EFFECT OF PLATELET RICH PLASMA ON CHONDROCYTE NUMBER IN CHEMICALLY INDUCED ANIMAL MODEL OF OSTEOARTHRITIS

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ABSTRACT

Objective: To study the histomorphological effects of platelet rich plasma infusion on number of chondrocytes in articular cartilage of monosodium iodoacetate induced osteoarthritic rat knee model.
Study Design: Experimental study.
Place and Duration of Study: Department of Anatomy, Army Medical College, Rawalpindi from March to May 2018.
Methodology: Thirty two healthy adult male rats of 3-4 months of age weighing 200-300 gm were taken. Animals were divided into two groups A and B as control and experimental with 8 and 24 animals respectively. Group B was further subdivided into B1, B2 and B3 with 8 animals in each group. All rats were injected with 50 µl of monoiodoacetate solution in the right knee. Group B1 along with group A was sacrificed on day 14 for establishing the presence of histological features of osteoarthritis (OA). Group B2 was injected with single dose of platelet rich plasma on day 18. Group B3 was not given any treatment. All animals were sacrificed 4 weeks after the injection of platelet rich plasma (PRP) in group B2. Number of chondrocytes was observed. Results were analyzed using SPSS v 21.
Results: Significant decrease in chondrocyte count was noted in group B3 as compared to group B2 in which PRP therapy was given.
Conclusion: Platelet rich plasma infusion prevented chondrocyte apoptosis in the treated group as compared to the untreated group.

Keywords: Chondrocyte, Mono iodoacetate, Osteoarthritis, Platelet rich plasma.

INTRODUCTION

Osteoarthritis (OA) is the most common joint disorder affecting hip, knee and other joints of the body. Knee OA affects 28% of the urban and 25% of the Pakistani population with women of middle age being the prime target[1]. OA is a multifactorial disease affected by various risk factors like age, gender, obesity, ethnicity and activity. OA is characterized by classical features at histopathological level including cartilage degradation, reduced bone mineral density, osteophyte formation, synovitis and capsule thickening[2]. Articular cartilage is a stiff and load bearing connective tissue which covers the bone ends making the smooth and frictionless movements possible at joints. Articular cartilage has low metabolic activity making it difficult to repair. It is devoid of perichondrium and has no vascular, neural and lymphatic supply. The structure of articular cartilage changes as we move deeper from the joint surface. On the basis of morphology of chondrocytes and matrix, articular cartilage structure can be divided into three zones; superficial (tangential), middle (transitional) and deep zone. These areas respectively constitute 10-20%, 40-60% and 30% of the articular cartilage volume. Middle zone of the articular cartilage acts as first line of defence against the compressive forces acting on joint surface. As we move from superficial to deep zones the arrangement of collagen fibres changes from parallel to perpendicular with reference to the surface with an increase in thickness of fibres. Chondrocyte count is most abundant in superficial zone with the cells being flat which become spherical and less dense in middle zone. In the middle zone chondrocytes

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are arranged in isogenous groups but their arrangement becomes perpendicular to joint surface once they enter the deep zone. Proteoglycan content is highest in the deep zone which provides maximum resistance against compressive forces. Tide mark separates deep zone from calcified cartilage which contains few hypertrophic chondrocytes[3].

Animal models are used to produce OA and can be classified as spontaneous or induced models. Osteoarthritic changes can be induced by surgical, chemical, mechanical and genetic modifications. Monosodium iodoacetate (MIA), papain, collagenase and carrageenan are the most commonly used agents for producing changes in knee joint. MIA, a derivative of acetic acid, causes chondrocyte apoptosis by inhibiting glyceraldehyde-3-phosphate dehydrogenase and is considered the most appropriate method for inducing OA changes[4]. MIA injected in a dose of 0.5mg in the knee joint showed apoptotic changes on the 1st post administration day with chondrocyte shrinkage, nuclear condensation, vacuolar degeneration and apoptotic bodies[5]. Teeple *et al*, noticed that MIA injection stimulates intra articular damage, matrix degradation and chondrocyte toxicity[6]. The effects produced by MIA are strongly related to its concentration with cell count decreasing in the central regions of the cartilage suggesting that the chondrocytes in the central region are more sensitive to MIA[7].

