ORIGINAL ARTICLE

Ameliorative Effect of Vitamin D on Articular Cartilage Thickness in Arthritic Rat Model

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ABSTRACT

Objective: To determine the protective effect of vitamin D on the articular cartilage of formalin-induced arthritis in rats.

Study Design: Lab–based Experimental study.

Place and Duration of Study: The study was carried out at the Department of Anatomy, Islamic International Medical College from September 2020 to September 2021.

Methods: We randomly split thirty adult male albino rats weighing between 250 and 300 grams into three groups. (10 in total). On days 1 and 3 of the experiment, formaldehyde was subcutaneously injected into the right paw of every animal, with the exception of those in group A. The usual rat food was given to the rats in Groups A and B. Throughout the trial, Rats in group C were given vitamin D 4000IU/kg orally by adding cholecalciferol (Vitamin D3) injection in a standard diet for 28 days. All of the animals were dissected after the experiment, and the right hind leg was taken out, processed, and stained with H&E for a microscopic assessment of the thickness of the unclarified cartilage. Version 21 of SPSS was used to analyze the results.

Results: The oral administration of vitamin D exhibited a significant increase in unclarified cartilage thickness on intergroup comparison.

Conclusion: Vitamin D is an effective antiarthritic agent in ameliorating the thickness of articular cartilage thickness in arthritic rat models.

Keywords: *Articular Cartilage, Osteoarthritis, Formaldehyde Induced Arthritis, Vitamin D.*

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Introduction

Osteoarthritis is a chronic, deteriorating disease causing articular cartilage loss, causing synovial joint damage, and affecting chondrocytes and matrix, making it the most common form of arthritis.[1] The chondrocytes undergo biochemical and mechanical stresses causing the breakdown of components of the matrix.[2] The decrease in the hydration of the matrix is associated with advancing age due to an increase in compressive stiffness. Loss of proteoglycans in the matrix leads to flaking or surface cracking. Matrix loss leads to deep fissures and cracks.[3] A granulation tissue called a pannus develops at the periphery of the cartilage which bridges across the fissures. The thinning of uncalcified cartilage is seen especially in the superficial and transitional zone.[4] Meanwhile calcified cartilage thickness increases due to

calcification of tidemark, an interphase between calcified and uncalcified layers of articular cartilage. Subchondral bone remodeling can be seen with the progression of cartilage damage into deep zone It is becoming a progressively widespread disabling disease with serious socioeconomic influence universally.[5] About 3.6% of North Pakistan's rural population and 3.1% of its urban population were estimated to have knee OA.[6]

Vitamin D is included in a class of fat-soluble prohormone. Early in the 20th century the discovery of the ameliorative effect of cod liver oil led to identification of vitamin D.[7] Nowadays vitamin D is considered to play a vital role in general nutrition and human disease states. Under the influence of ultraviolet light (UV) vitamin D3 is prepared in the skin from 7-dehydrocholesterol.[8] Vitamin D2 (ergocalciferol) is derivative of the ergosterol, a plant sterol. The biologically active form of vitamin D, D3, is produced by the liver and kidney from hydroxylated vitamin D2. The presence of vitamin D receptors (VDRs) in bone, cartilage, and muscle reflects the pathophysiological role of vitamin D metabolites in OA.[9]

Numerous studies have demonstrated that the majority of bodily tissues and cells contain vitamin D receptors (VDRs). This complex attaches to vitamin D3 response elements (VDREs) in the promoter region of several target genes when vitamin D interacts with vitamin D receptors (VDRs).[10] Stimulating the VDREs prevents the transcription of nuclear transcription factors like NFAT (nuclear factor of activated T cells) and NF-kB (nuclear factor kappa B pathway).[11] This study aims to investigate the impact of inadequate vitamin D levels on calcium metabolism, articular cartilage turnover, and osteoblastic activity. Additionally, it seeks to determine if vitamin D can reverse the histomorphological changes of articular cartilage in an arthritic rat model by measuring the thickness of the cartilage

Methods

The study was carried out at the Department of Anatomy, Islamic International Medical College from September 2020 to September 2021 after taking approval from the medical college's ethics review committee held on 3rd August 2020 vide letter no: Riphah/IIMC/IRC/20/158 and the sample was selected using a non-probability consecutive sampling technique. This investigation was conducted using thirty mature male Albino Sprague Dawley rats as a mammalian model, with each rat weighing 300 grams. The study excluded female rats and animals weighing less than 300 grams. Ethical review committee's guidelines were followed when caring for and handling the animals. Rats were kept under the supervision of the Animal House, in clean cages with standard temperature of 22 ± 0.5˚C in an air-conditioned room. In an air-conditioned environment with a standard temperature of 22 ± 0.5˚C, the animals were placed in hygienic stainless steel cages with a 12-hour light and dark cycle and 50% humidity. They were given food and water ad libitum for 7-days to acclimatize. Ten male rats were included in each group. Three groups: A control, B a negative control, and C two treatment groups. For a duration of 28 days, Group B (negative control) and Group A (control group) were administered a regular diet orally. For 28 days, the rats in group C received an oral dose of vitamin D (4000 IU/kg) by adding an injection of cholecalciferol (Vitamin D3) into their regular food.[12] All of the animals' The left hind paws of all animals were given the sub plantar injection of 0.1 ml of formaldehyde (2% v/v) on days 1 and 3 of the experiment to induce arthritis.[13] This was done one hour after oral administration of the vehicle/drugs. After 24 hours from the previous dose, the rats were euthanized by putting them to sleep with cotton balls drenched in chloroform.

