. तुम्ही तंबाखू खाता का: 1) हो. 2) नाही. 3)अगोदर करत होतो, आता नाही.

१७. कुठल्या प्रकारची तूम्ही तंबाखु वापरता का ? 1) गुटखा 2) पानपराग 3) जर्दा/खैनी 4) ईतर

१८. तुम्ही दारू घेताका ? 1) हो. 2) नाही.

१९. जर घेत असाल तर महिन्यामध्ये किती वेळा घेता : 1) 0–1 2) 1–3 3) 4–6 4) 7–12 5) > 12

२०. नोकरीच्या ठिकाणी तुम्हीयी शारिरीक किती हालचाल होते : 1) कमी. 2) साधारण. 3) जास्त.

२१. तुम्ही नोकरीच्या ठिकाणी जाता का ?

1) मोटर किंवा कामाच्या ठिकणी जाता का

2) सायकल, चालत जाताका (1 - 29 निमिष)

3) सायकल, चालत जाताका (>30 निमिष)

२२. तुम्ही योगा करता का: 1) हो. 2) नाही. 3) कधीतरी

२३. तुम्ही व्यायाम करता का : 1) हो. 2) नाही. 3) कधीतरी

२४. तुम्ही शाखाहारी आहात का : 1) हो. 2) नाही.

२५. तुमच्या जबलच्या नातेवाईकाध्ये सांधी दु:खी आहेका ? किंवा कुणाला होती का : 1) हो. 2) नाही.

जर उत्तर हो असेल तर ते कोण होते –

1.आई, 2. वडिल, 3.भाऊ बाहिण, 4.वडिलाचे भाऊ बाहिण, 5.आईचे भाऊ बाहिण

6.मूल 7.वडिलचे आई वडिल 8.आईचे आई वडिल, 9.तुम्हाच्या भावाच`मूल.

II) INFORMED CONSENT AND PATIENT INFORMATION SHEET

CONSENT FORM

K.L.E. UNIVERSITY'S

JAWAHARLAL NEHRU MEDICAL COLLEGE, NEHRU NAGAR, BELAGAVI – 590010

Project Title

"Histochemical Study of Mucopolysaccharides and Microscopic Structure of Osteoarthritic Menisci of the Human Knee Joint"

Research Scholar Name:- Mr. Sanjay Kumar Yadav (Tutor)

Supervisor Name:- Dr. V.S. Shirol, Professor, Dept. of Anatomy, JNMC, Belagavi.

Name of Participant:-

Introduction:-

I am going to give you information and invite you to be a part of this research study. You have to decide whether or not you will participate in the research. Before you decide, you feel comfortable with about the research.

There may be some words that you do not understand. Please ask me as we go through the information and I will take time to explain. If you have question later, you can ask them of me, the study doctor or staff.

Explanation of the sample collecting procedure:-

In this study, after your total knee joint replacement surgery or lower limb amputation surgery we will collect your menisci tissues for research purpose. These tissues are surgical waste. The entire procedure is totally noninvasive and painless.

If you agree and allow us to take the menisci tissue for the study purpose then you will be included. Any time during the specimen collection if you feel uncomfortable with about the research study then we will not take your specimen for the research purpose.

Possible risks to you: There will be no harm to the participant since it does not involve any interventions.

Possible benefits to you: You are not expected to get any benefit from being on this research study. The results of the research may provide benefits to the society in terms of advancement of medical knowledge.

Confidentiality: Your identity and the results of tests will not be revealed. All information collected, will be coded therefore, no one will know your identity. During publication, also the identity will not be disclosed.

Right to Refuse and Withdraw: The participation in this research is purely voluntary and you have the right to withdraw from this study at any time during the course of the study without giving any reasons.

Cost of participation: You will not be required to pay for the expenses of study. It will be borne by the principal investigator.

Authentication of publication:- The information obtained from this study, will be used to, write thesis, publish in scientific journals or presented at scientific meetings.

Legal rights: By signing this consent form, you are not waiving any of your legal rights

Questions: For further information / questions, you can contact us at the following address:

Principal Investigator: Mr. Sanjay Kumar Yadav,(Tutor), Ph. 9900591770

Guide: Dr. V S Shirol, Ph. 9449563520

Professor, Dept. of Anatomy, JNMC, Belagavi.

Chairman, Ethical Committee, KLE University, Belagavi: Dr. Anil P. Hogade

Informed Consent Statement:

I have read the information or it has been read to me in my own language. I have had the opportunity to ask questions about it and my questions have been answered to my satisfaction. I consent voluntarily to participate and give my samples for the purpose indicated above.

Name and signature / thumb impression of the participant:

Name _____

Signature _____ Date: _____

Name and signature of witness:

Name _____

Signature _____ Date: _____

Name and signature of the Investigator or representative obtaining consent:

Name _____

Signature _____ Date: _____

ಕೆ.ಎಲ್.ಇ. ವಿಶ್ವವಿದ್ಯಾಲಯ,
ಜವಾಹರಲಾಲ ನೆಹರು ವೈದ್ಯಕೀಯ ಕಾಲೇಜು, ನೆಹರು ನಗರ, ಬೆಳಗಾವಿ.

ಸಂಶೋಧನ ಶೀರ್ಷಿಕೆ

ಮಾನವನ ಕೀಲು ಸಂಧಿಗಳ ನ್ಯೂನ್ಯತೆಗಳ ಉತಾರ ಒಂದು ಸೋಕ್ಷ್ಮತೆಯ ಅಧ್ಯಯನ

ಸಂಶೋಧಕರು :

ಸಂಜಯ ಕುಮಾರ, ಯಾದವ, ಪ್ರಾಧ್ಯಾಪಕರು, ಶರೀರ ರಚನಾಶಾಸ್ತ್ರ ವಿಭಾಗ, ಜೆ.ಎನ್.ಎಂ.ಸಿ. ಬೆಳಗಾವಿ.

ಮೇಲ್ವಿಚಾರಕರು : ಡಾ॥ ವಿ.ಎಸ್.ಶಿರೋಳ, ಪ್ರಾಧ್ಯಾಪಕರು, ಶರೀರ ರಚನಾಶಾಸ್ತ್ರ ವಿಭಾಗ, ಜೆ.ಎನ್.ಎಂ.ಸಿ. ಬೆಳಗಾವಿ.

ಭಾಗವಹಿಸುವವರು :

ವಿವರಣೆ :

ನಾನು ಈ ಸಂಶೋದನೆಯಲ್ಲಿ ಭಾಗವಹಿಸಲು ಆಮಂತ್ರಿಸಿ ನಿಮಗೆ ಮಾಹಿತಿಯನ್ನು ಕೊಡುತ್ತಿದ್ದೇನೆ. ಅದರಿಂದ ನೀವು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನಿರ್ಧರಿಸಿ ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಸ್ವ-ಇಚ್ಚೆಯಿಂದ ಸಮ್ಮತಿಸಬೇಕು.

ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನಿಮ್ಮ ಕೆಲವು ಪ್ರಶ್ನೆಗಳಿರಬಹುದು ಅವುಗಳಿಗೆ ಅಧ್ಯಯನ ಮಾಡುವರು ನಿಮಗೆ ತಿಳಿಹೇಳುತ್ತಾರೆ.

ಅಧ್ಯಯನಕ್ಕಾಗಿ ಬೇಕಾಗುವು ಮಾದರಿಯ ವಿವರ :

ಈ ಸಂಶೋಧನೆಯ ಅಧ್ಯಯನದಲ್ಲಿ ಕೀಲುಗಳ ಮರು ಜೋಡಣೆಯಲ್ಲಿ ಶಸ್ತ್ರ ಚಿಕಿತ್ಸೆಯ ಪ್ರಕ್ರಿಯೆಯಲ್ಲಿ ಕೀಲುಗಳ ನಡುವಿನ 'ಮೆನಿಸ್ಕೈ' ಯನ್ನು ಸಂಶೋಧನೆ ತೆಗೆದುಕೊಳ್ಳಲಾಗುತ್ತದೆ. ಇದು ಶಸ್ತ್ರ ಕ್ರಿಯ ನಂತರ ಉಪಯೋಗವಿಲ್ಲದೆ ತೆಗೆದು ಹಾಕುವುದನ್ನು ಅಧ್ಯಯನಕ್ಕೆ ಬಳಸಲಾಗುತ್ತದೆ.

ನಿಮ್ಮ ಸಮ್ಮತಿ ಇಲ್ಲದೆ ಕೀಲುಗಳ ನಡುವಿನ ಭಾಗವನ್ನು ನಮ್ಮ ಸಂಶೋಧನೆಗೆ ತೆಗೆದುಕೊಳ್ಳಾಗುವುದಿಲ್ಲ.

ಆಗಬಹುದಾದ ತೊಂದರೆ : ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸುವವರಿಗೆ ಯಾವುದೇ ತೊಂದರಿ ಆಗುವುದಿಲ್ಲ.

ಆಗಬಹುದಾದ ಲಾಭಗಳ.

ಈ ಸಂಶೋಧನೆ ಅಧ್ಯಯನದಿಂದ ನಿಮಗೆ ಯಾವುದೇ ನೆರವು ಸಿಗುವುದಿಲ್ಲ. ಈ ಸಂಶೋದನ ಫಲಿತಾಂಶ ಸಮಾಜಕ್ಕೆ ಮತ್ತು ವೈದ್ಯಕೀಯ ಬುದ್ದಿ ಮತ್ತಗೆ ಉಪಯೋಗಿಸಲಾಗುತ್ತದೆ.

ಗೌಪ್ಯತೆ : ನಿಮ್ಮ ಮಾಹಿತಿ ಮತ್ತು ಪಲಿತಾಂಶವನ್ನು ಗೌಪ್ಯವಾಗಿಡಲಾಗುತ್ತದೆ.

