



## ACTION OF VITAMIN D ON SERUM LEVELS OF INTERLEUKIN -1 $\beta$ AND TUMOR NECROSIS FACTOR- $\alpha$ AMONG RHEUMATOID ARTHRITIS PATIENTS: A PROSPECTIVE STUDY.

### Medical Science

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### ABSTRACT

There are two types of functions of vitamin D. First one is skeletal and second one is non skeletal. Skeletal functions of Vit. D are mainly involved with the calcium metabolism and effector organs are kidney, gut and bone. Non- skeletal functions are evolutionarily more primitive but their importance has been realised very recently. Besides calcium metabolism Vit D is extensively studied for its beneficial effects on heart, diabetes, psoriasis, asthma etc.

The purpose of this study is to show whether Vit.D supplementation among diagnosed cases of Rheumatoid arthritis(RA) have any anti-inflammatory effect or not. For this purpose two inflammatory markers interleukin-1 $\beta$  (IL-1 $\beta$ ) and Tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) were estimated before and two months after supplementation of Vitamin D. The serum levels of both IL-1 $\beta$  and TNF- $\alpha$  were significantly lower after two months of Vitamin D supplementation ( $p < .005$  for both IL-1 $\beta$  and TNF- $\alpha$ ), indicating anti-inflammatory, systemic benefit of vitamin D supplementation.

### KEYWORDS

#### INTRODUCTION:

Although there is no established pathogenesis for the Rheumatoid arthritis (RA), currently the most acceptable theory portrays antigen-specific T-cells as the inciting agent for the recruitment of macrophages and synoviocytes which ultimately leads to the pannus formation and destruction of cartilage of the joint, soft tissue and subchondral bone(1). Some pauci T-cells variants (joint destruction without T-cell involvement) of RA also exists (2). But it is now well known that serum levels of inflammatory cytokines like Interleukin(IL-1) and Tumour Necrosis Factor- $\alpha$ (TNF- $\alpha$ ) increase in RA patients and these cytokines are related to the progression and remission of the disease (2,3).

Evolutionarily Vitamin D is one of the oldest hormone and it was first synthesized by the microorganisms- zooplanktons and phytoplanktons, and these organisms do not have the skeletal system(4). So, in the evolutionary point of view the first function of Vit D was not skeletal but it was non-skeletal. After its identification by Adolf Windaus various non skeletal functions of Vit D has been recognised by various authors and these non skeletal functions include protection from diabetes, cardiovascular accidents, asthma, psoriasis etc(5). One of the important extra skeletal function of Vitamin D is anti-inflammatory. The exact mechanism of anti-inflammation is not known but it is recognised that 1,25(OH)<sub>2</sub>D is an inhibitor of cell proliferation and an inducer of cell maturation (6,7). The mechanism of action of vitamin D is transcriptional and it acts on around 200 genes (8).

The purpose of this study was to show whether Vit.D supplementation among diagnosed cases of Rheumatoid arthritis(RA) who were on disease-modifying antirheumatic drugs (DMARDs) have any anti-inflammatory effect or not. There are various types of markers associated with inflammation in RA such as CRP, hepcidin and different cytokines. In this study two inflammatory markers interleukin-1 (IL-1) and Tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) were studied. There are two forms of IL-1,  $\alpha$  and  $\beta$ , and their functions are indistinguishable, for this study we estimated  $\beta$  form. We estimated the serum levels of IL-1 $\beta$  and TNF- $\alpha$  by ELISA before and two months after the Vit D supplementation.

#### MATERIALS AND METHODS:

The present study was conducted at the Department of Biochemistry, Patliputra Medical college, Dhanbad, Jharkhand during the period August 2018 and May 2019. Thirty cases of diagnosed rheumatoid arthritis, who were on treatment with various DMARDs but first time added with Vit D supplementation were taken for the study. Rheumatoid Arthritis cases were selected from the Department of medicine and Dept. of Biochemistry (referred to the Dept. of Biochemistry for investigations). All the cases were in the age group 25-45 years and females. As the disease prevalence is higher among

females, males were excluded from the study to avoid sex based mismatch.

The patients who have other diseases along with RA such as Hypertension, Diabetes mellitus, Tuberculosis, any chronic disease or on oral contraceptive pills were excluded from the study. RA patients with chronic kidney disease (eGFR less than 60) were also excluded. An informed consent was taken from all the cases before the participation to this study.

Blood samples were collected from the cases before and two months after the addition of supplementation of vitamin D3 (dosage 2000 IU/day or above) to their DMARDs. Taking all aseptic precautions, five ml of blood was drawn after peripheral venipuncture with a disposable syringe. The collected blood in plain vial were allowed to stand for 30 min at room temperature for the retraction of clot. Then samples were centrifuged at 3000 rpm for 10 min to separate the serum. Samples were stored at -20 degree C until assayed.

The micro ELISA plates for IL-1 $\beta$  and TNF- $\alpha$  coated with an antibody specific to IL-1 $\beta$  and TNF- $\alpha$  were used. Standards or samples were added then a biotinylated detection antibody specific for these two cytokines and avidin-horseradish peroxidase (HRP) conjugate was added and incubated. Free components were washed and absorbance was taken. Standard curve was plotted then serum levels of these cytokines were calculated.

#### RESULTS AND ANALYSIS:

All the statistical analysis was done by the software-R studio and R-language. Statistical parameters such as mean and standard deviation were calculated and data before vitamin D supplementation and after vitamin D supplementation were compared by using Welch's independent two sampled t-test for equality. All the important statistical parameters obtained from the study are shown in the fig. 1.

In this study we observed that the serum IL-1 and TNF-alpha levels were significantly decreased after vit. D supplementation in comparison to the levels before supplementation. The p values for IL-1 is 0.001 and for TNF-alpha is 0.003.

**Fig.1**

Paired Samples Statistics (pg/ml)					
		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	BS-IL-1	7.090	30	3.8986	0.7118
	AS-IL-1	5.807	30	3.3460	0.6109
Pair 2	BS- TNF- $\alpha$	15.830	30	7.5386	1.3764
	AS-TNF- $\alpha$	12.490	30	8.2827	1.5122

As there is significant decrease in inflammatory cytokines IL-1 and TNF- $\alpha$  after Vitamin D supplementation. It can be hypothesised from this study that Vitamin D supplementation may be used with DMARDs to decrease systemic inflammation and hence the severity of the disease in rheumatoid arthritis.

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