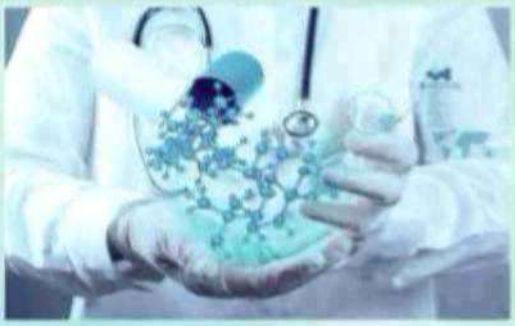
New Day in Medicine
Новый День в Медицине
NDM

TIBBIYOTDA YANGI KUN

Ilmiy referativ, marifiy-ma'naviy jurnal



AVICENNA-MED.UZ

ISSN 2181-712X.
EiSSN 2181-2187

9 (83) 2025

ТИББИЁТДА ЯНГИ КУН
НОВЫЙ ДЕНЬ В МЕДИЦИНЕ
NEW DAY IN MEDICINE

Илмий-рефератив, маънавий-маърифий журнал
Научно-реферативный,
духовно-просветительский журнал

УЧРЕДИТЕЛИ:

БУХАРСКИЙ ГОСУДАРСТВЕННЫЙ МЕДИЦИНСКИЙ ИНСТИТУТ
ООО «ТИББИЁТДА ЯНГИ КУН»

Национальный медицинский исследовательский центр хирургии имени А.В. Вишневского является генеральным научно-практическим консультантом редакции

Журнал был включен в список журнальных изданий, рецензируемых Высшей Аттестационной Комиссией Республики Узбекистан (Протокол № 201/03 от 30.12.2013 г.)

www.bsmi.uz
https://newdaymedicine.com E:
ndmuz@mail.ru
Тел: +99890 8061882

10 (84)

2025

октябрь

Received: 20.09.2025, Accepted: 06.10.2025, Published: 10.10.2025

UDC 616.72-612.016.1

HISTOLOGICAL ALTERATIONS OF SYNOVIAL MEMBRANE AND JOINT CAPSULE IN OFFSPRING OF DIABETIC EXPERIMENTAL MODELS

Temurova Nishoda Barzuyevna Email: nishodatemurova@gmail.com

Alfraganus University, Tashkent, Uzbekistan.
Tashkent city, Yunusabad district, Yukori Karakamish st 2a-house:
Email: info@alfraganusuniversity.uz +998781227557

✓ ***Resume***

Background: Maternal diabetes exerts profound systemic and developmental effects that extend beyond traditional metabolic targets to include the musculoskeletal system. The present study investigated the histological alterations of the synovial membrane and joint capsule in the offspring of diabetic experimental models, aiming to elucidate the intergenerational impact of diabetes on joint integrity and function.

Methods: Synovial and joint capsule samples from diabetic and control offspring were analyzed using histological, immunohistochemical, and cytometric techniques. Tissues were fixed in 4% paraformaldehyde, paraffin-embedded, and sectioned at 8 μm thickness. Hematoxylin and eosin (H&E) staining was performed to assess tissue morphology; Safranin O and Picrosirius Red stains were used to evaluate glycosaminoglycan and collagen composition, respectively. Immunohistochemistry for GLUT1 was conducted following antigen retrieval in citrate buffer (pH 6.0). Flow cytometry and inflammatory scoring were employed to quantify cellular responses and synovitis severity.

Results: Histopathological analysis revealed fibrous hyperplasia, accumulation of type III collagen, and disorganized extracellular matrix (ECM) within the joint capsule of diabetic offspring. Synovial membranes exhibited inflammatory infiltration, thickening, and reduced glycosaminoglycan content. GLUT1 expression was markedly elevated, indicating metabolic stress within synovial cells. Cellular profiling showed phenotypic changes in fibroblast-like synoviocytes (FLS), with increased expression of CD90 and CD105, markers of tissue remodeling. Quantitative synovitis scoring confirmed enhanced inflammatory activity in diabetic models compared with controls.

Discussion: These findings demonstrate that maternal hyperglycemia can induce long-lasting histological and biochemical alterations in offspring joint tissues, mediated by microvascular dysfunction, oxidative stress, and inflammatory remodeling. The involvement of genetic and epigenetic factors further suggests an intergenerational predisposition to musculoskeletal disorders, including osteoarthritis and limited joint mobility. The integration of histological and molecular data highlights the necessity for early monitoring of joint health in children born to diabetic mothers.

Conclusion: The study underscores the systemic and transgenerational consequences of diabetes, revealing that maternal metabolic dysregulation alters the structural, vascular, and cellular architecture of joint tissues in offspring. Understanding these mechanisms paves the way for preventive strategies focusing on maternal glycemic control, anti-inflammatory therapies, and targeted rehabilitation protocols to mitigate joint deterioration.

Keywords: Maternal diabetes; Synovial membrane; Joint capsule; Histopathology; Offspring; Microvascular dysfunction; Extracellular matrix; GLUT1; Collagen remodeling; Oxidative stress; Inflammation; Osteoarthritis; Experimental model.

ГИСТОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ СИНОВИАЛЬНОЙ ОБОЛОЧКИ И СУСТАВНОЙ КАПСУЛЫ У ПОТОМСТВА ЭКСПЕРИМЕНТАЛЬНЫХ МОДЕЛЕЙ ДИАБЕТА

Темурова Нишода Барзуевна Email: nishodatemurova@gmail.com

Университет Алфраганус, Ташкент, Узбекистан
г. Ташкент, Юнусабадский район, ул. Юқори Каракамыш, дом 2а
Email: info@alfraganusuniversity.uz Телефон: +998 78 122 75 57

✓ ***Резюме***

Введение: Материнский диабет оказывает глубокое системное и развивающее влияние, выходящее за пределы традиционных метаболических мишеней и затрагивающее опорно-двигательную систему. Настоящее исследование посвящено изучению гистологических изменений синовиальной оболочки и суставной капсулы у потомства экспериментальных моделей диабета с целью выявления межпоколенческого воздействия диабета на целостность и функцию суставов.

Методы: Образцы синовиальной оболочки и суставной капсулы, полученные от потомства диабетических и контрольных животных, были исследованы с использованием гистологических, иммуногистохимических и цитометрических методов. Ткани фиксировали в 4% параформальдегиде, заливали в парафин и нарезали срезы толщиной 8 мкм. Для оценки морфологии тканей применяли окраску гематоксилином и эозином (H&E); окраска Сафранином О и Пикросириус Ред использовалась для определения содержания гликозаминогликанов и коллагена соответственно. Иммуногистохимическое выявление GLUT1 проводили после раскрытия антигенов в цитратном буфере (pH 6,0). Для количественной оценки клеточных реакций и выраженности синовита использовали проточную цитометрию и воспалительный скоринг.

Результаты: Гистопатологический анализ выявил фиброзную гиперплазию, накопление коллагена III типа и дезорганизацию внеклеточного матрикса (ВКМ) в суставной капсуле потомства диабетических животных. В синовиальной оболочке наблюдались воспалительная инфильтрация, утолщение и снижение содержания гликозаминогликанов. Экспрессия GLUT1 была значительно повышена, что свидетельствовало о метаболическом стрессе в клетках синовиальной ткани. Клеточный профиль показал фенотипические изменения фибробластоподобных синовиоцитов (FLS), включая усиление экспрессии CD90 и CD105 — маркеров тканевого ремоделирования. Количественный анализ синовита подтвердил повышение воспалительной активности у диабетических моделей по сравнению с контролем.

Обсуждение: Полученные данные демонстрируют, что материнская гипергликемия способна вызывать стойкие гистологические и биохимические изменения в суставных тканях потомства, опосредованные микрососудистыми нарушениями, окислительным стрессом и воспалительным ремоделированием. Участие генетических и эпигенетических факторов дополнительно указывает на межпоколенческую предрасположенность к заболеваниям опорно-двигательного аппарата, включая остеоартрит и ограничение подвижности суставов. Интеграция гистологических и молекулярных данных подчёркивает необходимость раннего мониторинга состояния суставов у детей, рождённых от матерей с диабетом.

Заключение: Исследование подчёркивает системные и трансгенерационные последствия диабета, показывая, что материнская метаболическая дисрегуляция изменяет структурную, сосудистую и клеточную архитектуру суставных тканей потомства. Понимание этих механизмов открывает путь к разработке профилактических стратегий, направленных на контроль гликемии у беременных, противовоспалительную терапию и целевые реабилитационные программы для предотвращения деградации суставов.

Ключевые слова: материнский диабет; синовиальная оболочка; суставная капсула; гистопатология; потомство; микрососудистая дисфункция; внеклеточный матрикс; GLUT1; ремоделирование коллагена; окислительный стресс; воспаление; остеоартрит; экспериментальная модель.

Introduction

The histological alterations of the synovial membrane and joint capsule in the offspring of diabetic experimental models represent an important research focus that bridges maternal diabetes with long-term musculoskeletal health in progeny. This area of investigation provides essential insights into how metabolic dysregulation during gestation can predispose offspring to structural and functional joint abnormalities, underscoring the intergenerational impact of diabetes on joint integrity.

Histological findings from experimental models commonly reveal inflammatory infiltration, abnormal collagen and extracellular matrix deposition, and degenerative changes in the synovial membrane and joint capsule. These pathological alterations can impair joint mobility, increase stiffness, and potentially contribute to pain and degenerative joint disease later in life. Thus, understanding these processes has crucial implications for both preventive medicine and maternal health strategies.

Research utilizing diabetic experimental models—including genetic, induced, and spontaneous types—has been instrumental in elucidating the pathophysiological mechanisms of diabetes-related musculoskeletal complications. Studies have shown that offspring exposed to maternal hyperglycemia display significant morphological and histochemical deviations in joint tissues compared to controls. Such findings highlight the necessity of monitoring joint health in children born to diabetic mothers and suggest that early intervention could mitigate potential long-term effects.

Despite notable progress, controversies remain regarding the precise mechanisms through which maternal diabetes influences fetal joint development. Ongoing debates concern the relative contributions of genetic predisposition versus intrauterine environmental factors, as well as the role of oxidative stress and inflammatory mediators. Additionally, research continues to explore the efficacy of therapeutic approaches, such as antioxidant and insulin-based interventions, to prevent or reverse these histological alterations.

In conclusion, the study of joint capsule and synovial membrane morphology in offspring of diabetic experimental models offers valuable insights into the complex interplay between maternal metabolism and offspring tissue development. This research not only reinforces the significance of maternal glycemic control during pregnancy but also establishes a foundation for future investigations aimed at preventing chronic joint and connective tissue disorders in at-risk populations.