Dorsal recumbent was how the animals were positioned. The dissection was done with sterile tools. The right hind limb skin was shaved below the knee joint, and the right hind limb was amputated just proximal to the ankle joint using a bone cutter as shown in Figure 1. After that, it was preserved in 10% formalin and cleaned with saline. For ten days, the bone was submerged in formic acid to decalcify it. The ankle joints were decalcified and then transected in the frontal plane to produce two about equal sections. The paraffin wax was melted and used to implant the tissues. At roughly 200 mm intervals, these blocks were sectioned serially. For histological examination, the blocks were divided into 5-mm-thick pieces using a microtome.

Hematoxylin and eosin were used to stain the slides. The thickness of the uncalcified cartilage (UCC) was measured by utilizing Image J software to examine each slide under 40 X magnification. A line perpendicular to the joint surface was drawn, along with three lines parallel to and on either side of it. The measurement of uncalcified cartilage was obtained from the surface of the cartilage to the tide mark. Four measurements were made for each sample to corroborate the thickness measurements. All readings' means were recorded.

The data were entered and interpreted using SPSS version 21, and the results were presented as mean ±SD. One Way Analysis of Variance (ANOVA) was used to compare the mean thickness between the treatment group, negative control group, and control group. The post hoc Tukey's test was utilized to compare groups with one another. A *p*-value of less than 0.05 was regarded as significant.

Results

The mean uncalcified cartilage thickness in the H&E-stained segment of the ankle joint was determined to be 104um in control A, 23.52um in negative control B, and 42.29um in treatment group C, respectively. In group C, the cartilage thickness increased with the restoration of surface discontinuity, but in group B, it decreased with surface discontinuity and cartilage erosions. When group B and group C's mean values were compared, a significant mean difference (*p*-value <0.01) was discovered. Additionally, there was a notable distinction between groups A and B & C. (Figure, 1, Table 1).

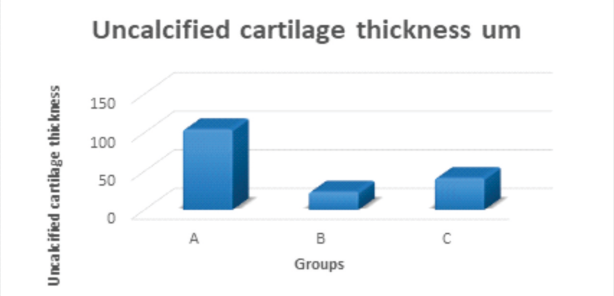

Fig 1: Bar chart showing mean thickness of uncalcified cartilage thickness in control group A, negative control B, and treatment group C

Table 1: Mean measurement of uncalcified cartilage thickness among control group A, negative control group B and treatment group C of albino rats by Post hoc tukey test

Parameters	Group A Mean± SD	Group B Mean± SD	Group C Mean± SD	*P*-value
Uncalcified cartilage thickness	104.4±0.4	23.52±0.4	41.29±0.4	<0.01
Post-Hoc Analysis Intergroup comparison	B vs A <0.01	B vs C <0.01	C vs A <0.01	

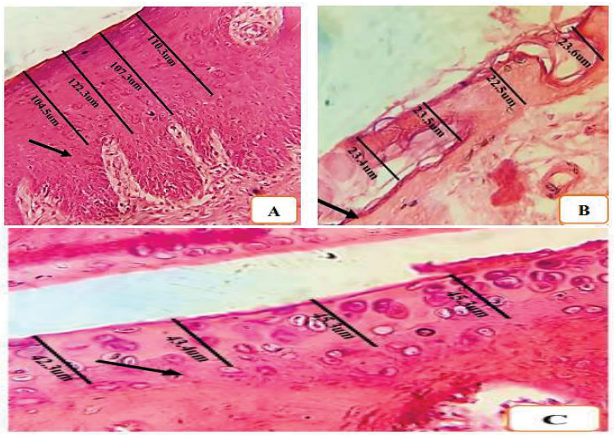

Fig 2: Photomicrograph of coronal section of ankle joint showing unclarified cartilage thickness in control A, negative control B andtreatment group C & amp; D (H & amp;E stains at 40x10x). Black lines are showing thickness of cartilage from surface to tide mark. Black arrow shows tidemark

