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Original Research Article

Assessment of Arthritis and Vitamin D in Experimental Mouse Model

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Abstract

Background: Rheumatoid arthritis is an autoimmune disease that causes inflammation in joints tissue and is characterized by concomitant destruction of bone and cartilage. Total 1% of the worldwide population suffers from this disease. Rheumatoid arthritis (RA) is a chronic inflammatory disease of unknown aetiology marked by asymmetric, peripheral polyarthritis. Aetiology of RA is still unknown, and many environmental and genetic factors play a role in the development of this disease. Vitamin D is necessary for healthy bone, as it is helps in absorption of calcium from the gut. Deficiency of vitamin D has been implicated as common cause of diseases such as Osteoarthritis, rickets etc. Vitamin D deficiency is highly prevalent in the general population particularly in patients with musculoskeletal and autoimmune diseases. Vitamin D level and the link to RA disease can open up new gateway for the better understanding of this disease. The results would also help in better understanding of the role curcumin in the treatment of arthritis. The present study was designed to study the level of vitamin D and uric acid with arthritis mice and compare it with healthy controls. Thus, the present study was undertaken to study the association of vitamin D and Uric acid with Rheumatoid arthritis Swiss albino mice.

Aim: The aim of the study was any change in Vitamin D level in experimental mouse model of arthritis as compared to normal.

Methods: We planned an animal study as mice mimics the better phenotypic arthritis symptoms. Swiss albino mice (12 - 14 weeks old) were taken for this study. Adjuvant induced arthritis (AIA) mice were prepared by injecting Freund's complete adjuvant (FCA) intra-dermally in the sub planter surface of the right hind paw, also a booster dose was given of FCA in order to produce autoimmune disease.

Results: After 47 days, the biochemical investigations; rheumatoid arthritis factor (RA factor) test was found mild positive in normal healthy animals, on the other hand arthritic samples exhibited strong agglutination reaction. Serum uric acid and Vitamin D levels were also estimated.

Conclusion: We observed the significantly elevated levels of uric acid in arthritic mice as compared to the normal mice, while there was no significant change in vitamin D levels in arthritic mice as compared to normal mice.

Keywords: Rheumatoid arthritis, Vitamin D, inflammation, autoimmune disease

Introduction

Rheumatoid arthritis is an autoimmune disease that causes inflammation in joints tissue [1] and is characterized by concomitant destruction of bone and cartilage [2].

The development of arthritis is markedly suppressed in interleukin-1β deficient collagen induced arthritis (CIA) [3] and human T cell leukemia virus type I transgenic mice [4].

Rheumatoid synovitis generates cartilage breakdown [5], bony erosions, and loss of function of the involved joints [6]. But besides articular involvement, cardiovascular disease generated by accelerated, premature atherosclerosis represents a serious complication of RA. It is known that in RA patients, cardiovascular disease represents the cause of 40–50% of the deaths in this group of population [7,8].

Vitamin D (25-hydroxyvitamin D) alters the expression of genes that affect cellular functions such as proliferation, differentiation, apoptosis, and angiogenesis [9].

Calcitriol (1α, 25-dihydroxyvitamin D_3 (1,25 $(OH)_2D_3$), the most active form of vitamin D, is a pleotropic hormone with a wide range of biological activities. The biologically active form of vitamin D_3,1α,25$(OH)_2D_3$ (1,25D_3), is obtained by 25-hydroxylation of vitamin D_3 in the liver and 1α-hydroxylation in the kidney and liver [10]. Vitamin D3 can be either synthesized in the human skin upon exposure to the UV light of the sun, or it is obtained from the diet. If the photo conversion in the skin due to reduced sun exposure (e.g., in wintertime) is insufficient, intake of adequate vitamin D from the diet is essential to health. Severe vitamin D deficiency can lead to a multitude of avoidable illnesses; among them are well-known bone diseases like osteoporosis and a number of autoimmune diseases [11,12]. In the context of autoimmune diseases, administration of vitamin D prevented the onset of experimental autoimmune encephalomyelitis (EAE), a rodent model of MS [13]. In an animal model of Alzheimer Disease, dietary supplements with vitamin D enhanced learning and memory compared to healthy controls [14].