ಹಿಂತೆಗೆಯುವಿಕೆ : ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಭಾಗವಹಿಸಲು ನೀವು ಇಚ್ಚಿಸದೆ ಇದ್ದರೆ ಯಾವುದೇ ಕಾರಣವಿಲ್ಲದೆ ಹಿಂತೆಗೆಯಬಹುದು.

ಭಾಗವಹಿಸುವ ಖರ್ಚು : ಈ ಸಂಶೋಧನೆಯ ಖರ್ಚು –ವೆಚ್ಚುಗಳನ್ನು ಸಂಶೋಧಕರು ವಹಿಸಿಕೊಳ್ಳುತ್ತಾರೆ.

ಪ್ರಕಟಣೆ : ಈ ಸಂಶೋಧನೆಯ ಅಧ್ಯಯನವನ್ನು ನಿಯತಕಾಲಿಕ ವೈದ್ಯಕೀಯ ಪತ್ರಿಕೆಗಳಲ್ಲಿ ಪ್ರಕಟಿಸಲಗುವುದು.

ಕಾನೂನಿನ ಹಕ್ಕುಗಳು : ಈ ಸಮ್ಮತಿ ಪತ್ರ ಸಹಿ ಮಾಡುವುದರಿಂದ ನೀವು ಯಾವುದೇ ಕಾನೂನಿನ ಹಕ್ಕುಗಳಿಂದ ವಂಚಿತರಾಗುವುದಿಲ್ಲ.

ನಿಮ್ಮಲ್ಲಿ ಹೆಚ್ಚುವರಿ ಪ್ರಶ್ನೆಗಳಿದ್ದರೆ : ಈ ಕೆಳಗೆ ನಮೂದಿಸಿದ ಸಂಶೋಧಕಗನ್ನು ಕೇಳಬಹುದು.

ಸಂಶೋಧಕರು : ಸಂಜಯ ಕುಮಾರ ಯಾದವ. – (ಅಧ್ಯಾಪಕರು)

ಶರೀರ ರಚನಾಶಾಸ್ತ್ರ ವಿಭಾಗ, ಜೆ.ಎನ್.ಎಂ.ಸಿ. ಬೆಳಗಾವಿ.

(ಮೋ) 9900591770.

ಮಾರ್ಗದರ್ಶಿ : ಡಾ॥ ವಿ.ಎಸ್. ಶಿರೋಳ,

ಶರೀರ ರಚನಾಶಾಸ್ತ್ರ ವಿಭಾಗ, ಜೆ.ಎನ್.ಎಂ.ಸಿ. ಬೆಳಗಾವಿ.

(ಮೋ) 9449563520.

ಚೇರಮನ್, ಎಥೀಕಲ್ ಕಮೀಟಿ, ಕೆ.ಎಲ್.ಇ.ವಿಶ್ವವಿದ್ಯಾಲಯ, ಬೆಳಗಾವಿ : ಡಾ॥ ಅನೀಲ ಪಿ. ಹೂಗಾಡೆ, (ಮೋ) :

ಮಾಹಿತಿಯುಕ್ತ / ತಿಳುವಳಿಕೆಯುಕ್ತ ಸಮ್ಮತಿ ಪತ್ರ

ನಾನು ಸ್ವ-ಇಚ್ಛೆಯಿಂದ ನನ್ನ ಮಗನನ್ನು/ಮಗಳನ್ನು ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಭಾಗವಹಿಸಲು ಸಮ್ಮತಿಸುತ್ತೇನೆ. ನಾನು ಈ ಮೇಲೆ ಕೊಟ್ಟ ಮಾಹಿತಿ ಓದಿದ್ದೇನೆ. ಅಥವಾ ಈ ಮಾಹಿತಿಯನ್ನು ನನಗೆ ತಿಳಿಯುವಂತಹ ಭಾಷೆಯಲ್ಲಿ ಓದಿ ತಿಳಿಸಲಾಗಿದೆ. ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ನನಗೆ ಸಂಪೂರ್ಣವಾಗಿ ತಿಳಿಸಲಾಗಿದೆ ಮತ್ತು ನಾನು ಈ ವಿಷಯಕ್ಕೆ ಸಂಬಂಧಿಸಿದಂತೆ ಸಂಬಂಧಪಟ್ಟ ಪ್ರಶ್ನೆಗಳನ್ನು ಯಾವ ಸಮಯಕ್ಕೆ ಬೇಕಾದರೂ ಕೇಳಬಹುದು.

ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗಹಿಸುವವರ ಸಂಪೂರ್ಣ ವಿವರ:

1. ಪಾಲಕರ ಸಹಿ _____
   ಮತ್ತು ಹೆಸರು _____
   ಫೋನ ನಂ _____

2. ಸಂಶೋಧಕನ ಸಹಿ _____
   ಮತ್ತು ಹೆಸರು _____
   ತಾರೀಖು _____
   ಸ್ಥಳ : _____

3. ಸಾಕ್ಷಿಯ ಸಹಿ _____
   ಮತ್ತು ಹೆಸರು _____
   ತಾರೀಖು _____
   ಸ್ಥಳ : _____

संमत्ती पत्र

शिर्षक : गुडध्याच्या चक्रतीमधील दोषांचा अभ्यास करणारे संशोधन

संशोधाकचे नांव : श्री. संजय कुमार यादव, (ट्यूटर), शरीर रचना शास्त्र विभाग, के. एल.ई, जे.एन.एम.सी.बेळगावी.

मार्गदर्शक : डॉ. वी.एस.शिरोळ. (प्राध्यापक) शरीर रचना शास्त्र विभाग के.एल.ई, जे.एन.एम.सी. बेळगावी.

सहभागीचे नांव :

संशोधनाची ओळख :

वरील संशोधनमध्ये आम्ही तुम्हाल स्वईच्छेने सहभाग घेण्यासाठी आमंत्रीत करीत आहोत. या संशोधक तुम्हाला ह्या संशोधनाबध्दल समजावून सांगतील व तुमच्या सर्व शंका व प्रश्नांची उत्तरे देतील. तुम्ही या संशोधनामध्ये भाग घेण्यापूर्वी खालील सर्व माहिती वाचूनच नंतर सहभागी व्हा. संशोधकाला तुम्ही हे पत्रक नीट वाचून दाखवा असे सांगू शकता.

प्रयोगाची पध्दती:

या संशोधनामध्ये तुमच्या गुडघा बदलण्याच्या शस्त्रक्रिये नंतर तुमचा गुडघ्या मधील जी चकती शस्त्राकिय्रे नंतर टाकली जाईल ती आम्ही आमच्या संशोधना साठी वापरू आमच्या संशोधनाच्या प्रक्रिये मध्ये तुम्हाला काहिही हानी होत नाही जर तुम्ही आम्हाला तुमच्या गुडध्यातील काढलेली चकती आमच्या संशोधनासाठी वापरू दयायला तयार असाल तर तुम्हाला आमच्या संशोधनामध्ये सहभागी केले जाईल. या संशोधनादरम्यान तुम्हाला जर सहभाग नको वाटल्यास, तुमच्या चकतीचा वापर आम्ही करणार नाही.

संशोधनामध्ये सहभागी होण्याचे फायदे:

या संशोधनामधून तुम्हाला काहीही फायदा मिळू शकणार नाही परंतु या संशोधनामुळे समाजातील वैद्यकीय ज्ञान वाठण्यासाठी मदत होईल.

संशोधनामध्ये सहभागी होण्याचे तोटे : या संशोधनामध्ये तुम्हाला कोणात्याही प्रकारची हानी होणार नाही.

संशोधनातून बाहेर पडण्याचा हक्क :

या संशोधनामध्ये भाग ध्यावा किंवा नाही हे तुमच्या वैयाक्तिक इच्छेवरती आधारीत आहे. तुम्हाला जेव्हा वाटेल तेव्हा तुम्ही यासंशोधनातून माघार घेऊ शकता. तुम्हाला कोणतीही जबरदस्ती नाही.

गोपनियता:

तुमच्याकडून मिळालेली संपूर्ण माहिती गुप्त ठेवंण्यात येईल. तुमची ओळख नियमाप्रमाणे कोड करण्यात येईल आणी कोणालाही तुमच्या परवानगी शिवाय दाखविण्यात येणार नाही.

सहभागाचा खर्च :

या संशोधनासाठी लागणारा सर्व खर्च संशोधक करतील. तुम्हाला कोणताही खर्च करावा लागणार नाही.

नियम / अधिकार :

या संशोधनामध्ये सहभागी झाकयामुळे तुमच्या कोणत्याही आधिकाराना हानी होणार नाही.

प्रकाशनाचे आधिकार :

या संशोधनाचा वापर वैद्यकिय संस्थेमध्ये शिकविण्यासाठी आणि वैद्यकीय पुस्तकांमध्ये प्रकाशित करण्यासाठी होईल. तुमची वैयक्तीक माहिती गुप्त ठेवण्यात येईल याची आम्ही पूर्ण हमी देतो.

संपर्क :

तुम्हाला कोण्त्याही प्रकारची माहीती किंवा प्रश्न विचारयाचे असतील तर तुम्ही खाली दिलेल्या पत्यावरती किंवा फोनवरती कधीही संपर्क करू शकता.

संशोधक : श्री. संजय कुमार यादव – (ट्यूटर)

शरीर रचना शास्त्र विभाग, के. एल.ई, जे. एन. एम. सी. बेळगावी. फोन.नं:९९००५९१७७०

मार्गदर्शक: डॉ. वी.एस.शिरोळ. प्राध्यापक शरीर रचना शास्त्र

विभाग के. एल.ई, जे. एन. एम. सी. बेळगावी.

फोन.नं:९४४९५६३५२०

चेरमन, इथीकल कमीटी, के. एल.ई, विश्वविद्यालय, बेळगावी:

डॉ. अनिल पी. होगाडे

(फोन.नं)----------

मान्यत पत्रक

मी सहभागी, लिहुन देतो कि संशोधकानी या संशोधनाची संपूर्ण माहीती अम्हाला सोप्या भाषेत समजवुन सांगितली आहे. आम्हाला या संशोधना मधील परिणाम व दुष्परीणाम सर्व नीट समजले आहे त्यामुळे मी स्वईच्छेने या संशोधनामध्ये सहभागी होत आहे आणि आमच्या समावेश करण्यात यावा.

