Original Article

Effect of Intra-Articular Injection of Peripheral Blood Mononuclear Cells Versus Platelet-Rich Plasma on Restoration of Collagen Fibers of the Articular Cartilage in a Rat Model of Knee Osteoarthritis

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ABSTRACT

Introduction: Osteoarthritis (OA) is a very common cause of disability. Chondrocytes stress and degradation of the extracellular matrix are characteristics of its pathogenesis. This can present clinically as pain and the joint shows inflammation and loss of its normal function due to cartilage degeneration, bone remodeling together with osteophytes formation.

Aim of the Work: This work was aimed to evaluate the effect of PBMNCs versus PRP on the restoration of collagen fibers content in the articular cartilage of the knee joint in a model of osteoarthritis in rats by using Mallory trichrome stain.

Materials and Methods: 39 male adult albino rats were divided into donor and experimental groups that were subdivided into four groups: Group I represent as a control group, group II served as a model for induction of knee osteoarthritis, group III received single intra-articular injection of PBMNCs and group IV received single intra-articular injection of PRP. Knee joint specimens were processed for light microscopic examination (LM) to be stained with Mallory trichrome stain then the mean area percentage of collagen fibers was statistically analyzed.

Results: Group II showed a homogenous decrease in collagen content in all cartilage zones. Group III demonstrated a moderate increase in collagen content mainly in the non-calcified zone but to a lesser extent in the radial and calcified zone. Group IV revealed a mild increase in the collagen content of the articular cartilage. Statistical analysis of the area percentage of collagen fibers confirmed LM findings.

Conclusion: Local intra-articular injection of PBMNCs restored the collagen fibers of the articular cartilage in a model of rat knee osteoarthritis better than those treated by PRP.

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Key Words: Collagen, osteoarthritis, PBMNCs, PRP.

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INTRODUCTION

The most common type of arthritis is osteoarthritis (OA) of the knee which is defined by the degradation of articular cartilage progressing to the development of osteophytes, or bone outgrowths. OA is the most prevalent cause of joint pain and physical incapacity in old age. The main value of its treatment is to reduce pain, slow disease progression, and enhance joint function and the quality of life[1,2].

During the progression of OA, pathological variations have been revealed to influence the whole joint. The initial sign of evident degeneration characteristic of osteoarthritis is the fibrillation at the articular cartilage surface due to damage of collagen fibrils. Formation of osteophyte, abnormal remodeling of subchondral bone, inflammation of synovial membrane, degeneration of menisci and ligaments, and hypertrophied joint capsule are also observed. In addition to infiltration of inflammatory cells, neovascularization as well as fibrosis in the infrapatellar pad of fat. Risk factors for the development of osteoarthritis include obesity, age, genetic factors, gender, excessive mechanical loading, and inflammation. Presumably, the mutual effects of obesity, aging, and an increasing number of joint damages in the world, this oppressive syndrome is predictable to become more widespread[3].

Mononuclear cells extracted from peripheral blood are composed of lymphocyte mixture (T lymphocytes, B lymphocytes and natural killer cells), monocytes and a slight percent of the endothelial progenitor cells. There are three major mechanisms of their action on injured tissues: formation of new blood vessels that is defined as angiogenesis, macrophage polarization from the M1 macrophage phenotype to M2 as well as paracrine stimulation. The resulting biochemical and cellular adaptations after mononuclear cells injection favor tissue regeneration and healing. Peripheral blood mononuclear cells injection is greatly associated with the production of paracrine and pro-angiogenic factors such as vascular endothelial growth factor, b-Fibroblast growth factor and angiopoietin-1[4].

Platelet-rich plasma (PRP) is achieved a great attention because of the high levels of growth factors and cytokines stored in platelets α-granules, that provide homeostasis of the articular cartilage. These active proteins can affect and

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promote a good joint environment, supporting the repair of a homeostatic balance in damaged joints. The effect of PRP injected intra-articularly via different bioactive molecules results in reducing the catabolic, inflammatory, and degradation processes, so this offering functional improvement and symptom relief[5].

Based on all these facts, this study was carried out to compare between the therapeutic effect of peripheral blood mononuclear cells (PBMNCs) and PRP in restoring the collagen fibers in knee joint of osteoarthritis rat model.