So we saw above that RA is associated with vitamin D deficiency and this study was planned to prove this association if any.

Material and methods

a. Study Design: Type of Study: Case Control with artificially induced arthritis in mice as the diseased group (case) and normal mice as control.
b. Study Period: 6 months
c. Subjects: Swiss albino mice
d. Animals: Aged Swiss albino mice (12 - 14 weeks old) weighing between 28 –30 g was procured from NABL-compliant Institutional Animal facilities and were used throughout the experiments. Animals were kept under standard husbandry conditions (Temperature 22 ± 2°C; relative humidity 50-55%; 12h. light and dark cycle. Animals were fed pellet diet (Lipton, India) and water *ad libitum*. The entire work was conducted with consent from the Institutional Animal Ethics Committee for the purpose of control and supervision on experiments on animals (CPCSEA) India

e. Induction of Arthritis in Mice: Adjuvant induced arthritis (AIA) mice were prepared by injecting (0.01 mL) Freund's complete adjuvant (FCA) (Sigma, USA) intra-dermally in the sub planter surface of the right hind paw. First injection was given on day 0 and a booster injection on day 12.

Animals were observed for at least 47 days (15)

f. Animal groups: Animals were divided in two groups of 6 mice each as follows;

Group 1 Healthy controls;

Group 2 Adjuvant induced arthritic mice.

g. Assessment of joint edema: The joint edema was measured with the help of Vernier calipers till day 47.

h. Collection of Blood: Blood was collected from the retro-orbital complex in the eye of the mice, in plain vial. The samples were centrifuged at 2000 rpm for 10 min and serum was collected and used for investigation.

i. Estimation of RA factor: RA factor was assayed qualitatively using kit method as described (16).

j. Estimation of Uric acid: Uric acid was estimated by colorimetric method, using Uricase-POD reagent on semi-autoanalyser.

k. Vitamin D estimation: Vitamin D was estimated in serum by ELISA kit (Calbiotech).

Methodology- The joint edema was measured with the help of Vernier calipers till day 47. After 47 days, we investigated for the biochemical investigations; rheumatoid arthritis factor (RA factor) test was found mild positive in normal healthy animals, on the other hand arthritic samples exhibited strong agglutination reaction

Results

In the present study, we have assessed the joint edema, serum uric acid, Rheumatoid Arthritis Factor and vitamin D in normal and arthritic mice.

After injecting the Freund's Complete Adjuvant (FCA) on day 0, we observed redness and inflammation at the site on injection (right hind paw) (Fig 1). After the booster dose of FCA on day 12, we again observed the enhanced redness and inflammation at the injected site (right hind paw). We continuously observed the right hind paw of normal as well as arthritic mice (Fig 2 and 3).

The joint edema was measured with the help of Vernier calipers till day 47. After 28 days of FCA injection, the mice demonstrated the secondary signs of inflammation such as high swelling and distorted phalanges (Fig 3).

Arthritic mice showed a significant increase in the joint measurement as compared to the normal mice (Table 1, Fig 4)