१. **सहभागी:** हस्ताक्षर / अंगठा

नांव :

फोन :

२. **संशोधक :** हस्ताक्षर

नांव :

फोन :

३. **साक्षी:** हस्ताक्षर / अंगठा

नांव :

फोन :

III) ETHICAL CLEARANCE LETTER

KLE UNIVERSITY

(Formerly known as KLE Academy of Higher Education & Research, Belagavi)
[Declared as Deemed-to-be-University u/s 3 of the UGC Act. 1956 vide Government of India Notification No.F.9-19/2000-U.3(A)]
'Accredited **'A' Grade** by NAAC (2nd Cycle) Placed in **Category 'A'** by MHRD (GoI)

Office of the **Director, Academic Affairs**

JNMC Campus, Nehru Nagar, Bealavi-590 010, Karnataka State, India

☎: 0831-2444444/2493779 **FAX:** 0831-2493777 **Web:** http://www.kleuniversity.edu.in **E-mail:**diracademic@kleuniversity.edu.in

Ref.No.KLEU/EC/17-18/D- 97 16th May 2017

To,
Mr. Sanjay Kumar Yadav
Part Time Research Scholar,
2016-17 batch, Faculty of Medicine
J. N. Medical College, **Belagavi**

Dear Research Scholar,

Sub:- Regarding Ethical Clearance.

The KLE University **Ethics Committee on Human Subjects** for Ph. D Research Project met on **22nd March 2017** to consider your application for approval of the research project **"Histochemical Study of Mucopolysaccharides and Microscopic Structure of Osteoarthritic Menisci of the Human Knee Joint.".**

As there are no ethical issues involved in your proposed research project, the committee has provided approval for this research project.

You are requested to report to Ethical Committee in case of the following:

4. Any deviation from or change of the protocol.
5. All serious adverse events.
6. Any changes in study documents.

(Dr. Anita Dalal)
Member Secretary,
Ph.D. Ethical Committee(Human),
K.L.E. University,
Belagavi.

(Dr. Anil Hogade)
Chairman
Ph.D. Ethical Committee(Human),
K.L.E. University,
Belagavi.

(Dr A. S. Hogade)

CC to: - The Director Academic Affairs, KLE University, Belagavi.
- The Director Research Foundation, KLE University, Belagavi.
- The Registrar, KLE University, Belagavi

IV) PUBLICATIONS

Original Article

Microscopic Structural Changes in Osteoarthritic Menisci of the Human Knee Joint

Sanjay Kumar Yadav, Veereshkumar S. Shirol, Ramesh Chavan[1], Shilpa M. Bhimalli

Departments of Anatomy and [1]Pathology J. N. Medical College, KAHER, Belgaum, Karnataka, India

Abstract

Background: Osteoarthritis (OA) is a degenerative joint disease for which there is currently no cure. It is characterized by degeneration of articular cartilage and changes in other joint tissues, including subchondral (substance) bone and menisci. It is one of the leading causes of chronic disability. Patients affected by this disease experience pain and loss of function. OA can be caused by a variety of factors, including diet, injury, stress, and genetic abnormalities. However, the molecular mechanisms driving the disease onset and progression are not fully understood. Therefore, this study is undertaken to estimate a large number of human OA menisci for microscopical structural changes in osteoarthritic menisci by histological techniques. **Materials and Methods:** Medial and lateral osteoarthritic menisci were collected from 110 human knee joints. After collecting the meniscal samples were stored in 10% formalin for 3–5 days. For each meniscus, three separate (anterior, middle, and posterior) parts were processed. The menisci were sectioned in two places vertically at 45° and 135° angles relative to the sagittal plane. After that, each part was sectioned along the horizontal plane from the inner border to the outer border. Then, tissues were fixed in 10% buffered formalin for 24 h. Tissue samples were brought in for routine tissue processing and studied for histological stain with hematoxylin and eosin (H and E) and Alcian blue pH 2.5, to find surface integrity, cellularity, fibrous organization and collagen orientation, and mucoid degeneration. **Results:** Meniscal degeneration begins with the tissue material rather than the surface. Tissue fibrillation and tears were first observed at the inner border, spread over time to the articular surface of the meniscus, and progressed to complete destruction or loss of meniscal tissue. The left side knee menisci have more OA than the right side. OA cases were more common in both legs, in the age group 60–69 years. Women and Hindus have higher OA cases than men and other religions, respectively. Nonvegetarian and physically inactive individuals were more susceptible to OA, and B +ve and O +ve were more prone to OA than other blood groups. **Conclusion:** Significant cellular and matrix differences were observed in the meniscus during degeneration. These findings may contribute to further understanding of knee OA and the search for biological treatments. OA was associated with religions, family history, dietary habits, exercise, blood types, and age groups. Hence, there is a need for a program on the care of dietary habits and physical activities for reducing the progression of OA.

Keywords: *Histological structural, human knee joint, menisci, osteoarthritic*

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Introduction

The meniscus is a complex fibrocartilaginous tissue that is critical in the knee joint for shock absorption, load distribution, stability, and protection of articular cartilage.[1] Each knee has two menisci: i.e., (i) medial meniscus and (ii) lateral meniscus. The medial meniscus is semicircular and significantly wider posteriorly than anteriorly. However, the lateral meniscus is almost round and roughly uniform in width from anterior to posterior. It occupies a larger portion of the articular surface (80%) than the medial meniscus (60%) and is more mobile.[2]

The developing meniscus is very cellular and vascular. As the fetus continues to develop, the cell structure of the meniscus gradually decreases, accompanied by an increase in the content of collagen arranged in a circumference. In adulthood, only peripheral 10%–30% of menisci have a blood supply.[2] In a normally aligned knee, approximately 70% of the load is conducted through the medial tibiofemoral compartment and 30% through the lateral compartment.

Osteoarthritis (OA) is a degenerative joint disease, for which there is currently

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no cure. It is characterized by degeneration of articular cartilage and changes in other joint tissues, including subchondral bone and menisci.[3] The knee meniscus plays an important role in the complex biomechanics of the knee joint. Meniscal injury, partial or total meniscectomy, or meniscal degeneration are thought to contribute to the development or progression of knee OA. Most of what is known about microstructural changes in the OA meniscus comes from animal studies.[4] This study was conducted to understand human meniscus changes caused by knee OA. Osteoarthritic menisci may exhibit changes in surface integrity, cellularity, fibrous organization, and collagen orientation, which could be interpreted as cellular and structural evidence for the development of knee OA.

OA is one of the leading causes of chronic disability. OA most commonly affects middle-aged and older adults, although younger adults may be affected by injury or overuse. Patients affected by this disease experience pain and loss of function.[5] OA can be caused by a variety of factors, including diet, injury, stress, and genetic abnormalities. However, the molecular mechanisms driving disease onset and progression are not fully understood.[3] Therefore, a study was conducted to examine the microscopical structural changes in the OA meniscus by histological technique.

Materials and Methods

Medial and lateral osteoarthritic menisci were collected from 110 human knee joints of both sexes, 65 women and 45 men, aged 50–84 years. These menisci specimens were collected from consecutive OA patients who undergo total knee joint replacement surgery, and who have undergone lower limb amputation surgery from the Orthopedics department unit of KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi, Karnataka, India. Patients were excluded in case of any malignancy in the menisci or torn menisci and injuries to the menisci. Ethical clearance was obtained by the KAHER'S, Ethics Committee on human subjects. After collecting the meniscal samples were stored in 10% formalin for 3–5 days. After that menisci were cut in a standardized way. For each meniscus, three separate (anterior, middle, and posterior) parts were processed. The menisci were sectioned in two places vertically at 45° and 135° angles relative to the sagittal plane. After that, each part was sectioned along the horizontal plane from the inner border to the outer border. Then, tissue of medial and lateral meniscal samples was fixed in 10% buffered formalin for 24 h. Tissue samples were brought in for routine tissue processing and studied for histological stain with H and E and Alcian blue pH 2.5.

Histological processing and staining

Tissue samples of the medial and lateral menisci were taken and routinely processed in the histology laboratory. After fixation, dehydrated with graded alcohol, then cleared with xylene and infiltrated with paraffin. Tissue was embedded with paraffin and blocks were prepared. After that using the rotatory microtome, 5 μm sections were cut from each region of the medial and lateral menisci and stained with H and E to assess surface integrity, cellularity, fibrous organization, and collagen alignment. Alcian blue pH 2.5 stain was applied for the evaluation of mucoid degeneration.

Histological staining techniques were used to stain the following parts of the menisci of both legs.

For left knee joint
- Left leg medial meniscus anterior part (LMA)
- Left leg medial meniscus middle part (LMM)
- Left leg medial meniscus posterior part (LMP)
- Left leg lateral meniscus anterior part (LLA)
- Left leg lateral meniscus middle part (LLM)
- Left leg lateral meniscus posterior part (LLP).

For right knee joint
- Right leg medial meniscus anterior part (RMA)
- Right leg medial meniscus middle part (RMM)
- Right leg medial meniscus posterior part (RMP)
- Right leg lateral meniscus anterior part (RLA)
- Right leg lateral meniscus middle part (RLM)
- Right leg lateral meniscus posterior part (RLP).

