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# Can Vitamin D Slow Progression of Osteoarthritis?

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## Abstract

Osteoarthritis (OA) is a chronic and prevalent joint disease resulting in degenerative changes in the cartilage. Now-a-days vitamin-D is emerging as an important component which has a wide biological effects. The studies are evaluating the beneficial effects of vitamin-D in osteoarthritis therefore the present investigation was planned to analyze i) levels of vitamin-D in selected controls and OA patients, ii) monitor gene expression changes in CYP2R1, CYP3A4, CYP27B1, CYP24A1 and CYP27A1, whose products are involved in vitamin D metabolism. Our result shows that there was no significant difference in the vitamin-D levels in OA versus controls. The mean vitamin D levels in controls was 35.9 ng/ml (3 had ViD<20g/ml) and in OA patients was 35.66 ng/ml (3 had Vit D<20ng/ml). However gene expression of CYP2R1, CYP3A4 was reduced CYP24A1, CYP27B1 showed no variation in expression and CYP27A1 was upregulated in OA patients as compared to control. We could not observe significant difference in the levels of vitamin D in control and patient showing that onset of primary OA may not be because of vitamin D deficiency or vitamin D may not be responsible for symptoms of OA. However its supplementation may have therapeutic benefits to all including control and patients as vitamin D levels are not optimum in both. Lower gene expression of cytochrome p 450 genes suggest some effects on OA patients but these are related to age or post-menopausal stage or OA is not clear as in our study we were unable to obtain primary OA patients without any comorbidity with <55 years of age. OA in less than 55 years are mostly associated with comorbid conditions as diabetes, hypertention, thyroid, obesity, chronic gastrointestinal disturbance, kidney or liver disease, with trauma etc.

**Keywords:** Osteoarthritis; Vitamin D; Cytochrome P 450

**Abbreviations:** OA-Osteoarthritis; CYP-Cytochrome P 450; BMI-Body mass index

## Introduction

Vitamin D has an important role in calcium and bone metabolism. It is emerging as an important component having diverse biological actions in a number of target tissues. Vitamin $D_3$ is the endogenous form produced by humans. Photoisomerisation of 7-dehydrocholesterol (DHC) by ultraviolet B (UVB) radiation in cutaneous tissues produces previtamin D [1]. Previtamin D forms vitamin $D_3$ [cholecalciferol] by thermal-dependent isomerization [2]. This cholecalciferol is biologically inactive and needs two sequential hydroxylations to achieve its active form. Therefore cholecalciferol binds to vitamin D binding protein (DBP) and is transported to the liver.

Main circulating form, 25-hydroxyvitamin D (25-OH-D3) is produced by sequencial 25-hydroxylations and then 1α,25-dihydroxyvitamin D3 (1,25-(OH)2$D_3$)[3]. The initial reaction of 25-hydroxylation is primarily managed by cytochrome P450 enzyme as CYP2R1 in the liver [4]. Another hydroxyl group is added to the 1st carbon in kidney as well as other extrarenal sites by CYP27B1 [5-7] resulting in the production of biologically active 1a,25-dihydroxyvitamin D3 (calcitrol;1α,25$(OH)_2D_3$). The 25$(OH)D_3$ form is normally measured in serum to determine vitamin D levels in patients. All of the vitamin D-related CYPs catalyze single or multiple hydroxylation reactions on specific carbons of the vitamin D substrate using a transient, heme-bound, oxygenated-iron (Fe-O) intermediate. Amongst the 25-hydroxylases as CYP2R1, CYP27A1, CYP3A4 and CYP2D25, the most relevant physiological enzyme is microsomal CYP2R1 [8]. Microsomal CYPs as CYP2R1 requires a single general-purpose protein, NADPH-cytochrome P450 reductases.

Osteoarthritis is degenerative and progressive joint disease which normally affects weight bearing joint [9]. Inflammation is now emerging as an important factor in the pathogenesis of OA leading to loss of cartilage [10-11]. Evidences suggest that OA not only affects articular cartilage but also affects sub chondral bone and synovial membrane lining in the joint [9].The deficiency of vitamin D and development and progression of OA is controversial. The literature has conflicting data with regard to vitamin D deficiency and progression of OA and vitamin D supplementation and relief from pain in OA patients. Therefore the present study was undertaken to access the levels of vitamin D in control and primary OA patients without any comorbidity and analysis of expression of vitamin D metabolism related genes.