Various treatment modalities are used for managing OA symptoms which include pain killers, anti inflammatory drugs, osteotomy, intra articular knee injections and ultimately knee replacement surgery. At present, platelet rich plasma (PRP) is the new approach being used for treating OA and has been reported with relief of symptoms and repair of injured tissues. PRP is defined as a concentrate of autogenous growth factors derived from blood which influences healing of musculoskeletal system by promoting cell multiplication, collagen synthesis and new vessel formation. American Red Cross society declared that plasma sample having platelet count two fold or more above the baseline or greater than 1.1 x 10^6 platelets/µl is labelled as PRP[8]. Marx *et al*, proposed that platelet count of 10 lakh/ml in 5ml of the plasma promoted bone and soft tissue healing and could be labelled as PRP however Rughetti *et al*, tossed the working definition of PRP with platelet count of 1million/ml[9]. PRP prepared from differential centrifugation of blood contains growth factors contained in α granules of platelets and promotes angiogenesis, collagen synthesis and cell proliferation[10]. Fortier *et al*, found that chondrocytes and mesenchymal stem cells (MSCs) exposed to PRP showed increase in cell count along with proteoglycan and collagen type-II synthesis[8].

The aim of this study was to establish the histopathological effect of PRP on chondrocyte count in articular cartilage of MIA induced osteoarthritic rat knee model.

MATERIAL AND METHODS

This study was approved by Ethical Review Committee of Army Medical College Rawalpindi and was conducted from March 2018 to May 2018 in the department of Anatomy, Army Medical College Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad. Thirty two male Sprague Dawley rats with average weight of 200-300 gm were taken, housed in separate well ventilated cages. Twelve hours light and dark cycles were maintained at standard room temperature of 20-26°C. Standardized laboratory diet and water ad libitum was provided to all animals. Both MIA and PRP were administered using insulin syringes.

MIA was used for inducing osteoarthritic changes in knee joint. Solution was prepared by dissolving 80 mg of the chemical in 2 ml of 0.9% saline yielding 2 mg of MIA in 50 µl. PRP was prepared by double centrifugation method proposed by Zhang *et al*[11], using hettich EBA 20 benchtop centrifuge machine. 3 ml of venous blood was collected through intracardiac route and 0.05 ml of 0.1M sodium citrate solution was added as anticoagulant[11]. First centrifugation was carried out at 500g for ten minutes. Clear serum

and buffy coat were removed using 20 gauge lumbar puncture needle and subjected to second centrifugation at 2200g for 10 minutes. Platelet poor plasma was removed and PRP was collected

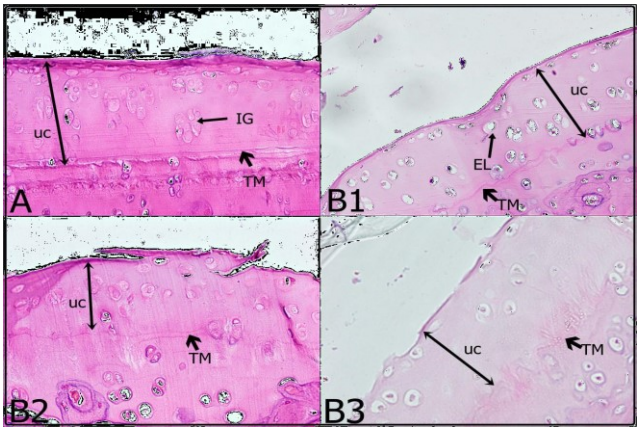

Figure-1: Photomicrograph showing articular cartilage histology. (A) From control group showing normal histology depicting un calcified cartilage (UC), calcified zone and tide mark (TM). B1 showing osteoarthritic changes i.e. surface irregularities and decrease in chondrocyte number. B2 showing articular chondrocytes in group receiving PRP. B3 showing articular cartilage of untreated group.

and activated using 0.05 ml of 10% $CaCl_2$[12].

