Low levels of Vitamin D are associated with non-alcoholic fatty liver disease.

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KEYWORDS

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ABSTRACT

Background: Vitamin D is the modified steroid synthesised in the skin under the influence of sunlight, nutritionally important form in humans are ergocalciferol and cholecalciferol. Both of them are considered to be provitamins and the active form of vitamin D is synthesised from them. Specific transport protein called vitamin D- binding protein binds cholecalciferol and moves it from skin to intestine and to the liver where it gets hydroxylated at twenty fifth position and forms 25-hydroxycholecalciferol. NAFLD and vitamin D insufficiency are frequently linked, and the two have even been linked to the severity of the illness. Vitamin D's anti-inflammatory, anti-fibrotic, and metabolic qualities offer tenable ways that it could influence the different stages of liver disease progression and severity.

Material and method: The present study was carried out in the Department of Biochemistry at Index medical college hospital and research centre in Indore, Madhya Pradesh. A total of 246 subjects were selected for study. Out of 125 were having normal echotexture of liver and121 were having NAFLD. Informed consent was taken from all the participants included in the study.

Results and conclusion: In the present study there is association between NAFLD & serum levels of liver enzymes. Also there is a significant association between random blood sugar levels and HbA1c levels. And Vitamin D levels in both NAFLD (21.96±13.060 and NON-NAFLD group (28.72±23.33) is insufficient which indicates Vitamin D deficiency is quite rampant in Indore region of Madhya Pradesh, India. There are many reasons for it being so common in our country. Increased indoor lifestyle, thereby preventing adequate exposure to sunlight. This is mainly in the urban population due to modernization and the present study concludes that there is a relationship between serum vitamin D levels and a higher risk of NAFLD.

Introduction:

The first stage of NAFLD, known as non-alcoholic fatty liver, is characterized by the buildup of excess liver fat without excessive alcohol use or a secondary cause. Patients are diagnosed when there is observable lipid accumulation in at least 5% of their hepatocytes; however, the absence of distinctive symptoms makes diagnosis difficult [1,2]. Poor hepatocyte metabolism, specifically from excessive fatty acid (FA) uptake, is the cause of nonalcoholic fatty liver disease (NAFLD) [3]. Reduced VLDL synthesis and secretion by hepatocytes, increased de novo lipogenesis, or decreased fatty acid oxidation are additional potential causes [3,4]. NAFLD has a complicated and multifaceted etiology. There are theories that explain how NAFLD develops and progresses. The "two-hit hypothesis" states that the liver is sensitized to the first hit and is prepared for the second hit by factors like obesity, high-fat diets, insulin resistance (IR), and hepatic lipid accumulation from a sedentary lifestyle. The second hit triggers fibrogenesis and inflammatory reactions. However, the "multiple-hit hypothesis" was created because this theory is insufficient to explain the different molecular and metabolic alterations in NAFLD. This hypothesis takes into account a number of effects, including insulin resistance, adipose tissue hormones, nutritional factors, gut microbiota, and genetic and epigenetic factors that combine to cause NAFLD in genetically predisposed individuals [5]. Because non-alcoholic fatty liver disease (NAFLD) is closely linked with metabolic diseases including obesity and type 2 diabetes (T2D), its prevalence is estimated at almost 25% worldwide. [6] Though other chronic diseases, including sleep apnea, colorectal cancers, osteoporosis, psoriasis and various endocrinopathies (e.g., polycystic ovary syndrome), NAFLD is also under increasing evidence to be connected to. [7]

Endogenous synthesis of vitamin D is possible. Ergocalciferol (vitamin D2) comes from plants, while cholecalciferol (vitamin D3) comes from animals. [8] When exposed to sunlight, the skin produces about 90% of the necessary vitamin D. [9] Pre-vitamin D3 is created in the plasma membrane of epidermal cells by UV-B photons reacting with pro-vitamin D3, a precursor in the cholesterol biosynthesis pathway. Pre-vitamin D3 is quickly converted to vitamin D3 and moved to the extracellular space, where it attaches itself to a protein called vitamin D-binding. [10] It is then taken to the liver, where it undergoes hydroxylation to form 25(OH)D. [11] Following exposure to UVB rays, skin epidermal cells can transform a cholesterol-like precursor (7-dehydrocholesterol) into pre-vitamin D, which subsequently isomerizes to vitamin D3. Vitamins D3 and D2 have no biological activity. To become its active forms, they require additional enzymatic conversion. The primary circulating form of vitamin D, 25(OH)D (calcidiol), which has a half-life of two to three weeks, is first produced by 25-hydroxylation in the liver. After that, it undergoes 1-alpha-hydroxylation in the kidneys to become 1,25(OH)2D (calcitriol), which is its most active form and has a half-life of 4 to 6 hours. Growth hormone, hypophosphatemia, and parathyroid hormone (PTH) are some of the mediators that drive this process. [12,13] It is well known that vitamin D controls the metabolism of calcium and phosphate. Normal calcium and phosphate levels in the blood are necessary for bone mineralization, muscle contraction, nerve transmission, and overall cellular function in every cell in the body. It is a hormone that regulates inflammation, cell division, and proliferation in addition to being vital for the maintenance of a healthy mineralized skeleton. [9, 10, 14, 15] Via the nuclear vitamin D receptor (VDR), the active form of vitamin D promotes calcium absorption in the duodenum and raises calcium influx in the kidney's distal tubules; the latter is particularly controlled by parathormone levels. [16] It is estimated that thirty to fifty percent of people are vitamin D deficient, and both vitamin D deficiency and insufficiency are acknowledged as global health concerns. [17]