MATERIALS AND METHODS

This work was done on thirty-nine male adult albino rats weighing 110-140 gm, their age ranges from 100- 120 days. The animals were divided into two major groups: donor group and experimental groups.

Donor group: included 12 rats that were divided into two equal subgroups; one for donation of PMNCs and the other for donation of PRP.

Experimental groups: included 27 rats and were sub-divided into four main groups:

Group I: included 9 rats served as a control group and was sub-divided into three equal subgroups: Subgroup Ia did not receive any medication. Subgroup Ib received unilateral intra-articular injection of 50 ul sterile saline solution in the right knee joint. Subgroup Ic received unilateral intra-articular injection of 50 ul phosphate-buffered saline solution in the right knee joint.

Group II: included 6 rats which received single unilateral intra-articular injection of 50 ul monosodium iodoacetate (MIA) in the right knee joint at a dose of 6 mg/kg of body weight dissolved in sterile saline solution for induction of osteoarthritis [O.A][6]. The rats were sacrificed two weeks after the day of MIA injection (1st day of the experiment).

Groups III and IV received intra-articular injection of MIA at the same dose and site as group II for osteoarthritis induction.

Group III: included 6 rats that received unilateral intra-articular injection of 50 ul peripheral blood mononuclear cells [PBMNCs] in the right knee joint two weeks after MIA injection and then sacrificed 3 weeks later[7].

Group IV: included 6 rats that received unilateral intra-articular injection of 50 ul platelet-rich plasma [PRP] in th right knee joint two weeks after MIA injection then sacrificed 3 weeks later[8].

A) Preparation of peripheral blood mononuclear cells (PBMNCs):

According to Rahmoune and Guest (2017)[9], density gradient centrifugation techniques with Ficoll-Hypaque were used to separate peripheral blood mononuclear cells from other components of whole blood. The materials needed were blood samples collected with EDTA as an anticoagulant, Dulbecco's phosphate-buffered saline, Ficoll-Paque Plus solution with a density 1.077 g/mL, 15 and 50 mL sterile Falcon tubes and Centrifugation machine.

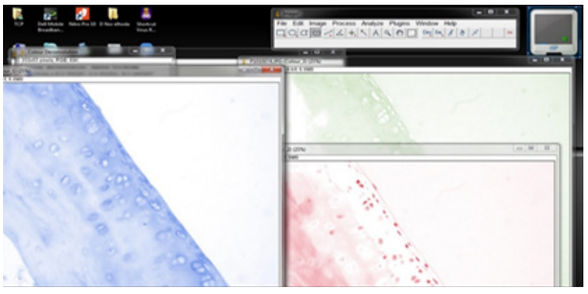
B) Preparation of platelet-rich plasma (PRP)

According to Dhurat and Sukesh (2014)[10], PRP was prepared by a process known as differential centrifugation. In the preparation of PRP, initial centrifugation is done to separate red blood cells at the bottom of the tube then, followed by another centrifugation to concentrate platelets, that were suspended in a small plasma volume (double centrifugation process).

All the experiments including preparation of PBMNCs and PRP were performed in the animal house and the tissue culture unit of Histology and Cell Biology Department, Faculty of Medicine, Tanta University. The animals were sacrificed at the end of the experiment, and the right knee joints from animal were dissected carefully and processed for light microscopic study after their decalcification. Serial 5um sections were cut and stained with Mallory trichrome. All the knee joints sections were examined and photographed using Olympus light microscope (Tokyo, Japan) coupled to an Olympus digital camera (DXC1850P, Tokyo, Japan) in Faculty of Medicine exactly at Histology & Cell Biology department, Tanta University.

Morphometric study and statistical analysis

The image analysis system was done by image analysis software program (Image J. version 1.46)[11] that help to measure the mean area percentage of collagen fiber contents in sections stained with Mallory Trichrome (photograph 1) (10 non-over lapping fields for each group) in all groups (X400).



Photograph 1: Shows the method used to measure the area percentage of collagen fibers.

SPSS software version 13 (SPSS Inc., Chicago, IL, USA) was used to statistically analyze the data, then compared via one-way analysis of variance (ANOVA) test trailed by Tukey's test to compare the various groups with each other. The results were conveyed as mean ± standard deviation (SD). The differences were considered statistically non-significant if probability value $p > 0.05$, significant if $P < 0.05$ and highly significant if $P < 0.001$[12].