Discussion

The degenerative condition known as osteoarthritis causes the articular cartilage to gradually wear away over time. It is the most prevalent type of arthritis that damages synovial joints both structurally and functionally.[14] Depending on the severity of the ailment, a mix of medication, lifestyle changes, and other therapies may be used for osteoarthritis treatment.[15] Nutritional supplements are becoming more and more well-known for the safe and efficient treatment of arthritis because of the severe side effects and expensive expense of pharmaceutical therapy.[16]

The articular cartilage of the rat ankle joint showed multiple histological changes as a result of the sub plantar infusion of formaldehyde. Significant

uncalcified cartilage thinning was seen when H&E-stained slices of the negative control group were examined.[13] Table 1 and Figure 2 of our study demonstrate that the oral administration of vitamin D in group C resulted in a considerable increase in the thickness of uncalcified cartilage.

The sub plantar injection of formaldehyde resulted in various histological alterations in the articular cartilage of the rat ankle joint.[17] These results were consistent with earlier rodent investigations that reported comparable structural alterations in the articular cartilage of an arthritic rat model. Significant weakening and degeneration of uncalcified cartilage was seen upon examination of H&E stained sections of the negative control group.[18] When vitamin D was taken orally, uncalcified cartilage thickness increased significantly. In the current study, oral vitamin D supplementation at a dose of 4000IU/kg for four weeks significantly reduced the thinning of articular cartilage in an arthritic rat model. However, in a 2019 study, Marta Anna found that exogenous vitamin D supplementation at a dose of 4000IU for ten weeks significantly increased the thickness of articular cartilage in a healthy rat model.[9] In 2017, Rachel J. found that vitamin D insufficiency was linked to a decrease in articular cartilage thickness in OA patients.[8] Cecilia Pascual-Garrido in 2016 also revealed decrease matrix staining due to matrix loss in vitamin D deficient arthritic rat model in 4 weeks.[19] Antiarthritic and anti-inflammatory properties of vitamin D on articular cartilage is reflected by study conducted by Katarzyna Patrycja Dzik in 2016 which suggests that insufficient levels of vitamin D cause atrophy and mitochondrial impairement of paraspinal muscle.[20] Jane Fletcher in 2019 also highlighted the antiinflammatory effects of vitamin D and demonstrated its function as a TNF-alpha inhibitor.[21] Thus vitamin D has got potent antiinflammatory properties making it effective antiarthritic agent. The present study showed that the oral administration of vitamin D restored the thickness of articular cartilage in arthritic rat model. According to Yongji Wang, vitamin D enhances the proliferation of chondrocytes due to presence of VDR on the osteoblasts and cartilage chondrocytes from the tissue of newborn mice calvaria.[22]

Few studies are available regarding anti-arthritic effect of vitamin D. In this study, restoration of articular cartilage thickness is observed in the group receiving oral vitamin D. This is consistent with study conducted by E. C. Castillo in 2012 on arthritic rat model where vitamin D attenuated articular cartilage thickness given at the dose of 4IU/kg for 20 days orally.[23] Jie-Chao Gu stated in 2022 that people with type 2 diabetes who have low vitamin D levels are in extremely inflammatory and oxidative states.[24] Present study demonstrated positive effects of vitamin D on hyaline cartilage of arthritic rat model. This is in line with the research where vitamin D insufficiency was shown to have negative effects on the hyaline cartilage of normal, healthy rats by Pascual-Garrido in 2016.[19] This study revealed restoration of surface discontinuity of articular cartilage of arthritic rat model as shown in figure 1 thus stating its ameliorative effect in osteoarthritis. According to Vikrant Rai lower serum level of vitamin D were associated with increased chondrocyte clustering and cartilage matrix loss in arthritic knee joints.[7] Thus vitamin D deficiency caused deterioration of articular cartilage in arthritis so its oral administration restored the damage as shown in this study. The limitation of this study is the lack of biochemical analysis like IL-6 and TNF alpha levels to support the antiarthritic effect of vitamin D.

In conclusion, it was discovered that Vitamin D is a potent antiarthritic agent that reduces cartilage thickness in a model of arthritic rats. Using an arthritic rat model, this study evaluated the potential medical benefits of vitamin D.[15] The findings inform that oral administration of vitamin D can be used as a medication to address OA-related inflammation.[25] Future research could explore the effect of vitamin D on histomorphological changes in synovial membranes and adjacent bony surfaces of arthritic joints.

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Authors Contribution

AS: Idea conception, study designing, data collection, data analysis, results and interpretation, manuscript writing and proof reading
HB: Idea conception, study designing, data collection, data analysis, results and interpretation, manuscript writing and proof reading
TK: Data collection, manuscript writing and proof reading
AR: Data collection, manuscript writing and proof reading
US: Data collection, manuscript writing and proof reading
MK: Data collection, manuscript writing and proof reading