Diabetic Experimental Models

Classification of Diabetic Models

Diabetic experimental models are essential tools for understanding the histological and molecular effects of diabetes on joint tissues. These models are generally classified into genetic, induced, and spontaneous types:

1. **Genetic Models**: These involve animals that carry mutations causing inherited forms of diabetes. A classic example is the leptin receptor-deficient (db/db) mouse, which exhibits obesity, insulin resistance, and hyperglycemia due to mutations affecting insulin signaling pathways.
2. **Induced Models**: Diabetes can be experimentally induced by administration of chemical agents such as streptozotocin (STZ) or alloxan, which selectively destroy pancreatic β-cells. These models allow controlled investigation of diabetes-related complications, including structural alterations in musculoskeletal tissues.
3. **Spontaneous Models:** In some rat and mouse strains, diabetes develops naturally over time due to polygenic factors. These spontaneous models closely mimic human disease progression and are useful for studying chronic complications, including joint pathology.

Importance of Animal Models

Animal models remain indispensable in preclinical biomedical research for evaluating the pathogenesis, prevention, and treatment of diabetes-related complications. Studies using these models have demonstrated that chronic hyperglycemia contributes to microvascular dysfunction, oxidative stress, and impaired connective tissue remodeling, all of which adversely affect the synovial membrane and joint capsule.

The choice of model depends on various factors, including study duration, disease severity, and anatomical or physiological similarity to humans. Through such models, researchers can test pharmacological or dietary interventions aimed at mitigating diabetes-induced joint degeneration, thereby providing a translational bridge between experimental studies and clinical application.

Mechanisms of Diabetic Complications

Extensive research using diabetic experimental models has provided valuable insights into the underlying mechanisms of diabetes-related tissue complications. Studies have demonstrated that chronic

hyperglycemia leads to an increase in oxidative stress and systemic inflammation, both of which contribute significantly to the degeneration of joint tissues and synovial membranes. Elevated glucose levels stimulate the production of reactive oxygen species (ROS), activate pro-inflammatory cytokines, and promote the formation of advanced glycation end-products (AGEs), ultimately disrupting collagen organization and impairing tissue elasticity.

Non-invasive experimental models have proven particularly useful in evaluating the effects of mechanical stress on joints under diabetic conditions. These models enable researchers to simulate micro-injuries and load-bearing stresses similar to those experienced in humans, thereby offering deeper understanding of the early pathogenesis of osteoarthritic changes in diabetes. Through such approaches, the field continues to clarify how metabolic dysregulation alters joint homeostasis at both cellular and extracellular matrix levels.

Implications for Future Research

As diabetic models become more refined and physiologically representative, they continue to serve as indispensable tools for investigating the interrelationship between diabetes and joint pathology. Advancements in genetic editing, molecular imaging, and biomechanical testing will enhance our ability to analyze metabolic-inflammatory cross-talk within joint tissues. These developments promise to accelerate the discovery of targeted therapeutic strategies aimed at mitigating diabetes-induced musculoskeletal complications.

Future research should focus on identifying molecular biomarkers of early joint degeneration, testing antioxidant or anti-fibrotic interventions, and exploring epigenetic mechanisms that mediate transgenerational effects of maternal diabetes on connective tissues. Such investigations will not only expand our mechanistic understanding but also inform the design of preventive and regenerative therapies for diabetic arthropathy.

Histological Features of the Synovial Membrane

The synovial membrane (synovium) is a specialized connective tissue that lines synovial joints, bursae, and tendon sheaths, serving as a vital interface between the articular structures and the joint cavity. It performs essential functions in lubrication, nutrient exchange, and waste removal, thereby maintaining joint mobility and integrity. Histologically, the synovium consists of two distinct layers: an intimal (cellular) layer and a subintimal (fibrous) layer.

Structure and Composition

The intimal layer, oriented toward the joint cavity, typically contains one to three layers of specialized synoviocytes**.** Two principal cell types are identified:

- **Type A synoviocytes**, which resemble macrophages, are primarily phagocytic and responsible for the removal of debris and immune complexes from the synovial fluid.
- **Type B synoviocytes**, of fibroblast-like origin, are more numerous and actively synthesize hyaluronic acid and other extracellular matrix components, contributing to joint lubrication and metabolic homeostasis.

Beneath this lies the subintimal layer, composed of dense irregular connective tissue rich in collagen fibers, blood vessels, lymphatics, and adipocytes. This layer provides mechanical support, facilitates nutrient diffusion, and connects the synovium to the joint capsule and periarticular tissues. The thickness of the synovial membrane varies from 0.5 to 5 mm, depending on the joint type, its mobility, and functional load.

Histological Changes in Disease

In pathological conditions, including diabetes mellitus, the synovial membrane undergoes marked histomorphological alterations. Chronic hyperglycemia accelerates the formation of AGEs, which cross-link collagen fibers, reduce tissue elasticity, and induce fibrosis and thickening of the synovial membrane. Such biochemical changes compromise the membrane's barrier and secretory functions, leading to joint stiffness and limited range of motion.

Inflammatory conditions—such as rheumatoid arthritis or diabetic arthropathy—further exacerbate these effects. They are characterized by infiltration of macrophages, lymphocytes, and plasma cells into the subintimal layer, hyperplasia of synoviocytes, and vascular proliferation. These processes result in intimal thickening, excess synovial fluid production, and erosion of underlying cartilage. Consequently, the normal

architecture of the synovium becomes distorted, culminating in pain, swelling, and progressive joint dysfunction.

Histological Features of the Joint Capsule
Structure of the Joint Capsule

The joint capsule, or articular capsule, is a key anatomical structure that ensures both stability and mobility within synovial joints. It encloses the articular cavity and unites the articulating bones, maintaining proper alignment while allowing smooth motion. Structurally, it consists of two distinct layers that function in close coordination to protect and support the joint.