Material and method:

This cross-sectional study was conducted at the department of biochemistry, index medical college. Participants for the study was chosen from both IPD and OPD, based on inclusion and exclusion criteria. Diagnosis of fatty liver disease was based on ultrasonography evidence of fatty infiltration of hepatocytes. Both males and females undergone upper abdominal ultrasonography were recruited for the study. Informed consent was obtained after explaining the study procedures and outcomes and privacy of data was maintained throughout the study duration. Sample size for the study was (n=246), out of which 121 participants were diagnosed with NAFLD and 125 participants are having normal echotexture of liver.

We have included all 246 participants (NAFLD and NON-NAFLD group) and their Random blood sugar, HbA1c, liver function test and Vitamin D levels were investigated.

Ethical clearance: Ethical and research committee of Index medical college hospital and research centre in Indore, Madhya Pradesh, gave approval to the research (***MU/Research /EC/Ph. D/2020/001: Dated Nov28, 2020***)

Inclusion criteria:

- Age >18 years
- People who consented to participate in the study

Exclusion criteria:

- Hypercalcemia
- Kidney disease
- Malabsorption
- Prior diagnosis of liver disease
- Medical conditions requiring daily use of calcium, antacids, or medications known to affect bone metabolism or interact with vitamin D

Statistical analysis:

All estimated results were expressed as mean ±SD. Mean values will be assessed for significance by unpaired student –t test. A statistical analysis will be performed using the Statistical Package for the Social Science program (SPSS, 24.0). Frequencies and percentages will be used for the categorical measures. Correlation between them was done by Karl Pearson's correlation coefficient method and Probability values $p < 0.05$ will be considered statistically significant.

Result:

Table 1: Characteristics of study population (n=246)

Variables		No. of subjects
Age	18-30 yrs	50
	31-60 yrs	156
	>60 yrs	38
Sex	Male	110
	Female	136
BMI	18.5-22.5 Kg/m^2	74
	23-24.9 Kg/m^2	60
	>25 Kg/m^2	112

Figure 1: Age wise distribution

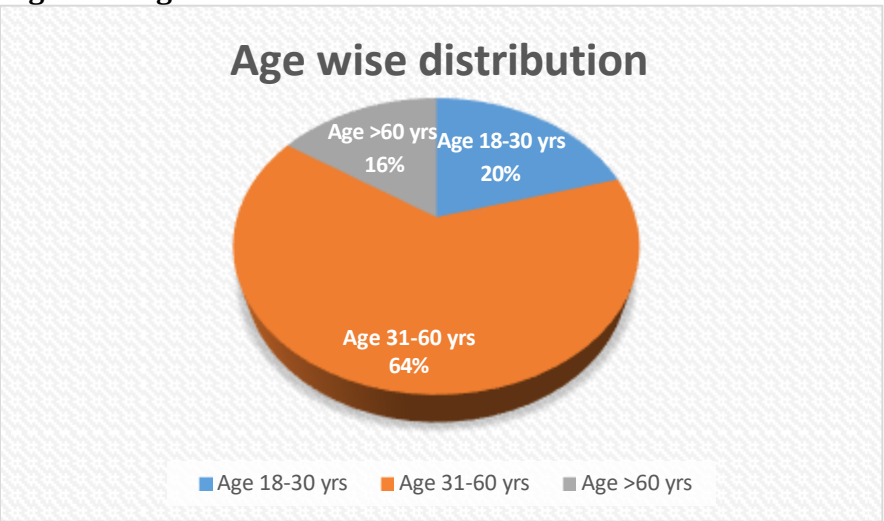

Figure 2: Gender wise distribution.

Figure 3: BMI wise distribution.