Development of histological scoring/grading system

The scoring system reported in this study was developed after reviewing slides from patients of different ages with OA. For the histological evaluation of the meniscus, criteria were selected that were significantly associated with major changes by age and disease [Table 1]. These criteria include: (i) characteristics of the tissue surface (smoothness or degree of fibrillation, indentations, and undulations); (ii) cellularity (normal, hypercellular, hypocellular, and acellular); (iii) organization of the collagen matrix and fibers including hyalinization, cyst formation, chipping, and tears; (iv) intensity of Alcian blue pH 2.5 staining for mucoid degeneration. After evaluating each category, a total score was calculated. Grade 1 represents normal tissue with scores ranging from 0 to 3, Grade 2 indicates mild degeneration with scores ranging from 4 to 6, Grade 3 indicates a moderately degenerated tissue score of 7–9, and Grade 4 represents the severe degeneration scores ranging from 10 to 12.

For the histological evaluation of meniscus specimens, degeneration grade was assessed by the "C. Pauli" microscopic grading system.
- The range of possible total scores was 0–12 was converted into four grades G1 = 0–3, G2 = 4–6, G3 = 7–9, and G4 = 10–12
- G1 – represents – Normal
- G2 – represents – Mild degeneration
- G3 – represents – Moderate degeneration
- G4 – represents – Severe degeneration

- All images were captured with an Olympus BX-41 microscope equipped with Gryphax software elements. (U-TV1X-2) T7 Tokyo, Japan.

Validation of the grading systems

To validate the microscopic grading system proposed in this study, 110 pairs of menisci (lateral and medial) from the left and right legs, of which the lateral and medial each had three distinct sections (anterior, middle, and posterior), so the total number of slides 660 were graded by Pathologist of KAHER'S, J. N. Medical College, Belagavi.

Statistical analysis

Descriptive statistics were used to generate histological scores and distributions to summarize mild, moderate, and severe meniscal OA. A nonparametric method (Chi-square test) was used to see the association between the sides and OA. Furthermore, there is a 5% significance level. Analyses were performed using MS Excel and SPSS version 22 (IBM, Bangalore, Karnataka, India).

Results

Analysis of Osteoarthritis menisci

Microscopic evaluation of menisci from osteoarthritic knee joints (Grade II, III, and IV) revealed fibrocartilaginous disruption. The degenerated extracellular matrix shows fine fibrillation and loss of structure, the fusion of the spaces occupied by the cells of the meniscus and matrix. Great variability in cell distribution was observed with hypercellular, hypocellular, and acellular areas, as well as areas containing large and abundant cell groups. Clusters of abnormal cells were found near the surface of the meniscus, typically associated with a worn area [Figure 1]. Variability was observed in the intensity pattern of mucoid degeneration with light and moderate staining areas.

Table 1: Scoring system

	Score
1. Surface	
I. Smooth or linear	0
II. Slight abrasion or cell cluster	1
III. Moderate abrasion or markedly undulation	2
IV. Severe abrasion or disruption (calcification, cell cluster)	3
2. Cellularity	
I. Normal	0
II. Diffuse hypercellularity	1
III. Diffuse hypo/acellular regions	2
IV. Hypo cellular (lacuna, pycnotic cells)	3
3. Collagen fiber organization	
I. Collagen fibers organized, homogeneous of extracellular matrix	0
II. Collagen fibers organized, diffuse foci of hyalinization	1
III. Collagen fibers unorganized, confluent foci or band of hyalinization, fraying	2
IV. collagen fibers unorganized, fibrocartilaginous separation, severe fraying, and tears	3
4. Mucoid degeneration (Alcian blue ph. 2.5)	
I. None	0
II. Slight	1
III. Moderate	2
IV. Severe	3

Criteria and scores for histological assessment of menisci developed by C. Pauli (2011), have been used to evaluate: I. Surface, II. Cellularity, cellular morphology, III. Collagen fiber organization, IV. Mucoid degeneration

Histological assessment of meniscus in LMA

Histological evaluation of the left leg medial meniscus (LMA) of the anterior part is shown in Figure 1, where (1) Indicates bands of degenerative changes, (2) Degenerated extracellular matrix substance (mucoid) is observed, (3) Diffuse hypercellularity with inflammation is observed, and (4) Separation of fibrocartilage with organized collagen fibers.

Histological assessment of meniscus in LMM

Histologic evaluation of the meniscus in the middle medial meniscus (LMM) of the left leg is shown in Figure 1, where (1) indicates moderate abrasion, fraying and waviness, few indentations, and few clefts, (2) the disorganized cellular pattern showed hypocellular to acellular areas and cell clusters, and (3) indicates the presence of degenerated extracellular matrix; most of the collagen fibers appear unorganized.

Histological assessment of meniscus in LMP

Figure 1 shows the histological evaluation of the meniscus in the left leg medial meniscus posterior part (LMP), where (1) indicates moderate-to-severe abrasion with a tear, (2) diffuse hypercellularity is seen, and (3) fibrocartilage separation in the presence of disorganized collagen fibers.

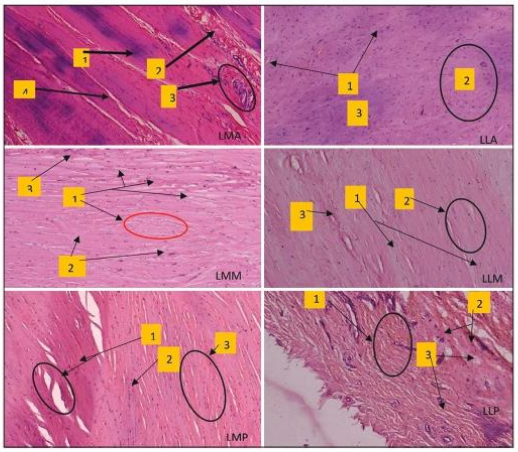

Figure 1: Hematoxylin and Eosin (H and E), ×10 (Olympus Microscope

Histological assessment of meniscus in LLA

Figure 1 discloses histological assessment of meniscus in left leg lateral meniscus anterior part (LLA), where 1 indicates slight abrasion and wrinkle, 2 indicates diffuse hypercellularity, and 3-indicates loose and degenerative changes.

Histological assessment of meniscus in LLM

Figure 1 reveals a histological assessment of meniscus in the left leg lateral meniscus middle part (LLM), where 1 represents a slightly worn and undulating surface, 2 represents a normal distribution of cells; cells appear to be more arranged between fibers, and 3 degenerated extracellular matrix substance and collagen fibers are unorganized.

Histological assessment of meniscus in LLP

Figure 1 shows a histological assessment of the meniscus in the left leg lateral meniscus posterior part (LLP), where 1 indicates severe abrasion, tear, and disruption, 2 depicts diffuse hypercellularity with clusters of cells, and 3-Fibrocartilage dissociation (edema, mucosal degeneration, cystic fissures, and disjointed collagen fibers.

Alcian blue at pH 2.5 staining intensity

LMA, LMP, LLA, and LLP showed slight staining intensity to Alcian blue 2.5, with a score of 1, while LMM and LLM showed moderate staining intensity for Alcian blue 2.5 scored as 2 [Figure 2].

Figure 3 shows the distribution of OA menisci percentage on the side of the legs. Approximately 51% of people have left leg OA and 49% in the right legs. Among 110 OA patients, about 59% and 41% of OA problems are in females and males, respectively [Figure 4].

OA are varying by age and side, as shown in Figure 5. Minimum case of OA was found in 50–59 and 70+ years, whereas OA cases were higher in the age group 60–69 years in both left and right side legs.

Sex, religions, family history, and vegetarian food

The distribution of OA in this series is shown in Figure 4. In the gender group, women have a higher incidence of OA than men, i.e., (59% of women and 41% of men). Among religions, Hindus get a more percentage of OA, than other religions (Muslims and Christians), (57% Hindu, 41% Muslim, and 2% Christian). Of these, 64% had no family history of OA, while 36% had a family history of OA. Whereas for dietary habits, nonvegetarians (71%) were more susceptible to OA than vegetarians (29%).

Among different types of physical activity, i.e., (moderate, active, light, medium, and inactive), inactive people were more affected by OA (52.7%). In the blood group distribution, people with B +ve (30.9%) and

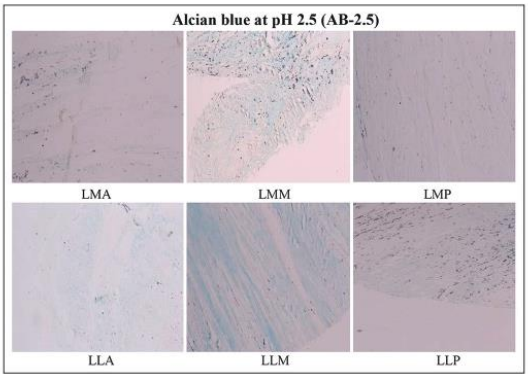

Figure 2: Mucoid degeneration: ×10

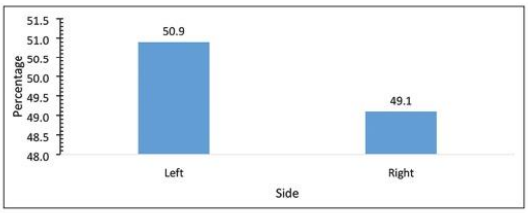

Figure 3: OA menisci by side of legs. OA: Osteoarthritis

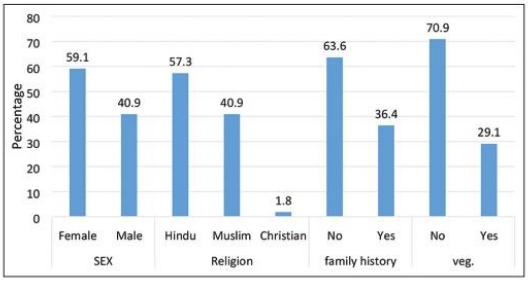

Figure 4: OA menisci by sex, religion, family history, and vegetarian food. OA: Osteoarthritis

O +ve (35.5%) blood types are more susceptible to OA than other blood types. In terms of age group distribution, people aged 60–69 were more susceptible to OA than other age groups [Figure 6].