## Materials and Methods

### Patient selection for gene expression profiling

The study was started after approval from Institutional Ethical Committee and written informed consent was obtained from all participants. 30 patients with OA (15F and 15M) with average age of 59.96 years were recruited from health centres. 30 controls (17F and

13M) with mean age 50.36 years were selected from neighborhood and offices who were independent from patients. All the controls and patients were non-hypertensive (blood pressure 120-130/78-90), non-diabetic and without anycomorbidity. Of these, 3 patients (all females) with knee OA and 3 asymptomatic independent controls (all females) were selected for gene expression analysis.

OA patients were screened according to radiological grading [12]. VAS score and ACR classification was followed for classification of OA [13]. Patients with OA were included who had grade II-III OA, knee pain (asymmetrical) of more than 6 months, stiffness (from 5-15 min), swelling, crepitation, tenderness on medial side of joint, X-ray had >1/3 decrease in joint space and/or presence of osteophytes and decreased range of motion in their knee joint. Their ligament stability (anterior cruciate, posterior cruciate) was normal. The participants who had comorbidty (trauma, any other disease of joint, smokers and obese) were excluded. Participants were matched for sex, weight and height (body mass index). None of the controls and patients had any comorbid disease.

Vitamin D (25-hydroxy vitamin D) was analyzed by ELISA on luminometer using commercial kits.

### RNA extraction and microarray analysis

Total RNA was extracted from whole blood with Qiagen RNA extraction kit according to manufacturer's instructions. Concentration of RNA was measured spectrophotometrically and its integrity was checked by agarose gel electrophoresis. This was used as a starting template to synthesize double-stranded cDNA with random hexamers tagged with T7 promoter sequence.

The fragmented DNA was labeled and used for overnight hybridization with Gene ST 1.0 arrays, followed by washing staining and scanning.

Microarray analysis was done by using Affymetrix Human Gene 1.0 ST arrays. The data QC and RMA normalization was performed for the arrays as recommended by Affymetrix. A fold change of ± 1 was used to select up and down regulated probe sets. The QC analysis was carried out using Affymetrix Expression Console (EC). The statistical analysis was performed using R-programming language and the biological analysis was carried out using GenowizTM software.

## Results

Vitamin D is emerging as a potential component affecting functioning of many metabolic pathways, thus has important role in various diseases.

| | Control (N=30) | Osteoarthritis patient (N=30) |
|---|---|---|
| Age | 50.36 ± 0.63 | 59.96 ± 0.79 |
| M/F | 13/17 | 15/15 |
| BMI | 23.14 ± 0.66 | 23.6 ± 0.83 |
| Vitamin D (ng/ml) | 35.9 ± 2.22 | 35.66 ± 2.00 |

**Table 1:** Shows the demographic parameters studied and vitamin D levels in control and patients. There is no significant difference in vitamin D level in control versus OA group. (N-number of participants; M-Males; F-Females; BMI-Body mass index).

In our study the mean serum levels of Vitamin D were 35.66ng/ml in OA patients and the levels were 35.9 ng/ml in controls. Only 3 OA patients and controls had vitamin D level <20ng/ml.

The minimum and maximum value of Vitamin D in controls ranged from 12 to 55 respectively whereas OA patients had values from 18-50. In our study we could not find significant difference in vitamin D levels in controls and patients (p>0.05) (Table 1).

The gene expression of CYP2R1, which is microsomal CytP450 in liver and testis is Vit D3-25 hydroxylase is downregulated in OA as compared to control (Figure 1). CYP3A4 expression whose protein product -24 and -25 hydroxylate Vit D2 more efficiently than D3 substrates is also downregulated. The expression of other cytochromeP450 enzymes as CYP27B1 and CYP24A1 were not altered in OA patients as compared to control (Table 2 and figure 1). Significantly upregulated gene expression was observed for CYP27A1 which is liver mitochondrial cytP450 and is better cholesterol 26-hydroxylase.