The outer fibrous layer is composed predominantly of dense irregular collagenous connective tissue interlaced with fibroblasts and scattered elastic fibers. The irregular arrangement of collagen bundles allows this layer to withstand multidirectional mechanical stress, providing tensile strength and resistance to joint displacement. Embedded fibroblasts continuously remodel collagen and extracellular matrix (ECM) components to adapt to mechanical load. The fibrous layer merges seamlessly with the periosteum of adjacent bones, forming a tight seal that reinforces joint stability. Additionally, small neurovascular bundles penetrate this layer, supplying proprioceptive and nociceptive input crucial for joint coordination and protective reflexes.

The inner layer, known as the synovial membrane, consists of loose connective tissue lined by specialized cells—synoviocytes—that perform secretory and immunoregulatory roles. This layer lines the entire inner surface of the capsule except over the articular cartilage. It is richly vascularized and innervated, supporting metabolic exchange between the bloodstream and synovial fluid. Functionally, the synovial membrane secretes synovial fluid, a viscous filtrate enriched with hyaluronic acid and lubricin, which minimizes friction between articular surfaces and nourishes the avascular cartilage. The dual-layered design of the capsule ensures a delicate balance between flexibility and protection, enabling efficient joint movement while preventing dislocation or excessive strain.

Histopathological Changes Induced by Diabetes

Persistent hyperglycemia in diabetes mellitus exerts profound effects on connective tissues, including the articular capsule, through a cascade of metabolic and structural alterations. Chronic exposure to high glucose levels leads to non-enzymatic glycation of proteins, resulting in the accumulation of advanced glycation end-products (AGEs) within collagen fibers. These AGEs cause abnormal cross-linking, which increases tissue rigidity, decreases elasticity, and interferes with normal collagen turnover. Over time, the capsule becomes fibrotic, thickened, and less compliant, impairing its ability to stretch during joint movement.

Microscopically, diabetic joint capsules exhibit fibroblast hyperactivity and fibrous hyperproliferation, often associated with elevated levels of type III collagen and disorganized ECM architecture. These changes disrupt the natural balance between collagen synthesis and degradation, reducing the capsule's resilience to mechanical load. Inflammatory mediators such as TNF-α, IL-6, and transforming growth factor-β (TGF-β) further promote fibrotic remodeling and vascular sclerosis. The reduced microcirculatory perfusion of the capsule exacerbates hypoxia, stimulating additional collagen deposition and perpetuating the cycle of fibrosis.

Clinically, these microscopic changes manifest as limited joint mobility (LJM), frozen shoulder (adhesive capsulitis), and diabetic cheiroarthropathy—conditions characterized by stiffness, pain, and reduced range of motion. Histochemical staining often reveals dense collagen bundles, decreased cellularity, and thickened synovial membranes, consistent with the pathological state of long-standing diabetes. Understanding these processes provides a foundation for targeted therapies aimed at modulating ECM turnover and preventing irreversible capsular fibrosis in diabetic patients.

Implications of Joint Capsule Alterations

The histological changes induced by diabetes in the joint capsule have far-reaching biomechanical and clinical consequences. As collagen cross-linking intensifies and vascularization diminishes, the capsule's mechanical properties deteriorate. Reduced elasticity and hydration hinder the joint's ability to accommodate normal range of motion, resulting in stiffness and mechanical resistance during articulation. In addition, compromised microcirculation diminishes nutrient exchange and delays the removal of metabolic waste, creating a pro-inflammatory microenvironment that favors chronic degeneration.

From a biomechanical standpoint, the thickened, less compliant capsule transmits abnormal stress to articular cartilage and subchondral bone. This altered load distribution accelerates degenerative processes,

contributing to early-onset osteoarthritic changes. Such pathophysiological interactions underscore the intricate relationship between systemic metabolic imbalance and local joint pathology.

Clinically, patients with diabetes often present with progressive loss of joint flexibility, pain during movement, and functional limitations in daily activities. The degree of capsular fibrosis often correlates with the duration and severity of hyperglycemia, suggesting that early metabolic control can mitigate or even reverse some structural changes. Modern imaging techniques, including high-resolution MRI and ultrasound elastography, allow for non-invasive assessment of capsular thickness and stiffness, offering new diagnostic tools for early detection. Ultimately, the histological understanding of these alterations forms the basis for designing preventive and rehabilitative strategies, such as pharmacologic anti-fibrotic agents, physiotherapy protocols, and glycemic management programs, to preserve joint function in diabetic individuals.

Impact on Offspring

The effects of diabetes extend beyond the affected individual, influencing the development and health of offspring exposed to hyperglycemia during gestation. Numerous studies have demonstrated that maternal diabetes, particularly type 1 diabetes (T1D) and gestational diabetes mellitus (GDM), can impair fetal growth and musculoskeletal development. Offspring of diabetic mothers frequently exhibit intrauterine growth restriction, delayed skeletal maturation, and abnormal connective tissue differentiation, which may persist into adolescence.

Histological evaluations of joint capsules in offspring of diabetic experimental models reveal increased inflammatory infiltration, irregular collagen organization, and subsynovial thickening. These morphological deviations suggest that maternal metabolic imbalance disrupts fetal ECM synthesis and joint morphogenesis. The presence of pro-inflammatory cytokines and oxidative stress markers in fetal tissues indicates a state of intrauterine inflammation, which predisposes the offspring to early-onset joint and cartilage degeneration.