RESULTS

Group I (control group) revealed the normal appearance, arrangement, and normally distributed collagen fibers in the different zones (calcified and noncalcified) and regions

(pericellular, territorial, and interterritorial matrices) of the articular cartilage evidenced by the homogenous diffuse bluish staining of the matrix. (Figures 1,2).

Group II (osteoarthritis group) demonstrated homogenous decrease in collagen content in all cartilage zones of the weight-bearing tibial articular cartilage (Figure 3). Regarding regions of the matrix, there was faint bluish staining of decreased collagen content mainly in the interterritorial matrix and mild bluish staining of collagen content in the pericellular and territorial matrices (Figure 4).

Group III (PBMNCs treated group) showed moderate bluish staining of increased collagen content mainly in the non-calcified zone (tangential & intermediate) but to a lesser extent in the calcified zone (Figures 5,6).

Group IV (PRP-treated group) showed moderately increased collagen content evidenced by heterogenous bluish staining of the interterritorial matrix of the intermediate and radial zones while it was very weak or absent in the calcified zone (Figure 7). Multiple vascular spaces with increased collagen content around them were seen in association with diminished collagen content in the nearby thin articular cartilage (Figure 8).

Quantitative and Statistical Analysis of the mean area percentage of collagen fibers

The percentage of mean area of collagen fibers content in Mallory trichrome stained sections was significantly decreased in group II in comparison with groups I & III. No significant change between group II & group IV. As regards group III, there was no significant change in the mean area percentage of collagen fibers between this group and group I. While the area percentage between group III and both group II & IV was significantly increased. Regarding group IV the area percentage was significantly decreased when compared with groups I & III while no significant change in comparison with group II. All these data were illustrated in (Table 1, Bar Chart 1).

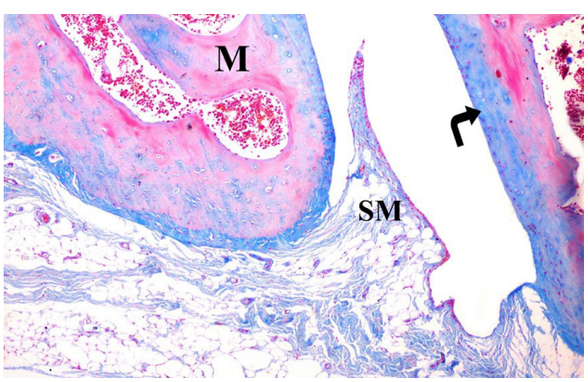


Fig. 1: Shows homogenous diffuse bluish staining of the collagen fibers in all zones of articular cartilage (curved arrow). Notice fold of the synovial membrane (SM) between the articular cartilage and the meniscus (M) (Mallory trichrome stain, tibial articular cartilage of group I ×100).

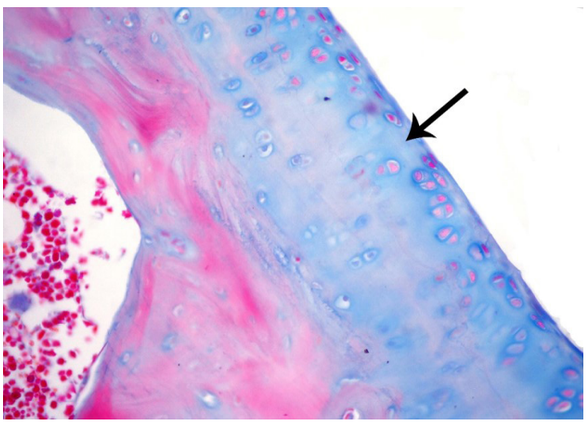


Fig. 2: Shows bluish staining of the collagen fibers in the pericellular, territorial and interterritorial matrices (arrow) of tangential, intermediate, and radial zones (Mallory trichrome stain, tibial articular cartilage of group I ×400).