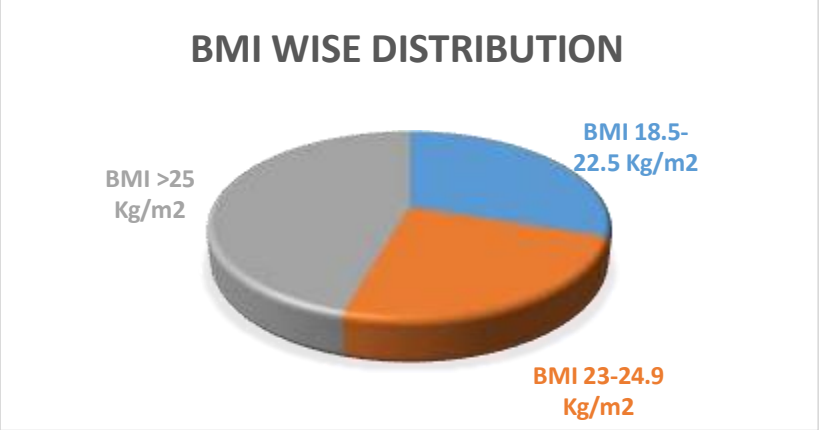

Table 2: NAFLD status in study population (n=246)

Variables		NAFLD Status	
		Yes	No
Age	18-30 yrs	20	32
	31-60 yrs	86	70
	>60 yrs	15	23
Sex	Male	43	67
	Female	78	58
BMI	18.5-22.5 Kg/m^2	22	52
	23-24.9 Kg/m^2	37	23
	>25 Kg/m^2	62	50

Figure 4: NAFLD status in different age groups.

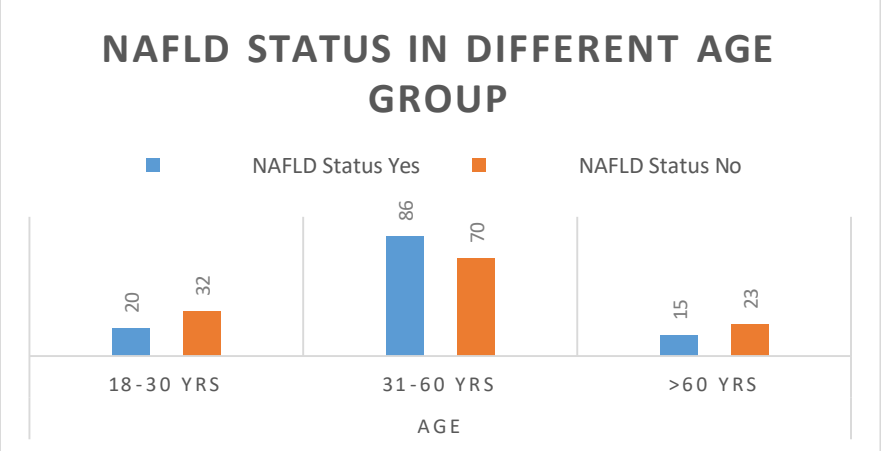

Figure 5: NAFLD status in different gender.

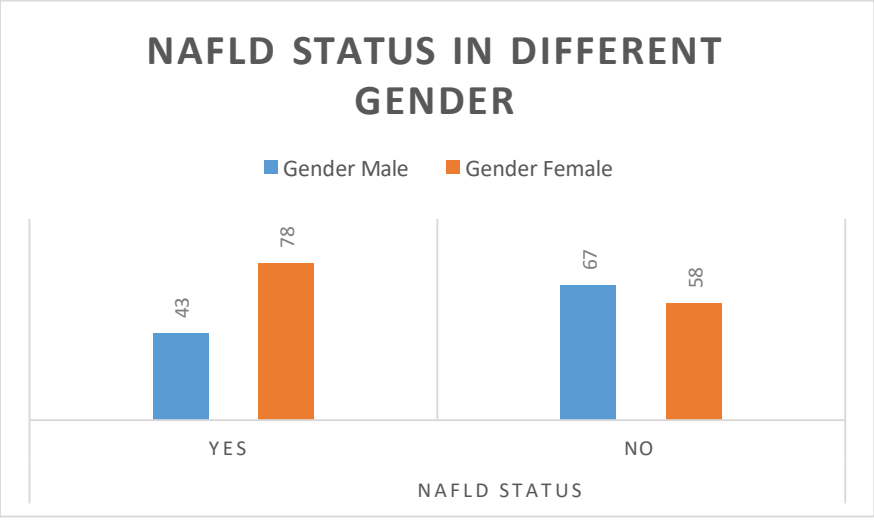

Figure 6: NAFLD status according to BMI.