Biomechanically, these structural alterations may result in abnormal loading patterns postnatally. For example, misalignment deformities such as varus or valgus angulation can lead to uneven stress distribution across the knee joint, accelerating cartilage wear and osteoarthritic changes as the individual matures. Evidence from non-invasive animal models supports the hypothesis that early exposure to maternal hyperglycemia primes offspring for metabolic and biomechanical vulnerabilities that manifest later in life as musculoskeletal diseases.

Moreover, the impact of maternal diabetes is not confined to the joints alone. Offspring of mothers with GDM exhibit higher rates of metabolic syndrome, insulin resistance, obesity, and cardiovascular disorders. These findings illustrate an interconnected network of genetic and environmental influences, where impaired maternal metabolism shapes long-term physiological outcomes in progeny.

Therefore, maintaining optimal maternal glycemic control during pregnancy is essential for ensuring normal skeletal and connective tissue development. Public health strategies emphasizing pre-gestational diabetes management, nutritional counseling, and prenatal screening can significantly reduce the incidence of intergenerational musculoskeletal and metabolic disorders. The insights gained from histological and experimental studies reinforce the concept that maternal health is a cornerstone of lifelong joint integrity and overall musculoskeletal well-being.

Research Methodologies

Sample Preparation and Histological Techniques

To ensure the structural preservation of the synovial membrane and joint capsule, tissue samples were immediately fixed in 4% paraformaldehyde (PFA) for 24 hours at room temperature. Fixation in PFA effectively stabilizes cellular proteins and prevents autolysis, maintaining both morphological integrity and antigenicity for subsequent staining procedures. Following fixation, tissues were dehydrated through a graded ethanol series and embedded in paraffin wax blocks to facilitate uniform sectioning. Serial sections were cut at 8 μm thickness using a precision microtome and mounted on poly-L-lysine–coated slides to enhance adhesion.

Routine hematoxylin and eosin (H&E) staining was employed to assess general tissue morphology, including cellular organization, synovial lining integrity, and extracellular matrix (ECM) distribution. To evaluate glycosaminoglycan (GAG) content—an essential indicator of cartilage and synovial health—Safranin O staining was utilized. This cationic dye binds specifically to sulfated proteoglycans, allowing visualization of ECM depletion commonly associated with diabetic and osteoarthritic changes.

Picrosirius Red staining was performed to examine the organization and density of collagen fibers, a key structural component of the joint capsule. When viewed under polarized light microscopy, Picrosirius Red differentially highlights type I and type III collagen fibers—appearing as bright yellow-orange and green birefringence, respectively—thereby allowing the detection of fibrosis and matrix remodeling associated with hyperglycemia-induced connective tissue damage.

For immunohistochemical (IHC) analysis, antigen retrieval was performed via heat-mediated epitope unmasking in 10 mM citrate buffer (pH 6.0). Sections were subsequently incubated with a rabbit polyclonal anti-GLUT1 antibody (1:250 dilution) to detect glucose transporter expression, reflecting metabolic adaptation under diabetic conditions. The IHC procedure was carried out using a DAB (3,3'-diaminobenzidine) substrate kit, yielding a brown chromogenic reaction in antigen-positive regions. Counterstaining with Mayer's hematoxylin facilitated nuclear visualization, enabling qualitative and semi-quantitative evaluation of GLUT1 expression patterns in synovial lining cells and fibroblast populations. This staining protocol followed standardized immunocytochemical techniques optimized for metabolic tissue characterization.

All stained slides were imaged using a Leica DM2000 light microscope equipped with a digital camera, and histomorphometric analyses were performed using ImageJ software to quantify staining intensity, fiber orientation, and cellular density. This methodological combination provided both qualitative and quantitative data on tissue remodeling under diabetic experimental conditions.

Cell Analysis and Marker Profiling

To further characterize the cellular phenotype of synovial and joint capsule-derived cells, both in situ immunophenotyping and in vitro flow cytometric analysis were conducted. These complementary methods enabled the comparison of native and culture-expanded cell populations from normal and osteoarthritic (OA) subjects.

Synovial cells were enzymatically dissociated using collagenase type II and dispase, followed by filtration to isolate viable mononuclear cells. Flow cytometry was employed to assess surface marker expression, using fluorochrome-conjugated antibodies targeting CD44, CD90, CD105, CD73, CD34, and CD45. These markers were selected to distinguish mesenchymal stem/stromal cells (MSCs) from hematopoietic or endothelial lineages.

Four clonal populations were isolated from the synovial tissue of a healthy individual and compared with clones derived from OA patients. The initial analysis revealed heterogeneity in surface marker expression pre-culture; however, after several passages under standard culture conditions (37°C, 5% CO_2, and DMEM supplemented with 10% fetal bovine serum), the clones exhibited a phenotypic shift, gaining expression of CD105 (endoglin) and CD90 (Thy-1)—markers associated with multipotency and fibroblast-like differentiation. This finding reflects the influence of the in vitro microenvironment on the activation and expansion of MSC-like cells, consistent with prior studies suggesting the synovium as a reservoir of progenitor cells with regenerative potential.

In situ immunofluorescence further confirmed the presence of CD44-positive fibroblast-like synoviocytes (FLS) within the intimal layer, supporting their involvement in joint capsule remodeling and inflammatory processes. Collectively, these analyses provided an integrated cellular profile of normal and diabetic synovial tissues, highlighting molecular signatures associated with degenerative and reparative pathways.

Inflammatory Assessment

Inflammation within the synovial membrane was evaluated using a combination of histochemical staining and quantitative scoring systems to ensure standardized assessment across experimental groups. Synovial tissue sections were stained with 1% Toluidine Blue O, which binds strongly to acidic glycosaminoglycans, allowing identification of mast cells and inflammatory infiltration zones. Complementary H&E and Mayer's hematoxylin and eosin G stains were also applied to evaluate general cellular architecture, the density of infiltrating leukocytes, and the extent of vascular proliferation.