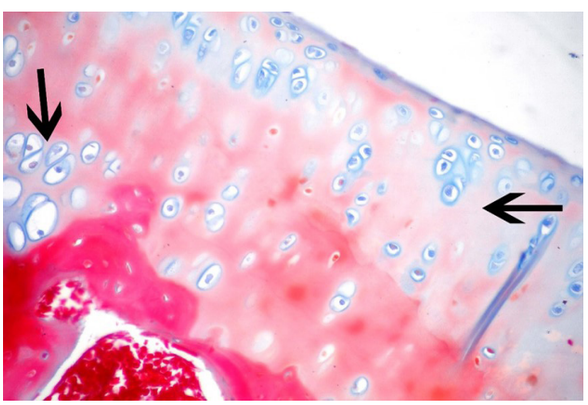


Fig. 3: Shows widespread faint bluish staining of decreased collagen content in all cartilage zones (arrows) (Mallory trichrome stain, tibial articular cartilage of group II ×400).

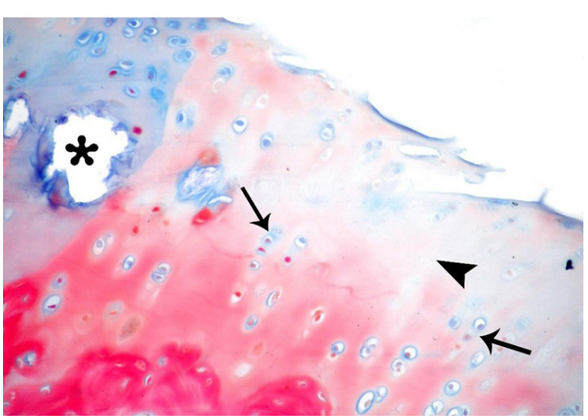


Fig. 4: Shows faint bluish staining of the collagen content mainly in the interterritorial matrix of the articular cartilage (arrowhead) while there is mild bluish staining of decreased collagen fibers in the pericellular and territorial matrices (arrows). Notice: a large irregular cavity in articular cartilage (asterisk) (Mallory trichrome stain, tibial articular cartilage of group II ×400).

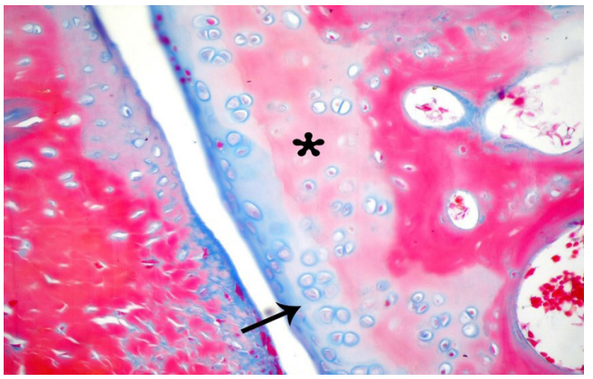


Fig. 5: Shows moderate bluish staining of increased collagen content mainly in the non-calcified zones (tangential & intermediate) (arrow) there is mild bluish staining of collagen fibers (asterisk) (Mallory trichrome stain, tibial articular cartilage of group III ×400).

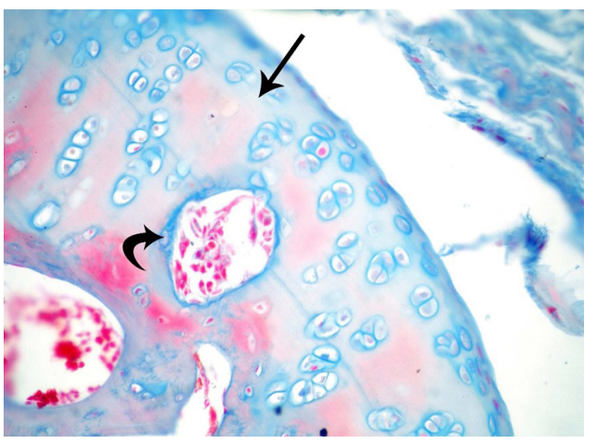


Fig. 6: Shows moderate bluish staining of increased collagen content in the articular cartilage (arrow). Notice good zonal organization of the articular cartilage cells despite the presence of bone marrow cavity (curved arrow) inside the articular cartilage substance (Mallory trichrome stain, tibial articular cartilage of group III ×400).