Animals were divided into control group A (n=8) and experimental group B (n=24). Group B was subdivided into groups B1, B2 and B3 (8 animals per group). All animals in group B were injected with 50 µl of MIA solution in the right knee. Group B1 along with group A was sacrificed after two weeks for establishing presence of osteoarthritic changes. Group B2 was injected with PRP while group B3 was left as such. All animals in group B2 and B3 were sacrificed four weeks after the administration of PRP. Gross parameters were noted and specimens obtained were fixed in 10% Neutral Buffered Formalin. Decalcification was done using 5% nitric acid solution and processed for paraffin embedding. Sections of 5µm thickness were obtained using rotary microtome and hematoxylin and eosin stains were used for routine histological study. For quantitative analysis, ocular micrometer was calibrated with stage micrometer. Four random fields were selected and chondrocytes count was done at 40 x magnification in 0.06mm^2 area excluding the calcified zone. Chondrocyte lacunae with absent nuclei were not counted.

Data were analysed by using SPSS (Statistical Package for Social Sciences) version 21. Chondrocyte count was expressed as mean ± standard deviation. Statistical significance was calculated between the groups using independent sample t-test. A *p*-value ≤0.05 was considered significant and confidence intervals were kept at 95%.

RESULTS

Histologically, control group showed normal articular cartilage (A), however osteoarthritic changes like surface irregularities and decreased number of chondrocyte were noted among group B1 (B1). Chondrocyte count was done in control group A and experimental groups B1, B2 and B3. The mean count ± SD were calculated. The mean chondrocyte count ± SD in groups B2 and B3 was found to be 38.12 ± 8.6 and 18.90 ± 13.8 respectively. The *p*-value between the experimental groups B2 and B3 was found to be 0.006 which was statistically significant.

Table: Comparison of mean values of chondrocyte count between control group A and experimental groups B1, B2 and B3.

Parameter	Group A Mean ± SD (n=8)	Group B1 Mean ± SD (n=8)	Group B2 Mean ± SD (n=8)	Group B3 Mean ± SD (n=8)	Group A vs B1 (*p*-value)	Group B2 vs B3 (*p*-value)
Chondrocyte count	63.12 ± 12.80	39.25 ± 10.36	38.12 ± 8.611	18.90 ± 13.80	0.001	0.006

DISCUSSION

In the present study, PRP prevented apoptosis of articular chondrocytes thus providing the chondroprotective effect. Extracellular matrix changes and decrease in chondrocyte number is the hall mark of OA. MIA is an alkylating agent which reacts with proteins in cysteine residues

and causes inhibition of glycolytic pathway ultimately leading to death of chondrocytes. Miyamato *et al*, showed that MIA induced osteoarthritic knee models in rats and rabbits exhibited chondrocyte death and loss of proteoglycan which progressed to cartilage destruction, subchondral bone exposure and functional impairment similar to humans[13]. Morais *et al*, induced osteoarthritic changes in rat knee using two mg of MIA and showed decrease in chondrocyte number due to death

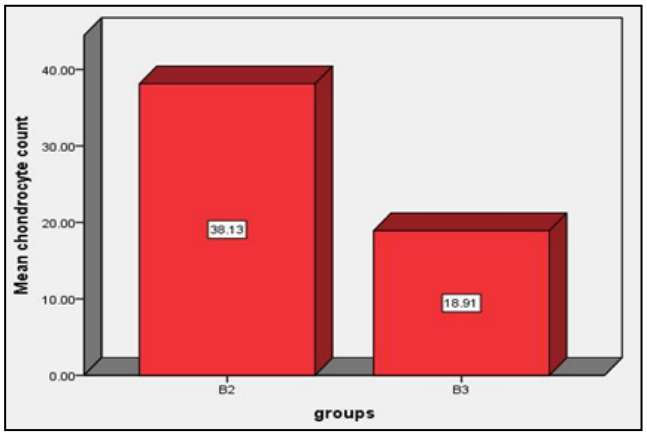

Figure-2: Bar chart showing the comparison of mean values of chondrocyte count of experimental groups B2 and B3.