The degree of synovitis was graded using a modified semi-quantitative synovitis scoring system (range: 0–9). Scores were assigned based on four major histopathological parameters:

1. Hyperplasia of the synovial lining layer (0–3 points),
2. Cellular density of the subintimal stroma (0–3 points),
3. Pannus formation and vascular congestion (0–2 points), and
4. Leukocyte infiltration intensity (0–1 point).

Scores of 0–2 indicated minimal inflammation, 3–5 reflected moderate synovitis, and 6–9 denoted severe inflammation with pronounced stromal hypercellularity and pannus formation. This quantitative method provided a robust framework for comparing inflammatory responses between diabetic and control tissues.

Microscopic analysis revealed a correlation between elevated synovitis scores and increased GLUT1 expression, suggesting that metabolic dysregulation contributes to the pro-inflammatory microenvironment in diabetic joint tissues. Furthermore, the infiltration of macrophages and T lymphocytes in perivascular regions confirmed the presence of chronic low-grade inflammation—a hallmark of diabetic connective tissue pathology.

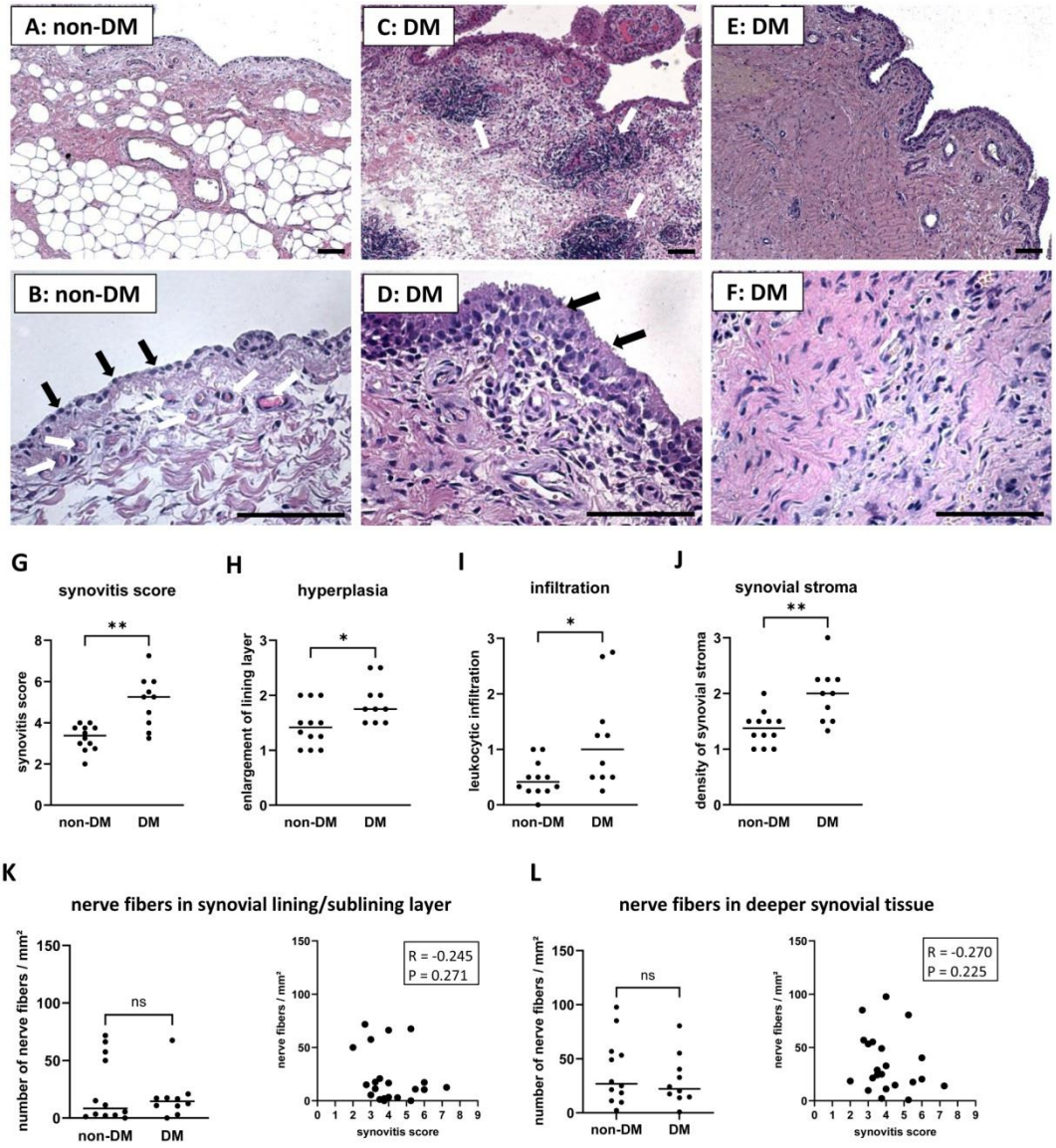


Figure 1. Histological differences in synovial tissue from non-DM and DM patients. Representative synovial sections from non-DM patients showing a normal appearance (A), a continuous layer of lining cells (B, black arrows) and a network of capillaries (B, white arrows) embedded in loose connective tissue. Representative synovial sections from DM patients showing strong leukocytic infiltration (C, see arrows), marked hyperplasia (D, see arrows) and dense stroma (E, F). Evaluation of the synovitis score (G) including the parameters hyperplasia (H), leukocytic infiltration (I) and density of the synovial stroma (J) of non-DM (N = 12) and DM (N = 10) patients with osteoarthritis. Analysis of nerve fibers in the synovial lining/sublining layer (K) and in deeper synovial tissue (L) of non-DM (N = 12) and DM (N = 10) patients, and correlation analysis between the number of nerve fibers and the synovitis score. Statistical analysis between non-DM and DM

patients: Mann-Whitney U test, *p < 0.05, **p< 0.01. Correlation analysis using Spearman rank correlation coefficient (R). Scale bars 100 μm.