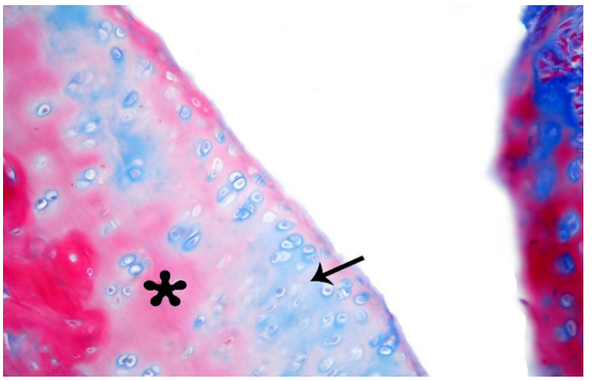


Fig. 7: Shows heterogenous bluish staining of moderate increase in collagen content in the interterritorial matrix of intermediate and radial zones (arrow) and absent in the calcified zone (asterisk) (Mallory trichrome stain, tibial articular cartilage of group IV ×400).

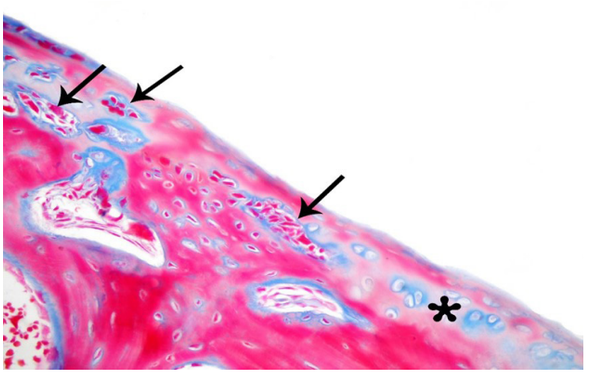
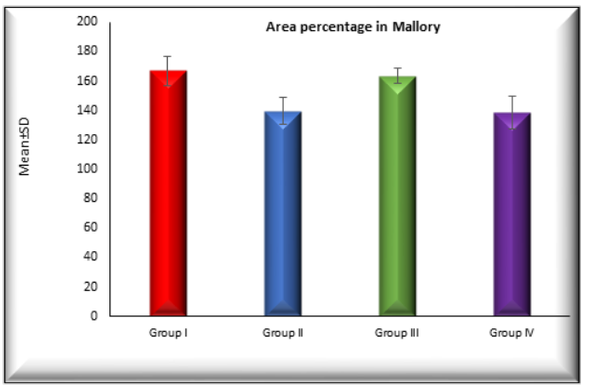


Fig. 8: Shows thinning of the articular cartilage with diminished collagen content (asterisk). The articular cartilage is invaded by multiple vascular spaces that are surrounded directly by increased collagen fibers (arrows) (Mallory trichrome stain, tibial articular cartilage of group IV ×400).

Table 1: The mean area percentage of collagen fiber content in different studied groups

Groups	Area percentage in Mallory						ANOVA	
	Range			Mean	±	SD	F	*P-value*
Group I	149.75	-	180.89	167.082	±	10.017		
Group II	127.89	-	150.35	139.734	±	9.137	51.887	<0.001*
Group III	156.69	-	173.11	163.440	±	5.214		
Group IV	114.81	-	151.55	138.606	±	11.140		
				TUKEY'S Test				
I&II	I&III	I&IV	II&III	II&IV		III&IV		
<0.001*	0.866	<0.001*	<0.001*	0.998		<0.001*		



Bar Chart 1: Mean area percentage of collagen fibers in the articular cartilage of different experimental groups.

DISCUSSION

Osteoarthritis (OA) remains the most challenging arthritic disorder and the most common joint disease, affecting more than 500 million individuals worldwide, of whom over 260 million have osteoarthritis of the knee joint. It is generated by a metabolic disturbance because of several extrinsic and intrinsic factors like trauma, or different forms of molecular dysfunction respectively. This disturbance induces the secretion of cytokines cascades and various inflammatory mediators resulting in nitric oxide production that leads to chondrocyte death and degeneration of the extracellular matrix (ECM)[13,14].

The present study focused on the role of peripheral blood mononuclear cells versus platelet-rich plasma in the restoration of collagen fibers of articular cartilage of osteoarthritic knee joint in adult male albino rat through light microscopic examination of Mallory trichrome stained sections. Monosodium iodoacetate (MIA) was used in this study for experimental induction of knee OA as Han *et al*., (2021)[15] who mentioned that monosodium iodoacetate (MIA) is a commonly used chemical model for knee OA induction, as it leads to progressive cartilage destruction and erosion in subchondral bone which mimic those of human knee OA.