| Genes | Gene symbol | Accession number | Fold change |
|---|---|---|---|
| Cytochrome P450 | CYP2R1 | NM_024514 | -0.94 |
| | CYP3A4 | NM_017460 | -0.70 |
| | CYP24A1 | NM_000782 | 0.0 |
| | CYP27A1 | NM_00784 | +2.53* |
| | CYP27B1 | NM_000785 | 0 |

**Table 2:** Shows the gene expression of cytochrome p450 genes involved in vitamin D biosynthetic pathway along with their excession numbers and fold change expression.* represent significant change (p<0.05)

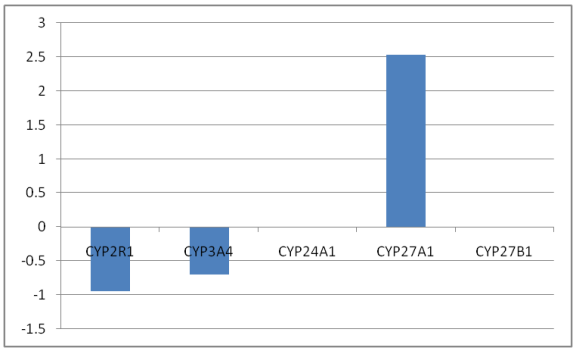


**Figure 1:** The figure shows the fold change in expression of genes involved in vitamin D metabolism in osteoarthritis versus control. CYP2R1, CYP3A4, CYP24A1 and CYP27A1 are 25-hydroxylases while CYP27B1 is 1-hydroxylase. CYP2R1 and CYP3A4 are non significantly down regulated. There is no change in the expression of CYP24A1 and CYP27B1 whereas significantly high gene expression was observed in CYP27A1 (p<0.05) in OA patients as compared to control.

## Discussion

In our study 25-hydroxylases as CYP2R1 and CYP3A4 were non-significantly downregulated. CYP2R1 is the important 25-hydroxylase.

CYP3A4 has been shown to be selectively induced by 1,25-$(OH)_2D_3$ in the intestine [14-16]. It 23R- and 24S- hydroxylates the already existing 25-hydroxyltes 1,25-$(OH)_2D_3$[14]. CYP3A4 was reported in drug-drug interactions involving Vit-D, in which coadministered drug causes accelerated degradation of Vitamin $D_2$ over $D_3$. These are involved in 25-hydroxylation of vitamin D. The expression of another 25-hydroxylase CYP27A1 was significantly higher. There may be a possibility that if one of the prime 25-hydroxylase is compromised, the alternative hydroxylase as CYP27A1 is higher. CYP27A1 which was highly upregulated in OA was considered to be D-25-hydroxylase [17], however it was found to be controversial as purified liver enzyme seemed to be better cholesterol-26-hydroxylase than VitD-25-hydroxylase and was proposed as bifunctional enzyme involved in bile acid and vitamin D metabolism [18]. CYP27A1 seems to be involved in the metabolism of Vit-D compounds as 1α-OH-$D_3$ and 1α-OH-$D_2$, when present at high concentration. The gene expression of CYP27B1 and CYP2D25 were not affected in OA as compared to control.

There could be another possibility that all patients choosen for gene expression study were elderly post-menopausal females. The exact involvement of hormones as estrogen and menopausal stage on OA and vitamin D needs to be established. An Egyptian study on newly diagnosed post-menopausal women showed lower $25(OH)D_3$ in their serum and was associated with knee OA when compared to healthy males [19]. The patients choosen in our study were only those who had only osteoarthritis without any co-morbid conditions. OA in less than 55 years are mostly associated with comorbid conditions as diabetes, hypertention, thyroid, obesity, chronic gastrointestinal disturbance, kidney or liver disease, with trauma etc and were therefore excluded from the study.

Blood concentrations of vitamin D over the range of 30 to 50 nmol/L are required for optimal bone health [20]. In the few studies that prospectively examined predictors of change in 25(OH)D, predictors included measures of body size [21,22], physical activity [21], vitamin D intake and exogenous estrogen dose [23]. Studies suggest that vitamin D may have implication on the radiographic manifestations of OA [24,25] or cartilage loss in knee joint [26]. Vitamin D have been shown to be associated with progression of knee OA [25,27].