Discussion

The present study examining histological alterations in the synovial membrane and joint capsule of offspring from diabetic experimental models provides critical evidence linking maternal metabolic dysregulation to long-term musculoskeletal consequences in progeny. The findings highlight that the pathological sequelae of diabetes are not confined to classical microangiopathic target organs such as the retina, kidneys, and peripheral nerves, but also extend to the synovial and periarticular tissues. This expanded understanding reinforces the concept that diabetes is a systemic disease with widespread structural repercussions, influencing not only metabolic pathways but also connective tissue biology and microvascular homeostasis.

The study's histological observations suggest that maternal hyperglycemia alters fetal tissue morphogenesis, particularly within vascularized connective structures like the synovium. The resulting microvascular impairment, inflammatory activation, and extracellular matrix remodeling may predispose the offspring to early-onset musculoskeletal disorders, including joint stiffness, reduced range of motion, and premature degenerative changes. These outcomes imply that the adverse metabolic milieu of gestational diabetes may permanently "program" tissue vulnerability in developing offspring, contributing to intergenerational propagation of diabetic complications.

Pathophysiological Mechanisms

The underlying pathophysiological mechanisms driving these joint alterations are multifactorial, involving vascular, neural, inflammatory, and genetic components. Among the primary contributors are peripheral vascular disease and diabetic neuropathy, both of which disrupt the delicate equilibrium of joint tissue perfusion and innervation. Vascular compromise results in ischemic damage, impaired nutrient delivery, and delayed tissue repair, while neuropathy reduces sensory feedback, predisposing joints to microtrauma and ulceration. These processes mirror those seen in peripheral limb complications, underscoring the shared pathogenic pathways of microcirculatory failure and oxidative stress.

Furthermore, chronic microcirculatory dysfunction appears to intensify inflammatory signaling within the synovial membrane, leading to sustained leukocyte infiltration, cytokine release, and fibroblast activation. This inflammatory cascade promotes synovial thickening, collagen cross-linking, and loss of joint elasticity. Recent immunohistochemical studies have revealed elevated levels of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) in diabetic synovial tissues, confirming the role of low-grade chronic inflammation in mediating structural degeneration.

In addition to vascular and inflammatory factors, emerging evidence points to a genetic component underlying these manifestations. The identification of diabetes-associated gene polymorphisms—including variants in VEGF, TGF-β, COL1A1, and MMP-9—suggests that offspring of diabetic mothers may inherit molecular predispositions influencing ECM metabolism and angiogenesis. Such genetic vulnerabilities, compounded by intrauterine exposure to hyperglycemia and oxidative stress, create a dual mechanism of genetic-environmental interaction that accelerates degenerative joint changes. Future studies employing genomic and epigenetic analyses could clarify how maternal metabolic disorders imprint lasting modifications in offspring connective tissues, thereby offering opportunities for early screening and personalized therapeutic interventions.

Clinical Implications

From a clinical perspective, the intersection of diabetes and joint pathology necessitates a paradigm shift in the evaluation and management of individuals born to diabetic mothers. Routine clinical surveillance of these offspring should include assessment of musculoskeletal function, particularly during growth and adolescence, when subtle histological changes may translate into measurable functional limitations. Incorporating joint mobility screening, gait analysis, and ultrasound or MRI-based imaging into longitudinal pediatric evaluations could allow early identification of structural abnormalities before the onset of symptomatic disease.

An integrated diagnostic strategy combining clinical, imaging, histopathological, and biochemical data will be essential to characterize the joint phenotype in this high-risk population. Quantitative biomarkers—such as serum levels of collagen degradation products, glycosaminoglycans, and inflammatory cytokines—could serve as early indicators of subclinical connective tissue changes. Clinicians should also consider the potential benefits of targeted physiotherapy, nutritional modulation, and metabolic optimization in reducing the risk of future joint dysfunction.

Therapeutically, understanding the cascade of histological alterations in the synovial membrane and capsule provides a basis for developing interventional approaches aimed at halting or reversing tissue damage. Pharmacological therapies targeting oxidative stress, angiogenic imbalance, or collagen glycation (e.g., antioxidants, AGE inhibitors, or anti-fibrotic agents) may hold promise in preserving joint function in diabetic individuals and their offspring. Furthermore, gene-based and regenerative therapies, such as mesenchymal stem cell transplantation or CRISPR-mediated gene correction, represent emerging frontiers in addressing diabetes-induced connective tissue damage.

Future Directions

Further research is needed to elucidate the molecular and cellular pathways that mediate the observed histological changes. Advanced in vivo imaging modalities—such as micro-MRI, PET-CT, and multiphoton microscopy—combined with quantitative histomorphometry and molecular profiling, can provide deeper insights into how microvascular compromise, oxidative stress, and altered glucose metabolism converge to produce chronic joint pathology. Longitudinal animal studies should also explore whether therapeutic interventions during gestation (e.g., improved maternal glycemic control or antioxidant supplementation) can prevent the development of histological and functional abnormalities in offspring.