Sections stained with Mallory trichrome in group II (osteoarthritis group) showed a homogenous decrease in collagen content in all cartilage zones. The synovial membrane showed increased collagen fibers. In addition, the statistical analysis approved that the mean area percentage of articular cartilage' collagen fibers was significantly decreased in group II when compared with groups I and III. While no significant change was observed in the mean area percentage between group II & group IV. The previously mentioned microscopic findings were also described by Estrada *et al*., (2001), Olli-Matti *et al*., (2017) and Wu *et al*., (2020)[16,17,18].

This study focused on the collagen fibers changes in tibial articular cartilage as it is the most affected part of the knee joint as Pitcher *et al*., (2016) and Ma *et al*., (2018) [19,20] who mentioned that the morphological changes of OA are measurable and quantifiable more in weight-bearing parts of the joint.

Several theories were postulated in trial to understand the mechanism of all previously observed findings, He *et al*.,(2020)[21] discussed the simplicity of articular cartilage tissue composition as it is formed of only a single type of cell called chondrocytes, wrapped in an copious ECM, without any blood vessels, nerves, or lymphoid tissue. Because ECM of cartilage is synthesized and secreted by chondrocytes, the pathogenesis of OA is consequently related to changes in activities of chondrocyte, involving deposition of matrix, cell proliferation, secretion of inflammatory cytokine, together with response to various signaling particles.

Additionally, Parrish (2017) and Kumar *et al*, 2018[22,23] mentioned that chondrocytes respond to injury by secretion of MMPs that degrade the type II collagen network. Synthesis of matrix metalloproteinases (MMPs) is affected by TGF-β that is secreted by chondrocytes and synovial cells. MMP-13 is the commonest proteinase in pathogenesis of osteoarthritis as well as MMP-1 and MMP-3. The previously mentioned types of MMPs can disrupt the collagen network. They added that the principal goal of MMP-13 is the destruction of collagen type II as it is the commonest form of collagen in the matrix of cartilage.

Furthermore, interleukin 6 (IL-6) is involved in the process of inflammation in OA. Binding of IL-6 to its receptors encourages inflammation and increases the production of MMPs via JAK/STAT pathway activation. receptors of IL-6 are found on the cell membrane of cartilage cells and present as soluble receptors in the synovial fluid[24,25].

Moreover, Zhang & Zeng (2019)[26] reported that TLR4 expression is increased in MIA-induced knee OA. This possibly activate the cascade of MyD88/NF-κB signals influencing the production of inflammatory factors (IL-6, IL-1β, and TNF-α) in tissue of articular cartilage together with their increase in synovial fluid leading to joint swelling and limited activity.

Vascular endothelial growth factor (VEGF) is secreted also via hypertrophic chondrocytes and synovial membrane fibroblasts, it impedes the formation and production of aggrecan and type II collagen fibers[27,28]. Moreover, synovitis leads to extensive secretion of proteolytic enzymes, causing destruction of the articular cartilage, whereas cartilage matrix catabolism expresses molecules that propagate synovial membrane inflammation and vice versa[29,30].

Sections of group III (PBMNCs treated group) revealed that there was moderate bluish staining of increased collagen content of the articular cartilage. No statistically significant change in the percentage of the collagen fibers' mean area between this group and group I, while it was significantly increased between group III compared with group II & group IV.

Collagen fiber content in this group was restored to its normal values as proved by statistical study of the mean percentage area for collagen fibers in sections of Mallory trichrome stain. The previously mentioned data were coping with Mizoguchi *et al*., (2018)[31] who studied the effect of PBMNCs on the treatment of cutaneous ulcers and reported that these cells produce interleukins and multiple growth factors like platelet-derived growth factor-BB and transforming growth factor that are required for the inflammatory phase of the normal healing process (proliferative and tissue remodeling phases). Moreover, they increase the concentrations of collagen mRNAs inside fibroblasts. So, it is attributed that the increased collagen synthesis was due to increased collagen mRNAs in chondrocytes.

Group IV (PRP-treated group) showed a moderate increase in the collagen content of the articular cartilage, but the cellular pattern of these zones displayed hypocellularity and disorganization. The area percentage of collagen fibers was significantly decreased when compared with control group in Mallory trichrome-stained sections. These results were observed similarly by Khatab *et al*., (2018), Asjid *et al*., (2019) and Chouhan *et al*., (2019)[32,33,34].