A Framingham study25 has shown that people with low and suboptimum vitamin D levels have 3 fold increased risk of developing progressive knee OA whereas females are more likely to develop severe OA than males [28]. The increased incidence of OA in females during pre-post menopause has led researchers to speculate the role of estrogen however, conflicting results are obtained by observational studies with endogenous or exogenous estrogen on OA [29-31]. The study by Sanghi et al. [32] suggest that there is small statistically significant clinical benefit to vitamin D treated patients with knee OA. However their study was done on patients who had vitamin D deficiency. A study in UK reported that 24% of elderly OA patients had deficient vitamin D levels (<40nmol/L (~16ng/ml) according to the National Diet and Nutrition Survey definition) [33]. Ireland study showed 70% vitamin D deficient (<21 ng/mL) and 26% severely deficient (<12 ng/mL) patients in rheumatology outpatient [34].

An important observation is that different cell types present inside osteoarthritic joint may experience negative effects at increased sensitivity to vitamin D[9]. Vitamin D is known to play a crucial role in the behaviour of metabolic processes in bone on which it has a range of effects in the pathophysiology of OA [9]. OA osteoblast proliferation is positively affected by vitamin D treatment [35]. However as there is no difference in the vitamin D level in control and patients therefore it is difficult to establish role of vitamin D in onset or progression of OA. A 22 year follow up study of 805 Finns revealed that serum levels of $25(OH)D_3$ were not associated with hip or knee OA incidences [36] supporting an earlier study where low vitamin D was not found to increase the risk of developing knee OA [25]. Konstari et al. studied a large cohort of 5,274 OA-free participants over a period of 10 years and observed that low serum $25(OH)D_3$ levels were not associated with higher risk of developing OA of knee or hip OA [37]. However, contrasting results are reported where low serum vitamin D was found to be associated with OA. Part study of Osteoporosis Fractures in Men in the US observed a high prevalence of vitamin D deficiency in patients with OA of hip and concluded that they were twice as likely to have hip OA [38]. An Iranian study also reported a stronger association between knee OA and serum $25(OH)D_3$ in younger participants and positive association in patients under 60 years of age [39]. Patients having lower vitamin D levels and low BMD were associated with increased radiographic knee OA incidences [27]. Cao et al. performed systemic review analysis and found 15 studies providing strong evidence for an association between vitamin D levels and cartilage loss in knee joint and moderate evidences in favor of positive association between $25(OH)D_3$ and radiographic changes in knee OA [26]. Low dietary and low serum vitamin D levels may be associated with the progression of OA of knee [25,27]. Ding et al. in their study as part of the Tasmanian Older Adult Cohort Study found both sunlight exposure and vitamin D levels to be associated with decreased knee cartilage loss [40]. Felson et al. found no association between worsening of joints affected with OA and lower vitamin D levels by magnetic resonance imaging done on two longitudinal studies with [41].

Serum 25-hydroxyvitamin D3 are suggested to be associated with progression of knee osteoarthritis as it plays an important role in maintaining quality of bone [24,25] as persistent deficiency may change line of force leading to deformity and probably plays a role in development of OA. Vitamin D, calcium and physical activity are considered to be the major contributors to bone health for individuals of all ages In our results we found that there is no statistical significant difference in vitamin D levels in control and OA suggesting that vitamin D deficiency may not be responsible for onset of OA, however control group who had lesser vitamin D levels reported pain in spine and hands, probably due to decrease in bone quality. Though reports suggest that nearly 1 billion people have vitamin D deficiency and insufficiency[20] but association of vitamin D with OA remains controversial [20,25,27,39,42]. Another interesting fact is the percentage of population which is deficient in vitamin D may not develop OA. Another important observation in our study was while recruiting patients only with knee OA the younger patients (age 40-55 years) had some or the other comorbid condition (as described in materials and methods). In only older patients (>55years) primary OA without any comorbidity was observed therefore we excluded the patients where OA was present with any other comorbid condition. These all studies suggest that initial vitamin D deficiency may not lead to onset or progression of OA but lead to problems in bone quality, multiple joint movement, knee alignment and laxity but in long run persistent deficiency of vitamin D may result in onset of OA in the weight bearing joint due to sub-optimal bone quality. The existence of any other clinical condition and vitamin D deficiency may trigger the onset of OA but vitamin D deficiency as the only confounding factor for development of OA needs to be analyzed in controlled randomized trials with huge selected cohort.

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