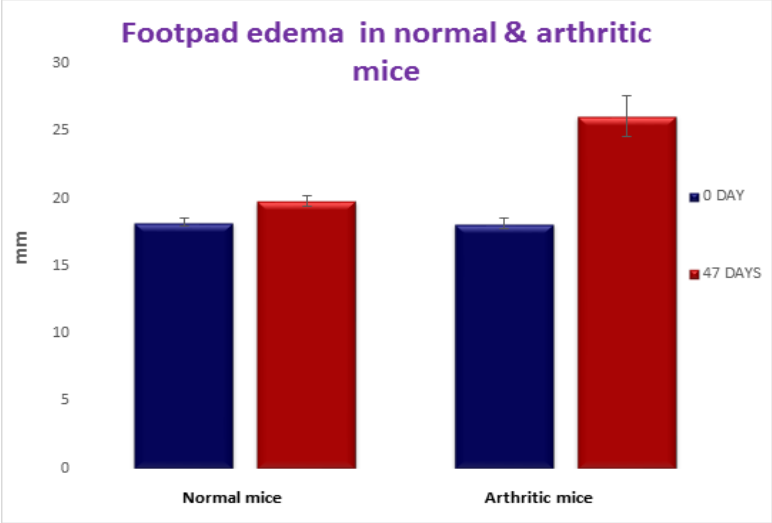

Figure 1: Footpad edema in normal & arthritic mice (0 day and 47 days)

Table 1: The edema (mean ± S.D.) in the footpad of normal and arthritic mice -

	Day 0	Day 47
Normal mice	18.2 ± 0.3 mm	19.8 ± 0.4mm
Arthritic mice	18.1 ± 0.4mm	26.1 ± 1.5mm

Uric acid level in normal mice were found 4.54 ± 1.66 (mean ± SD) and arthritic mice uric acid level were found 7.95 ± 1.06 (mean ± SD). The elevated values of uric acid in arthritic mice were found significant as compared to the normal mice.

Vitamin D was estimated by ELISA method and values in normal and arthritic mice were found 26.34 and 25.59 nm/ml respectively. There was no significant change in vitamin D value in arthritic mice as compared to normal mice. (Table 2 and Fig. 5 and 6)

Table 2: Uric acid and vitamin D levels in normal and arthritic mice

	Uric Acid (mg/dL)	Vitamin D (ng/mL)
Normal Mice	4.54 ± 1.66	26.34 ± 13.23
Arthritic Mice	7.95 ± 1.06	25.59 ± 10.64