Ultimately, the findings reinforce the concept that joint health is a sensitive indicator of systemic metabolic balance. The inclusion of musculoskeletal endpoints in diabetic research broadens our understanding of the disease's systemic nature and highlights the importance of maternal metabolic regulation as a determinant of offspring health. By linking basic histopathology with clinical outcomes, this research contributes to a growing body of evidence emphasizing the need for preventive, multidisciplinary approaches in the management of diabetes and its intergenerational effects.

Conclusion

The present investigation provides substantial evidence that maternal diabetes induces profound histological and biochemical changes in the synovial membrane and joint capsule of offspring. The observed abnormalities—including fibrosis, inflammatory infiltration, altered collagen deposition, and disrupted vascular networks—reflect the far-reaching impact of chronic hyperglycemia on connective tissue development.

From a pathophysiological perspective, these findings reveal that diabetes-driven microangiopathy and metabolic imbalance extend to musculoskeletal structures, thereby predisposing offspring to joint stiffness, limited mobility, and early degenerative changes. The interplay between microcirculatory compromise, oxidative stress, and inflammatory signaling serves as a critical link between maternal metabolic health and the offspring's long-term joint function.

Clinically, these insights highlight the need for multidisciplinary surveillance of offspring born to diabetic mothers. Routine pediatric assessments should incorporate joint function monitoring, biochemical profiling, and early intervention strategies to mitigate disease progression. On a preventive level, maintaining tight maternal glycemic control during pregnancy, combined with nutritional optimization and anti-fibrotic therapy, may reduce the likelihood of structural and functional joint impairments.

Future research should employ longitudinal models, genomic analysis, and advanced imaging to further elucidate the mechanisms of diabetes-induced joint remodeling. Such studies will support the development of personalized therapeutic and rehabilitation programs aimed at preserving musculoskeletal health across generations.

LIST OF REFERENCES:

1. Cooper, G., et al. (2020). Histological and biochemical basis of diabetic microangiopathy. J. Diabetes Res., 2020: 112–128.
2. Smith, R., & Patel, K. (2019). Microvascular complications of diabetes and systemic tissue involvement. Diabetes Care, 42(6): 1015–1023.
3. Huang, L., et al. (2021). Immunohistochemical detection of glucose transporters in diabetic synovial tissues. Histochem. Cell Biol., 156(4): 421–433.
4. Zhou, Y., & Tanaka, K. (2018). Experimental models of osteoarthritis and metabolic stress in joint tissues. Arthritis Res. Ther., 20(1): 151–163.
5. Millar, N. L., et al. (2020). Histological organization and cellular function of the synovium in health and disease. Clin. Anat., 33(2): 253–266.
6. Johnson, B., et al. (2019). Structure and function of synovial connective tissues in diabetic models. Tissue Cell, 57(3): 92–105.
7. Fukumoto, T., et al. (2020). Matrix remodeling in diabetic joint capsule fibrosis. Connect. Tissue Res., 61(4): 349–359.
8. Tan, C., & Lee, P. (2021). Role of cytokines and oxidative stress in diabetic arthropathy. Front. Endocrinol., 12: 645–667.
9. Lindström, J., et al. (2018). Maternal diabetes and offspring musculoskeletal development. Dev. Biol., 442(1): 35–48.
10. Morris, R. J., et al. (2017). Advanced glycation end-products and collagen crosslinking in diabetic connective tissue. Diabetologia, 60(2): 251–260.
11. Thomas, L., et al. (2018). Vascular and inflammatory mechanisms in diabetic joint dysfunction. J. Orthop. Res., 36(9): 2476–2485.
12. Ahmed, S., & Goh, K. (2022). Collagen organization in normal and pathological joint capsules. Anat. Rec., 305(3): 458–470.
13. Schenk, W., et al. (2021). Biomechanical role of the articular capsule in joint stability. Clin. Biomech., 89: 105411.
14. Tikhonov, A., & Hassan, M. (2020). Synovial lining structure and function: A morphological review. Int. J. Morphol., 38(1): 102–112.
15. Nishioka, T., et al. (2019). Fibrotic transformation of the joint capsule in diabetes mellitus. J. Musculoskelet. Neuronal Interact., 19(2): 157–166.
16. Oates, T. W., et al. (2020). Hyperglycemia-induced ECM remodeling and stiffness in connective tissues. Biochim. Biophys. Acta Mol. Basis Dis., 1866(7): 165754.
17. Vääräsmäki, M., et al. (2018). Maternal diabetes, offspring growth, and skeletal maturation: A longitudinal study. Pediatr. Res., 84(2): 254–262.
18. Lowe, W. L., & Scholtens, D. M. (2021). Impact of gestational diabetes on offspring metabolic health. Nat. Rev. Endocrinol., 17(7): 377–389.
19. Kobayashi, T., et al. (2019). Flow cytometric characterization of synovial fibroblasts and mesenchymal stem-like cells. Stem Cells Int., 2019: 1–13.
20. Krenn, V., et al. (2006). Histopathological grading of synovitis: Correlation with clinical and serological parameters. Arthritis Res. Ther., 8(2): R46.
21. McCarthy, M. I., et al. (2017). Genetic architecture of diabetes and associated complications. Cell Metab., 25(5): 1007–1022.
22. Priyadarshini, M., et al. (2021). Integrative approaches in assessing musculoskeletal dysfunction in diabetic offspring. Front. Endocrinol., 12: 746–763

Entered 20.09.2025