Table 2 reveals the histological grade distribution of the OA meniscus on the sides of the leg and its medial and lateral meniscal parts. In this study, 110 medial and lateral menisci were observed in the anterior, middle, and posterior parts. About 51% of the left OA meniscus and 49% of the right OA meniscus were observed [Figure 3]. The study showed 8.9% mild, 76.8% moderate, and 14.3% severe OA in the left leg medial meniscus anterior part (LMA), whereas 1.8% mild, 92.9% moderate, and 5.4% severe OA in LMM. In LMP, mild, moderate, and severe OA are 14.3, 75% and 10.7%, respectively [Table 2].

Table 2: Osteoarthritis menisci by side of legs and parts of medial menisci

OA	Left, *n* (%)	Right, *n* (%)	Overall, *n* (%)	χ^2 (df), *P*
Medial anterior				
Mild	5 (8.9)	2 (3.7)	7 (6.4)	1.309 (2), 0.520
Moderate	43 (76.8)	43 (79.6)	86 (78.2)	
Severe	8 (14.3)	9 (16.7)	17 (15.5)	
Medial middle				
Mild	1 (1.8)	1 (1.9)	2 (1.8)	0.196 (2), 0.907
Moderate	52 (92.9)	49 (90.7)	101 (91.8)	
Severe	3 (5.4)	4 (7.4)	7 (6.4)	
Medial posterior*				
Mild	8 (14.3)	1 (1.9)	9 (8.2)	6.057 (2), 0.048
Moderate	42 (75.0)	44 (81.5)	86 (78.2)	
Severe	6 (10.7)	9 (16.7)	15 (13.6)	

*The Chi-square statistic is significant at the 0.05 level. OA=Osteoarthritis

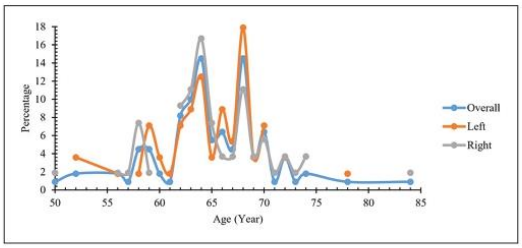

Figure 5: OA menisci by age and side of legs. OA: Osteoarthritis

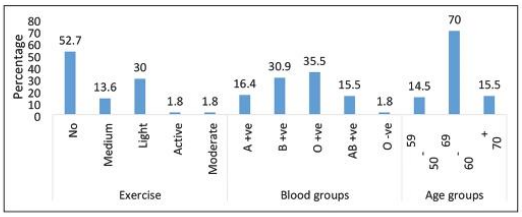

Figure 6: Menisci by exercise, the blood group, and age group. OA: Osteoarthritis

Left leg lateral meniscus anterior part (LLA) had 8.9% mild, 85.7% moderate, and 5.4% severe OA. However, LLM had 12.5% mild, 76.8% moderate, and 10.7% severe OA, whereas LLP had 12.5% mild, 83.9% moderate, and 3.6% severe OA [Table 3].

Right leg medial meniscus anterior part (RMA) had 3.7% mild, 79.6% moderate, and 16.7% severe OA, whereas RMM had 1.9% mild, 90.7% moderate, and 7.4% severe OA. Similarly, RMP had 1.9% mild, 81.5% moderate, and 16.7% severe OA [Table 2]. The right leg lateral meniscus anterior part (RLA) showed 98.1% moderate and 1.9% severe OA. Similarly, RLM had mild (7.4%), moderate (81.5%), and severe (11.1%) OA. However, RLP was 1.9% mild, 87.0% moderate, and 11.1% severe OA [Table 3]. The posterior part of the medial menisci of both legs is significantly associated with OA [Table 2], whereas, the anterior and posterior parts of the lateral menisci have a significant association ($P < 0.05$) [Table 3].

Discussion

Menisci are complex fibrocartilaginous tissues.[1] Several disruption and loss of articular fibrocartilage is the structural hallmark of OA. The menisci play an important role in both tibiofemoral compartments through load distribution and shock absorption. Microscopic analysis revealed a strong association between degenerated meniscus and OA.[6-10] Microscopic degeneration is a nearly constant finding in middle-aged and older patients and is common in both the lateral and medial menisci. In elderly participants with uniform degeneration of the matrix and collagen, other factors may contribute to severe meniscal tears. Meniscal degeneration is a complication of OA. However, inaccessible meniscal injuries can also lead to OA.[11] The mechanistic relationship between meniscus injury and knee OA is not fully understood. Experimental animal models are used to study meniscal changes during OA development.[12] Several authors have also described human meniscus degeneration.[13-17] but most grading systems for the meniscus are based on MRI.[18] However, this study provides a more systematic assessment of aging and OA meniscal changes at the microscopic level and validates more details about the severity of OA through a grading system.

Histological analysis (H and E staining) of the medial meniscus in the posterior part of the left and right legs observed moderate-to-severe abrasion, and tore on the surface, diffuse cellularity, fibrocartilage separation, the presence of disorganized collagen fibers, and rarely mucoid degeneration. In the lateral meniscus of both legs, there were slight abrasions and folds on the surface, diffuse cellularity, and loose fibers with degenerative changes observed in the anterior part, while in the posterior part of the lateral meniscus were seen severe abrasion, tearing, and destruction on the surface, diffuse hypercellularity with a cluster of cell and fibrocartilage dissociation, and

Table 3: Osteoarthritis menisci by side of legs and parts of lateral menisci

OA	Left, *n* (%)	Right, *n* (%)	Overall, *n* (%)	χ^2 (df), *P*
Lateral anterior*				
Mild	5 (8.9)	0	5 (4.5)	6.213 (2), 0.045
Moderate	48 (85.7)	53 (98.1)	101 (91.8)	
Severe	3 (5.4)	1 (1.9)	4 (3.6)	
Lateral middle				
Mild	7 (12.5)	4 (7.4)	11 (10.0)	0.794 (2), 0.672
Moderate	43 (76.8)	44 (81.5)	87 (79.1)	
Severe	6 (10.7)	6 (11.1)	12 (10.9)	
Lateral posterior*				
Mild	7 (12.5)	1 (1.9)	8 (7.3)	6.466 (2), 0.039
Moderate	47 (83.9)	47 (87.0)	94 (85.5)	
Severe	2 (3.6)	6 (11.1)	8 (7.3)	

*The Chi-square statistic is significant at the 0.05 level.
OA=Osteoarthritis

broken collagen fibers. In another study, the examination of articular cartilage in a human knee with OA and found that histological analysis (H and E staining) of cartilage from an OA donor showed joint swelling and edema and horizontal splitting cracks or flaps. The surface becomes dull and irregular and has minimal healing capacity. Moderate OA cartilage showed structural changes, including a reduction in superficial and medial cartilage thickness. The collagen network structure was damaged, resulting in reduced cartilage thickness. Chondrocytes can no longer maintain their repair activity, and cartilage tissue was subsequently lost. In severe OA cartilage surface, fissures were deep, cells in the peripheral zone disappear, and clonal, intermediate, and circular zones lack cells that were not arranged in the columns. The slight changes are detected in the cartilage surface, which is no longer smooth, and the subchondral bone shows fibrillation.[19] In other studies on animals, in the rabbit OA model, cell density in the meniscus increased or decreased depending on the area, and clusters of cells were frequently found in degenerated areas.[12,20] However, these changes were not evident in the human OA meniscus.[21,22]

In different parts of the meniscus of both legs, the medial meniscus had mild (14.3% on left and 1.9% on right), moderate (75.0% on left and 81.5% on right), and severe (10.7 on left and 16.7% on right) OA in the posterior part, shown significant at 5% level. While the lateral menisci in anterior and posterior part menisci were significant at the 5% level. In other studies of human OA menisci, histological changes in the anterior part segment of the OA meniscus were moderate.[23] Furthermore, in other studies conducted on human menisci, in moderate or severe OA, there was severe matrix destruction in one or more meniscal regions. The posterior part was most often affected microscopically. Biomechanically, during knee flexion, the femoral condyles roll back onto the tibial plateau and more force is transmitted to the posterior portion of the meniscus, usually subluxing the posterior part of the lateral meniscus during deep flexion.[24-27] On the other hand, the anterior part is always less affected. This suggests that the anterior part may be more resistant to degeneration or that the anterior part is subject to less damaging biomechanical stress. On macroscopic and histopathological examination, the inner borders of the medial and lateral menisci tend to degenerate.[4]

Apart from this, in the current study, the left leg has more OA than the right leg. OA cases in both left and right legs were higher in the 60–69 years of age group. While another study described, that the chance of developing OA increases with age by previously injured joint overload, joint misalignment, and obesity can all contribute to the development of OA. Age-related changes observed in the cells and extracellular matrix of joint tissue may increase the susceptibility of older adults to OA. Other risk factors for OA also exist. OA is characterized by an imbalance between catabolic and anabolic activity in the joints, and aging may contribute to this imbalance. Aging chondrocytes respond poorly to growth factor stimulation and are unable to maintain articular cartilage homeostasis. Chondrocyte loss due to increased susceptibility to cell death also appears to be important.[28]

This study shows OA is associated with gender, religion, family history, dietary habits, exercise, blood type, and age groups in India. However, in the previous study, OA was not correlated with religion, family history, food pattern, exercise, and blood groups.[1-18] The study found that across gender groups, women (59%) had more OA than men (41%), because risk factors for OA in women, as in men, are many like anatomical differences, obesity, previous trauma, genetic disorder, and hormonal issues. Menopausal women often gain weight, and increased joint stress leads to increase OA in women. Anatomically, women's hips are wider than men's. The angle formed by the hip bone being wider than the knee puts more stress on the outside of the knee. This "knock-kneed" posture, even mild, leads to OA in some women over time. Anatomical differences between males and females that play a role include a narrower femur, thinner patella, greater quadriceps angle, and differences in tibial condyle size.[29]