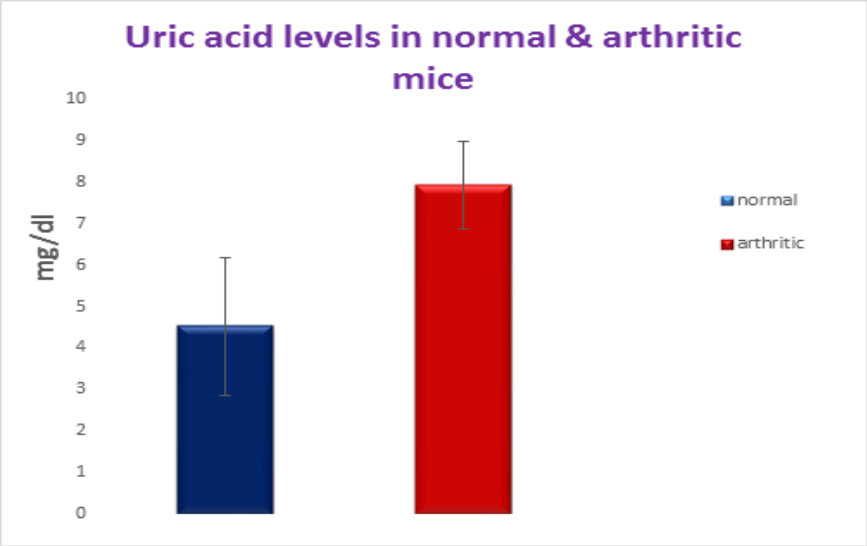

Figure 2: Assessment of Uric Acid levels in normal & arthritic mice

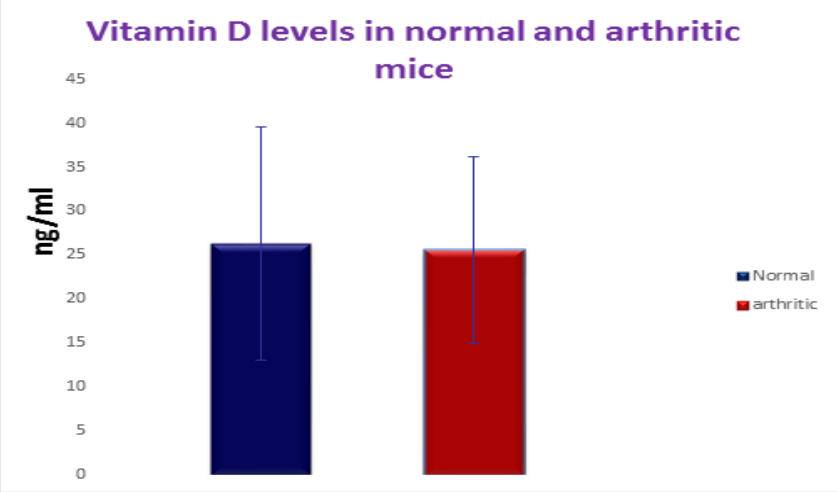

Figure 3: Assessment of Vitamin D levels in normal & arthritic mice

Rheumatoid Arthritis Factor

All the 5 Serum samples of normal mice showed positive reaction (mild agglutination), on the other hand among arthritic mice serum samples, three showed moderate agglutination reaction and two showed strong agglutination reaction.

	Sample	Agglutination reaction
Normal Mice	n-1	+
	n-2	+
	n-3	+
	n-4	+
	n-5	+
Arthritic Mice	a-1	++
	a-2	++
	a-3	+++
	a-4	++
	a-5	+++

From the results, we can conclude that during experimental arthritic condition, higher levels of serum Uric acid are observed while there was no change recorded in Vitamin D levels.

Discussion

This study was conducted for assessment of Vitamin D and Uric Acid level in arthritic mice and healthy mice, the finding of this study indicate higher levels of serum Uric acid in Arthritic mice (diseased condition).Vitamin D levels were not significantly changed in arthritic mice. The findings of this study indicated that neither the serum vitamin D levels nor the vitamin D deficiency in rheumatoid arthritis patients were significantly different from controls which is similar to a study done by Maurizio Rossini et al. involving 1,191RA patients and 1,019 controls. They reported that 55% of RA patients were not on vitamin D supplements, of these 52% patients had vitamin D deficiency (<20 ng/ml) as compared to healthy control (58.7%). In a study conducted by Turhano AD et al. too showed no significant difference in vitamin D levels between 65 RA cases and controls (17) and a study involving 108 RA cases and 239 healthy volunteers also showed no difference in the mean serum vitamin D levels between the two groups [18].

Uric acid is commonly raised in arthiritic conditions like gout and other inflammatory conditions of body [19]. Patients with RA with gout mostly are elderly men who have high RF titer and significant level of uric acid levels in serum than those suffering for only RA [20]. Uric acid is generally an end product of purine metabolism and it is raised in condition of raised non veg diets, excessive protein intake and pathological condition of excessive catabolism in cell or high cell turnover like posiarisis, tumors or other autoimmune conditions [21]. High plasma uric acid (UA) is a precipitating factor for gout and renal calculi as well as a strong risk factor for Metabolic Syndrome and cardiovascular disease [22].

Vitamin D is synthesised in our body at skin, liver and kidney level. Active and final step of its synthesis occurs in kidney hence a functional nephron is very important source of vitamin D. Vitamin D_3 (cholecalciferol) is synthesized in the skin by the reaction of 7-dehydrocholesterol with UVB radiation, present in sunlight with an UV index of three or more. First hydroxylation step occur in the liver by the vitamin D-25-hydroxylase (25-OHase) to convert vitamin D to 25(OH)D. However, 25(OH)D requires a further hydroxylation in the

kidneys by the 25(OH)D-1-OHase (CYP27B1) to form the biologically active form of vitamin D 1,25(OH)2D [23]. Deficiency of vitamin D has been incriminated in number of diseases but it role in RA is limited as proven in this study also. [24]

Conclusion

From the present study, we can conclude that;

(i) Higher levels of serum Uric acid are present in Arthritic mice (diseased condition).
(ii) There is no significant change in Vitamin D levels in Arthritic mice.

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