Osterman *et al*., (2015)[35] added that the most beneficial influence of PRP has been mediated via cyto & chemokines, adhesive proteins, growth factors and proteases in addition to various small-sized molecules like adenosine di-phosphate, Calcium, Serotonin, Epinephrine and Histamine. Furthermore, platelet-rich plasma injection is a harmless procedure as it is not associated with any reported deteriorated clinical features, infections, or serious complications. One of the optimistic influences of PRP is attributed to high concentrations of growth factors stored in the α-granules that are released from platelets after the injection of PRP into the joint. PRP has obvious mechanisms to improve the inflammatory and catabolic environment of OA by decreasing the proinflammatory gene expression and increasing the anti-inflammatory gene expression.

Li *et al*., (2005) &Mifune *et al*., (2013)[36,37] formerly stated that while transforming growth factor beta improves cartilage cells proliferation, it inhibits the terminal discrepancy of chondrocytes and helps them to persist in the pre-hypertrophic phase. Regarding the articular cartilage, it is reported that platelet-rich plasma stimulates extracellular matrix synthesis via chondrocytes.

From the present research, it could be concluded that single unilateral intra-articular injection of PBMNCs restores the collagen content of the articular cartilage in a model of rat knee osteoarthritis than single unilateral intra-articular PRP injection.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تأثير الحقن داخل المفصل للخلايا أحادية النواة في الدم المحيطي مقابل البلازما الغنية بالصفائح الدموية على استعادة ألياف الكولاجين بالغضروف المفصلي في نموذج لالتهاب مفصل الركبة بالجرذان

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المقدمة: التهاب المفصل العظمي هو سبب شائع جدًا للإعاقة. حيث أنه يتميز بإجهاد للخلايا الغضروفية وتدمير للبيئة النسيجة خارج الخلية و ذلك يظهر من خلال أعراض علي شكل آلام في المفاصل ، وفقدان لوظيفة المفصل الطبيعية بسبب تدهور الغضاريف ، وتغيير في تشكيل العظام ، مع تشكيل للنبتات العظمية.

الهدف: كان هذا العمل يهدف إلى تقييم تأثير الخلايا أحادية النواة للدم المحيطي مقابل البلازما الغنية بالصفائح الدموية على محتوى ألياف الكولاجين في مفصل الركبة في نموذج الجرذان من التهاب المفصل العظمي باستخدام صبغة مالوري ثلاثية الكروم.

مواد وطرق البحث: تم تقسيم ٣٩ جرذًا من الذكور البالغين إلى مجموعات مانحة وتجريبية تم تقسيمها إلى أربع مجموعات: المجموعة الأولى بمثابة مجموعة ضابطة ، المجموعة الثانية بمثابة نموذج لتحريض التهاب مفاصل الركبة ، المجموعة الثالثة تلقت حقن داخل المفصل بالخلايا أحادية النواة للدم المحيطي ، تلقت المجموعة الرابعة حقن داخل المفصل بالبلازما الغنية بالصفائح الدموية. تمت معالجة عينات مفصل الركبة للفحص المجهري الضوئي ليتم صبغها بصبغة مالوري ثلاثية الكروم وتحليلها إحصائيًا.

النتائج: أظهرت المجموعة الثانية انخفاضًا متجانسًا في محتوى الكولاجين في جميع مناطق الغضروف. بينما كشفت المجموعة الثالثة عن زيادة معتدلة في محتوى الكولاجين بشكل رئيسي في المنطقة غير المتكلسة ولكن بدرجة أقل في المنطقة الشعاعية والمتكلسة. أظهرت المجموعة الرابعة زيادة طفيفة في محتوى الكولاجين في الغضروف المفصلي. وقد تم اثبات تلك النتائج أيضا بالتحليل الإحصائي.

الخلاصة: الحقن الموضعي داخل المفصل للخلايا أحادية النواة للدم المحيطي أعاد ألياف الكولاجين للغضروف المفصلي لصورتها الطبيعية في نموذج الجرذ المصاب بالتهاب مفاصل الركبة بمعدل أكثر من تلك التي تم علاجها بواسطة البلازما الغنية بالصفائح الدموية.