The present study shows Hindus (57%) had higher rates of OA than Muslim religion (41%), whereas another study found the same that Muslims have a lower prevalence of OA than Hindus of the same race but with different religious practices. The Muslim way of praying since childhood, forcing the knees into deep flexion, may stretch the soft tissue surrounding the knee and decrease stiffness and contact pressure of the articular cartilage,[30] and in family history, 64% had no family history of OA. While other studies described that, people inherit an increased risk of developing OA. This predisposition can be passed down from generation to generation in families, but the pattern

of inheritance is unknown. In the first cross-sectional comparison, the author reported a higher prevalence and severity of cartilage defects in the offspring.[31] While the longitudinal study suggested that not only genetic but knee cartilage loss, changes in cartilage defects, and decreased physical performance all play a role in the development of knee OA, this is likely polygenic but reflects a shared environment. The authors concluded that offspring with a family history of knee OA have an increased risk of knee pain independent of structural factors, suggesting that genetic factors may be involved in the OA.[32]

Nonvegetarians (71%) were more susceptible to OA than vegetarians. According to Chang Xu *et al*., western unhealthy dietary patterns that include French fries, red and processed meats, poultry, refined grains, sugar-sweetened beverages foods, and pizza, were associated with increased radiographic and symptomatic knee OA progression, whereas adherence to promoting a healthy, prudent diet that includes fruits, vegetables, beans, fish, and whole grains were associated with decreased radiographic and symptomatic Knee OA progression in longitudinal cohorts of men and women.[33]

The study also showed that people who did not participate in physical activity were more likely to be affected by OA (52.7%). Other studies were concluded that physical activity increased the risk of OA if it was a part of sports activity that continually exposed normal joints to a high level of impact or torsional loading, and also if the activity consisted of sport that could lead to injury to supporting structures such as ligaments, tendons, and menisci. There is overwhelming evidence that mild-to-moderate physical activity does not cause or accelerate knee OA; in fact, exercise prevents its onset and is clearly effective in treating and managing OA-related pain and functional decline. According to modern literature, regular mild-to-moderate physical activity has preventive and therapeutic effects in patients with knee OA.[34,35]

In this study, B +ve (30.9%) and O +ve (35.5%) blood types are more susceptible to OA than people with other blood groups. Whereas another study found that the AB blood group is a risk factor for primary knee joint OA independent of age and gender. Blood group-related Le^Y antigen may play a role in the association between AB blood group and primary knee OA. Despite rigorous methodology, the inherent limitations of retrospective studies are unavoidable.

Prospective cohort studies should confirm the findings of this study.[36]

Conclusion

Degeneration of the meniscus begins in the substance of the tissue rather than the surface. Tissue fibrillation and tears are first observed at the inner border, spread over time to the articular surface of the meniscus, and progress to complete destruction or loss of meniscus tissue. OA menisci in both legs were significantly associated with medial menisci of the posterior part ($P < 0.05$), while lateral meniscus of the anterior and posterior parts was significantly associated with OA menisci. The left leg knee menisci have more OA prevalent than the right leg knee menisci. OA cases are more in both legs in the 60–69 years of age group. The OA cases are more in women and Hindus than in men and other religions, respectively. Nonvegetarians and not physically active are more likely to be affected by OA. Moreover in blood groups, B +ve and O +ve are more susceptible to OA than other blood groups. The findings of this study may help the physicians, clinicians, and policy planners to have programs on the care of dietary habits and physical activities for reducing the progression of OA.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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HISTOCHEMICAL STUDY OF ACID MUCINS IN OSTEOARTHRITIC MENISCI OF THE HUMAN KNEE JOINT

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ABSTRACT

Knowledge regarding changes of proteoglycans (acid mucins) in human osteoarthritis (OA) meniscus may help in understanding development of meniscal degeneration. Therefore, present study was planned to know changes in acid mucins in human knee OA menisci by histochemical analysis of different parts of medial and lateral menisci of both legs. Medial and lateral OA menisci were collected from 110 human knee joints of both sexes. Normal meniscal tissue of sheep was taken as control and studied for histological stain with alcian blue pH 2.5, to find acid mucins changes in OA menisci. Data were analyzed by bivariate and one-way ANOVA using MS-Excel. Osteoarthritis is more common in females than males. OA changes were found to be more on right side in females and on left side in males, while OA was more common in both legs in number of cases in 60-69 years. Further, decreased staining intensity for acid mucins was observed in different parts of medial and lateral OA menisci of both legs than control meniscus. A significant change in level of acid mucin was observed at anterior, middle, and posterior parts of medial and lateral OA menisci of both legs (P-value=0.0306). Significant changes in acid mucins in human OA meniscus provide information on scientific evidence of OA progression, which could help health professionals in development of structure-modifying drugs for OA therapy.

KEYWORDS

Histochemical, proteoglycans (acid mucins), osteoarthritic, menisci, human knee joint

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INTRODUCTION

The knee meniscus is a specialized tissue that plays an important role in power transmission, shock absorption, and joint stability, and contributes to joint lubrication.[1, 2] osteoarthritis (OA) is a progressively disabling disease caused by a pathological imbalance between degenerative and repair processes. Patients with knee meniscus injuries are at high risk of developing the disease,[3] up to 91% of patients with symptomatic knee OA have concurrent meniscal tears,[4] and it is one of the strongest risk factors for the development and progression of knee OA.[5] The probability of horizontal meniscus tear was 63% in patients with imaging evidence of OA, but only 23% in patients without imaging evidence of OA.[6] Multiple MRI (magnetic resonance imaging) studies have shown that meniscal degeneration is a common feature of OA, and meniscal degeneration is an important risk factor for the development of OA.[7-9] Consistent with the role of the meniscus in knee function, meniscal injuries are common in athletes and the general population. The complex role of meniscal tissue components in the etiology of the subsequent development of knee OA is not fully understood, and it is increasingly clear that the meniscus plays a critical role in the long-term health of the knee. To study meniscal degeneration during the development of knee OA and to prevent its progression, changes in meniscal tissue composition must be detected before gross morphological changes occur.[10,11]

Proteoglycans and other non-collagen proteins play an important role in stabilizing the extracellular network and are therefore important in maintaining the structural integrity and mechanical properties of the meniscus. Cartilage oligomeric matrix protein (COMP) is a component of the cartilage matrix and plays an important role in the construction of the extracellular matrix.[12-16] Procollagen I/II and chondrocytes, bound to collagen types I, II, and IX, play a role in the storage and release of hydrophobic hormones, and calcium-binding proteins.[17-19] The chemical composition of the meniscus also varies by different region, with predominantly type I collagen in the outer, more fibrous, area and a mixture of type I and type II collagens in the inner, more cartilaginous area.[20] The greater part of the remaining extracellular matrix (ECM) is composed of negatively charged glycosaminoglycans (GAGs),[21] which hydrate the tissue, contribute to its compressive properties, and also enable electrical activity.[22] Later a meniscus injury, an increase in GAG levels in the synovial fluid peak early and persist for four years after injury.[23] Mucins are called mucopolysaccharides, glycosaminoglycans, and mucosubstance. More recently the term glycoconjugates have been divided into proteoglycans and glycoproteins.[24]

Proteoglycans (mucoproteins) are formed from glycosaminoglycans (GAGs) covalently linked to core proteins. Mucopolysaccharides or glycosaminoglycans are long, unbranched polysaccharides composed of repeating disaccharide units. The repeating unit (except keratan) consists of amino sugars (N-acetyl glucosamine or N- acetylgalactosamine) with a uronic sugar (glucuronic acid or iduronic acid) or galactose. Mucopolysaccharides are highly polar and absorb water. Therefore they can be useful as lubricants or shock absorbers for the body. The highly negatively charged GAG chains allow the proteoglycans to seize water and divalent cations and confer space-filling and lubricating functions.[25,26]

Meniscal degeneration in OA has been extensively studied. It has been shown that there was a much less organized network of collagen in OA menisci compared to early OA, and that collagen content was reduced in advanced OA.[27,28] However, the literature review indicates that previous studies on the OA menisci were performed using animal models, and there were few previous studies on proteoglycans changes in human OA menisci. Therefore, the chemical changes in the OA menisci are likely to be localized to different regions.[29] So the present study was undertaken to know the acid mucins (proteoglycan) changes in human OA menisci in different parts with a large sample size to have an understanding of the degenerative disease process.

MATERIALS AND METHODS

Medial and lateral osteoarthritic menisci were collected from 110 human knee joints of both sexes. The design of the study was hospital based cross-sectional study, and the sample size was calculated by the below-mentioned formula;

$$n = \frac{Z^2_{1-\alpha/2} \times SD^2}{(0.2 \times SD)^2} \times 1.1 = 110$$

Where,

$Z_{1-\alpha}$ = 1.96 with 95% C.I

SD = Standard Deviation

d = Tolerable error

Sample size at 95% CI, 20% error, and 10% lost to data entry/data collection or outliers.

Menisci were collected from 65 females and 45 males, aged 50-84 years. These menisci specimens were collected from consecutive osteoarthritis (OA) patients who had undergone total knee joint replacement surgery, and lower limb amputation surgery from the Orthopedics department unit of KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi, Karnataka, India. Patients were excluded in case of any malignancy in the menisci or torn menisci and injuries to the menisci. Meniscal specimens were collected intraoperatively during total knee replacement (TKR) surgery. Normal meniscal tissue from 8 months old (n=1) male domesticated (cud-chewing) ruminant sheep (ovine) average weight 13 kg was used in this study. Sheep meniscal tissue was used as a control and showed no signs of knee-related musculoskeletal disease. It was obtained from a commercial source at Nehru Nagar in Belagavi and dissected after 4 hours of defeat.[30] Meniscal samples were stored in 10% formalin for 3 to 5 days. After that menisci were cut in a standardized way. For each meniscus, three separate parts (anterior, middle, and posterior) were processed. The menisci were sectioned in two places vertically at 45° and 135° angles relative to the sagittal plane. After that, each part was sectioned along the horizontal plane from the inner border to the outer border. Then tissue of medial and lateral meniscal samples was fixed in 10% buffered formalin for 24 hours. Tissue samples were brought in for routine tissue processing and studied for histological stain with a color intensity of Alcian blue pH 2.5.