and degeneration of chondrocytes along with osteophyte formation and bone degradation which was similar to our study[14]. Udo *et al*, induced osteoarthritis using different doses of MIA and concluded that induction of OA is both dose and time dependant[15]. Ferreira *et al*, showed that 1mg and 2mg did not induced bone destruction even after 4 weeks which was different than our findings[16]. All these researches showed use of wistar rats which were younger than rats used in this study. Miyamato *et al*, showed that 2mg of MIA injected into rat hip joint induced articular cartilage degeneration which was evident at day 14 which was similar to ours[13]. Guzman *et al*[17], observed that 1 mg produced extensive chondrocyte degeneration at day 1 with moderate collapse and loss of chondrocyte details by day 5 or 7 which was opposed to our research.

Cell/extracellular matrix interactions play a vital role in pathophysiology of OA. Chondrocytes are the only cells regulating the breakdown and synthesis of cartilage matrix. Cytokines; a family of proteins involved in immune responses along with growth factors maintain the balance between catabolic and anabolic processes within the matrix. Interleukins (IL) being pro inflammatory cytokines interfere with activity of growth factors and decrease the synthesis of aggrecan which is a prime component of matrix. Lipid peroxidation and radical oxygen species (ROS) induced by interleukins have also been found to cause matrix degradation[18]. These are believed to cause disruption of mitochondrial function by damaging mitochondrial DNA, lipids and proteins. Tumour Necrosis Factor (TNF)- and IL-1 increase nitricoxide (NO) and Reactive Oxygen Species (ROS) in OA chondrocytes causing mitochondrial dysfunction, ultimately leading to apoptosis[19].

Inhibition of chondrocyte apoptosis serves as a therapeutic strategy for treatment of OA. Moussa *et al*, concluded that PRP inhibits apoptosis of chondrocytes which coincided with our findings. PRP therapy has been found to induce proliferation of chondrocytes, decrease apoptosis and increased autophagy among chondrocytes[20]. PRP prevents aging of chondrocytes by increasing autophagy which prevents chondrocytes from excessive inflammation and nutritional deficiency. PRP isolated from autologous blood contains multiple growth factors which have been proved to promote chondrocyte proliferation and extracellular matrix secretion. PRP promotes expression of anabolic genes and reduces inflammatory stress ultimately leading to attenuation of post traumatic cartilage degene-ration. This study was also supported by Knut *et al*, who carried out a research on chondrocyte viability and concluded that PRP increased chondrocyte and tenocyte viability in vitro[21].

The present study has some limitations. First, MIA induced OA is a chemically induced model. To confirm the beneficial effects of PRP, this study should be conducted with other non

chemically induced models like immobilization and partial menisectomy which involves removal of all or some part of meniscus. Literature review suggested that MIA is used widely for induction of OA and demonstrates the characteristics of progressive inflammatory arthritis resulting in rapid destruction of cartilage structure[14]. The absence of proliferative effect noted in this study can be attributed to the fact that MIA continued to have its effect even after the administration of PRP. Second, this study was conducted with a single dose of PRP injection. Literature search suggested that chondrocyte proliferation increased in a dose dependant manner with increasing concentrations of platelets however our research was carried out using a single injection of PRP. This proposes that if chondrocyte count was done after two or three doses of PRP, increase in chondrocyte count would have been noticed.

CONCLUSION

Platelet rich plasma infusion prevented chondrocytes apoptosis in the treated group as compared to the untreated group.

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CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any authors.

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