Ethical consideration: Prior approval was taken from the institutional ethical committee (ref. no. KLEU/EC/17-18/D-97; Dated 16/5/2017). Informed written consent was obtained from the participants before initiating the data collection process. Privacy and confidentiality of information were maintained and informed about their freedom of choice.

Histological processing and staining: Tissue samples of the medial and lateral menisci were taken and routinely processed in the histology laboratory. After fixation, dehydrated with graded alcohol, then cleared with xylene and infiltrated with paraffin. Tissue was embedded with paraffin and blocks were prepared. After that using the rotatory microtome, 5 µm sections were cut from each different parts of the medial and lateral menisci and stained with alcian blue pH 2.5, and evaluated for proteoglycan (acid mucins).

Development of histological scoring/grading system: The scoring system reported in this study was developed after reviewing slides from patients of different ages with OA. For microscopic evaluation of meniscus, the staining color intensity of Alcian blue pH 2.5 for acid mucins was graded as a negative stain (- : 0%), weak or variable stain (± : <25%), slight or mild stain (+ : 26-50%), moderate stain (+ + : 51-75%), and strong stain (+ + +: 76-100%).[31,32] All images were captured with an Olympus BX-41 microscope equipped with Graphix software elements. (U-TV1X-2) T7 Tokyo, Japan. The histochemical grading of the meniscal OA was done by a Pathologist of KAHER's, JN Medical College, Belagavi.

Statistical analysis: Descriptive statistics were used to generate histochemical scores and distributions to summarize negative, weak, mild, moderate, and severe acid mucins in meniscal osteoarthritis. Further chi-square test was used to see the association between AB-2.5 Staining Intensity of Acid Mucins and sides of osteoarthritis menisci. One-way ANOVA (F-test) was applied to test various parts of Alcian Blue 2.5 Staining Intensity of Acid mucins in medial and lateral OA menisci of both left and right legs. Also, significance was seen at 5% level. Analyses were performed using MS Excel.

RESULTS

Meniscal samples were taken from 110 patients of different ages with OA in this study. In that 65 (59%) females and 45 (41%), males participated (Fig. 2). Among them 56 (51 %) have left leg OA and 54 (49 %) a right leg OA (Fig. 1). Further, 43% of females have left leg OA and 57 % have a right leg. On the other hand, 62 % and 38 % of males got left and right leg OA respectively (Fig. 3). In this study, the patient's sample was taken from the age group 50-84 years and minimum number of cases of osteoarthritis were found in

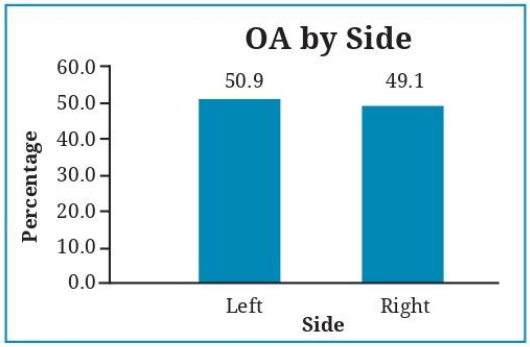


Fig. 1: Osteoarthritis (OA) menisci by side of legs

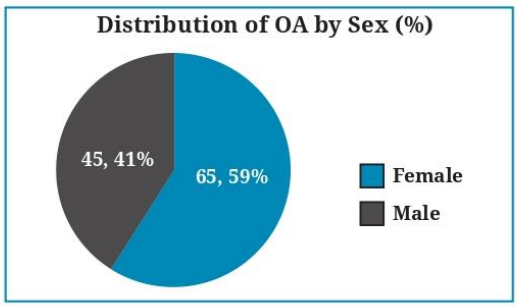


Fig. 2: Distribution of osteoarthritis menisci by sex

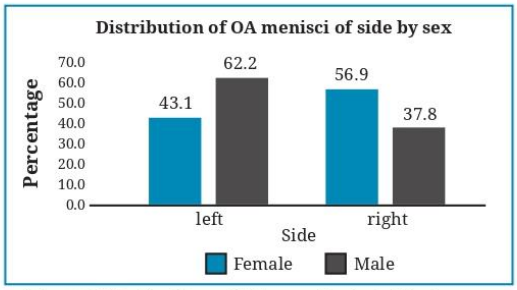


Fig. 3: Distribution of OA menisci of side by sex

50 – 59 and 70+ years, whereas OA cases were higher in the age group 60 - 69 years in both left and right legs of knee joint (Fig. 4).

Fig. 1 shows the distribution of Osteoarthritis (OA) menisci percentage on the side of the legs. Approximately 51 % of people have left leg OA and 49 % in the right legs. Among 110 OA patients, about 59% and 41% of OA problems are in females and males respectively (Fig. 2).

Fig. 3 shows the distribution of OA menisci of side by sex. 43% of females have left leg OA whereas 57% have a right leg OA. On the other hand, 62% and 38% of males got left and right leg OA respectively.

Osteoarthritis menisci are varying by age and side, as shown in Fig. 4. The minimum number of cases of osteoarthritis was found in 50–59 and 70+ years, whereas OA cases were higher in the age group 60-69 years in both left and right legs of knee joint.

Fig. 5a shows assessment of histological staining intensity of alcian blue pH 2.5 in the extracellular matrix of sheep meniscus used as control group at 3 regions (anterior, middle, posterior) of medial and lateral menisci of right leg: RMA, RMM, RMP, RLA, RLM and RLP showed strong staining with score (+ + +).

Fig. 5b shows an assessment of histological staining intensity of alcian blue pH 2.5 in the extracellular matrix of OA human meniscus used as a test group at 3 regions (anterior, middle, posterior) of medial and lateral menisci of both legs: figure 'A' score: negative (-), figure 'B' score: weak or variable stain (±), figure 'C' score: Slight or mild stain (+) and figure 'D' score: moderate stain (+ +).

Table 1a reveals a histochemical assessment of alcian blue pH 2.5 staining intensity level in the extracellular matrix of medial menisci of left and right legs. The study showed strong staining intensity of alcian blue pH 2.5 in the control and its score of three plus (+ + +) acid mucins. It is present in both legs of the medial and lateral menisci of sheep. The observation made in the left leg medial meniscus anterior part (LMA) was 1.8% negative, 51.8% weak, and 46.4% mild staining intensity of AB-2.5 while, it was 1.8% negative, 46.4% weak, and 51.8% mild in the left leg medial meniscus middle part (LMM). Similarly, the staining intensity in the left leg medial meniscus posterior part (LMP)

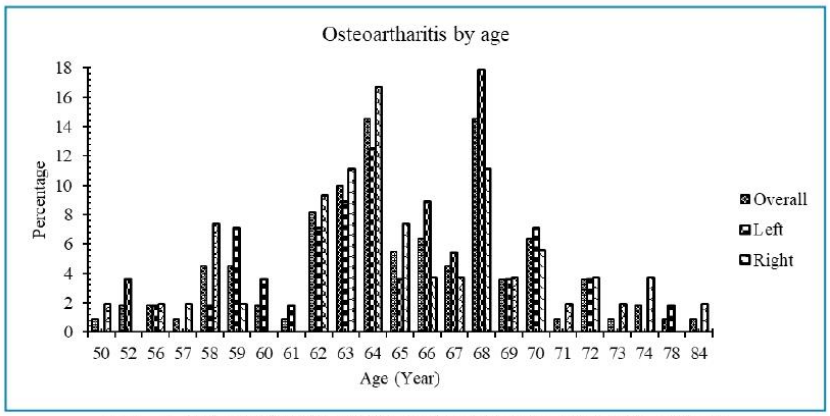
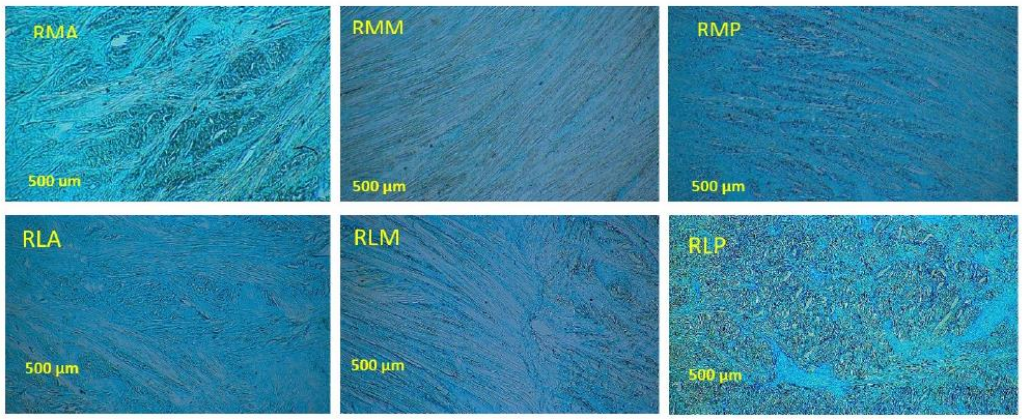


Fig. 4: Osteoarthritis (OA) menisci by age and side of legs



Note: RLA- Right leg lateral meniscus anterior part, RLM- Right leg lateral meniscus middle part, RLP- Right leg lateral meniscus posterior part, RMA- Right leg medial meniscus anterior part, RMM- Right leg medial meniscus middle part, RMP- Right leg medial meniscus posterior part

Fig. 5a. The intensity of acid mucins in control group: magnification 10x

Table 1a: Alcian blue pH 2.5 Staining Intensity of Acid Mucins in Medial Meniscus of left and right legs.

Alcian blue 2.5 staining intensity of acid mucins		Left leg		Right leg		Overall	
		n1	%	n2	%	n	%
Control (normal menisci)	Strong (+ + +)	1	100	1	100		100
Test (OA menisci)							
Medial anterior	Negative (-)	1	1.8	2	3.7	3	2.7
	*Weak (±)	29	51.8	27	50	56	50.9
	#Mild (+)	26	46.4	25	46.3	51	46.4
Medial middle	Negative (-)	1	1.8	0	0	1	0.9
	*Weak (±)	26	46.4	25	46.3	51	46.4
	#Mild (+)	29	51.8	29	53.7	58	52.7
Medial posterior	Negative (-)	1	1.8	1	1.9	2	1.8
	*Weak (±)	27	48.2	33	61.1	60	54.5
	#Mild (+)	25	44.6	20	37	45	40.9
	Moderate (++)	3	5.4	0	0	3	2.7

Note: - : - : 0 %, ±: < 25 %, +: 26 -50 %, ++: 51-75 %, +++: 76 -100 % acid Mucins, *,F= 20.35 (Weak), p=0.037 #; F=37.44 (Mild), p= 0.030, n1+n2=n

was 1.8% negative, 48.2% weak, 44.6% mild, and 5.4% moderate. Right leg medial meniscus anterior part (RMA) was 3.7% negative, 50% weak, and 46.3% mild staining intensity while, it was 46.3% weak, and 53.7% mild staining intensity in RMM. Similarly, the staining intensity in RMP was 1.9% negative, 61.1% weak, and 37% mild. Staining intensity in the medial OA menisci is significantly varying by parts ($p < 0.05$).

Table 1b. the result made in left leg lateral meniscus anterior part (LLA) had 64.3% weak, and 35.7% mild staining intensity of AB-2.5 while, it was 5.4% negative, 53.6% weak, and 41.1% mild in the left leg lateral meniscus middle part (LLM). Similarly, staining intensity in the left leg lateral meniscus posterior part (LLP) was 1.8% negative, 66.1% weak, and 32.1% mild. The right leg lateral meniscus anterior part (RLA) showed 3.7% negative, 51.9% weak, and 44.4% mild staining intensity.

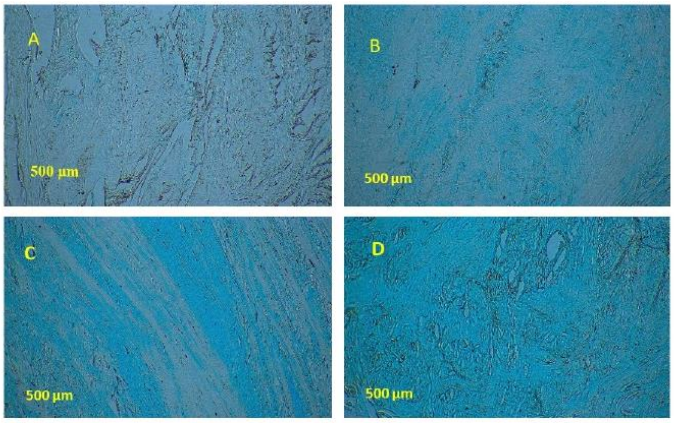


Fig. 5b: The intensity of Acid Mucins in the test group: magnification 10x

Table 1b: Alcian blue pH 2.5 Staining Intensity of Acid mucins in Lateral Meniscus of left and right legs.

Alcian Blue 2.5 Staining Intensity of Acid mucins		Left leg		Right leg		Overall	
		n1	**%**	**n2**	**%**	**n**	**%**
Control (Normal menisci)	**Strong (+ + +)**	**1**	**100**	**1**	**100**		**100**
Test (OA menisci)							
Lateral Anterior	Negative (-)	0	0	2	3.7	2	1.8
	*Weak (±)	36	64.3	28	51.9	64	58.2
	#Mild (+)	20	35.7	24	44.4	44	40
Lateral Middle	Negative (-)	3	5.4	2	3.7	5	4.5
	*Weak (±)	30	53.6	36	66.7	66	60
	#Mild (+)	23	41.1	16	29.6	39	35.5
Lateral Posterior	Negative (-)	1	1.8	1	1.9	2	1.8
	*Weak (±)	37	66.1	30	55.6	67	60.9
	#Mild (+)	18	32.1	23	42.6	41	37.3

Note: - : - ; 0 %, ±: <25 %, +: 26-50 %, ++: 51-75 %, +++: 76 -100 % acid Mucins, *F= 3.38 (Weak),p=0.048 # F= 3.80 (Mild); p = 0.0436, n1+n2=n

Table 2: Association of AB-2.5 intensity of Acid Mucins – medial and lateral menisci with side of the legs

AB-2.5 Staining Intensity of Acid Mucins		**Side of legs**				**Chi-square (df), P-value**
		Left		**Right**		
		n	**%**	**n**	**%**	
Medial menisci	Weak	27	48.2	26	48.1	3.058 (2), 0.217
	Mild	26	46.4	28	51.9	
	Moderate	3	5.4	0	0	
Lateral menisci	Weak	36	64.3	33	61.1	0.119 (1), 0.731
	Mild	20	35.7	21	38.9	

While, it was negative 3.7%, weak 66.7%, and mild 29.6% staining intensity in RLM. Similarly, the staining intensity in RLP was 1.9% negative, 55.6% weak, and 42.6% mild. Staining Intensity in lateral OA menisci significantly ($p < 0.05$) varied by parts.

Table 2 stated the association in the medial and lateral OA meniscus of AB-2.5 intensity of acid mucins on both sides of the legs. Medial menisci of left and right leg have 46.4 and 51.9% respectively mild intensity of acid mucins. However, lateral menisci of the left and right leg have respectively 35.7 and 38.9% mild intensity of acid mucins. Moreover, the sides of the legs do not show a significant association between the medial and lateral meniscus.

DISCUSSION

The knee menisci are specialized tissues that play a vital role in load transmission, shock absorption, and joint stability.[1] In accordance with the role of menisci play in knee joint function, meniscal injuries are common in athletes and the general population. The complex role of meniscal tissue composition in the etiology of meniscal tears and the subsequent development of knee OA is not entirely clear.[5,10] The ability to perform these mechanical functions is based on their cellular and chemical composition and, perhaps more importantly, on the organization and interactions of their constituents.[2] Mucopolysaccharides or Proteoglycans and other non-collagenous proteins play an important role in stabilizing the extracellular meshwork and therefore, are very important for the maintenance of the structural integrity and mechanical properties of the menisci.

In this study, for comparison purposes, sheep's normal meniscal tissue was taken as control of three different parts (anterior, middle, and posterior) of the medial and lateral menisci of both legs because gait analyses in this model have demonstrated a similar pattern of hind limb loading to humans, and post-surgical GRF (Ground reaction forces) changes comparable to OA patients.[33,34] Another study compared several viscoelastic properties of the bovine, ovine, and porcine menisci biomechanically with the human meniscus and reported that the ovine (sheep) model showed the greatest resemblance to the human meniscus. In addition, few studies compared ovine and rabbit menisci with human menisci using histology and scanning electron microscopy in terms of vascularization pattern, cell density, and extracellular matrix collagen and reported that sheep (ovine) menisci have greater structural similarity to the ultrastructure.[35,36]

In this study, decreased acid mucins (proteoglycan) staining intensity was observed in the various parts (anterior, middle, and posterior) of the medial and lateral menisci of both legs compared with normal control menisci. However, in other studies, normal meniscal tissue showed a predominance of acid mucins (80%) and sparse neutral mucins (20%), suggesting that the menisci are rich in acid mucins and play a major role in viscosity. About 80% of the total GAGs in the meniscus were identified as sulfated. Normal human meniscal proteoglycans contain approximately 40% chondroitin 6-sulfate, 10-20% chondroitin 4-sulfate, 20-30% dermatan sulfate, and 15% keratan sulfate.[37-39]

Severe collagen and proteoglycan loss occurred in OA cartilage, signifying that collagen and proteoglycan are more actively involved in the degenerative process and development of OA. The findings of Videman *et al*[40] on proteoglycan content (1979). reported that proteoglycan content in the menisci was increased after limb immobilization-induced OA in rabbits. Djurasovic *et al*[41] reported a decrease in proteoglycan content in the menisci of adult beagle dogs after OA was induced by limb immobilization. Adams *et al*[42] reported that proteoglycan content in the menisci decreased during the first trimester but gradually increased in the following months after induction of OA.

Peters and Smillie[43] reported elevated proteoglycan levels in the portion of the meniscus with degenerative tears in patients with meniscal injuries.[43] Herwig *et al*[39] reported that in patients with meniscal lesions, the proteoglycan content (µg/mg dry weight) in the meniscus increased with the severity of meniscal degeneration. In a study using human meniscus specimens obtained from OA patients, Ghosh *et al*[44] found increased proteoglycan content in the degenerative region of the OA meniscus compared to normal control menisci. Another study showing proteoglycan changes in human menisci by Alcian blue staining (pH 2.5) observed acidic mucin (proteoglycan) and a tendency to degenerate the meniscus due to reduced acidic mucin. Decreased matrix proteoglycans in degenerative human menisci due to decreased synthesis and development of OA.[32]

Osteoarthritis in the left leg was higher than in the right leg. Women are more likely to develop OA than men. OA changes were more

common in women on the right leg and men on the left leg, OA cases were higher in the age groups from 60-69 years in both left and right legs. The control group showed strong acid mucins staining intensity. Whereas, in the test group, the meniscus showed varying degrees of reduced (moderate to negative) staining intensity levels. Histochemical analysis of acid mucins staining intensity of the medial and lateral meniscus of the left and right legs showed a reduction compared to the control meniscus. The staining intensity of the medial and lateral menisci showed significant [$p<0.05$ (F-test)] differences in different parts of the OA meniscus in both legs. The changes of the acid mucins in human OA menisci provide information on the scientific indications of the progressive process of OA. Therefore, this study could help health professionals in the development of structure-modifying drugs for OA therapy.

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