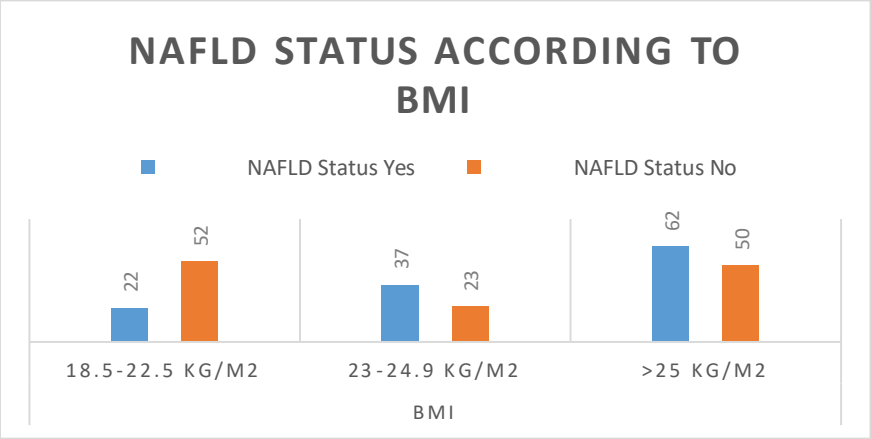

Figure 7a: Assessment of vitamin D deficiency in NON-NAFLD group.

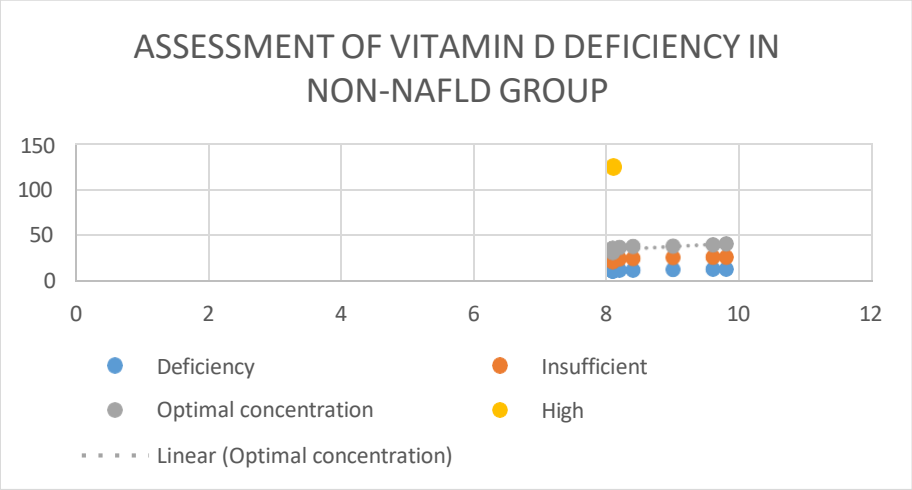

Figure 7b: Assessment of vitamin D deficiency in NAFLD group.

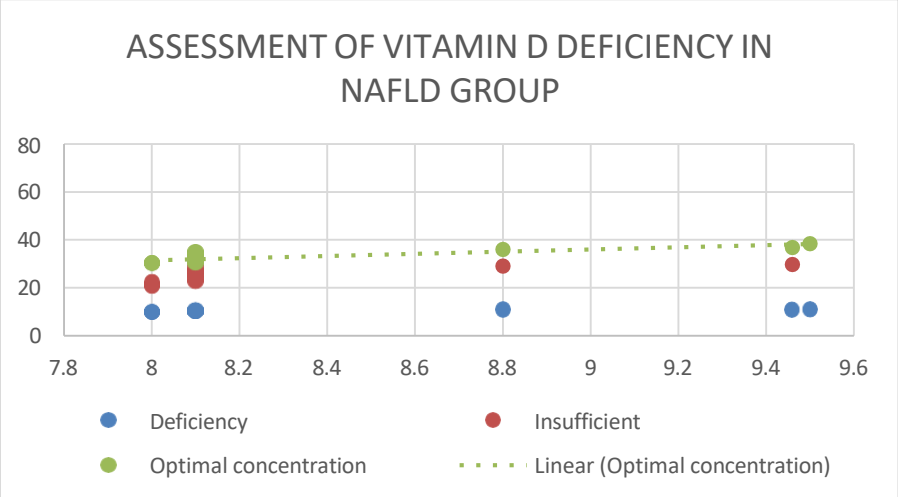

Figure 7a and 7b: Above graph have assessed the vitamin D deficiency criteria as the optimal blood concentration of >30ng/ml; 20-29ng/ml is considered as insufficient and 10-19ng/ml is vitamin D deficiency. A level below 10ng/ml indicates severe deficiency. Concentrations more than 150ng/ml is toxic.

Table 3: Chi-square test is performed to find out the association between NAFLD & serum levels of liver enzymes.

Variables	Mean±SD (NAFLD Group)	Mean±SD (Non-NAFLD Group)	t-value	p-value
ALT	70.107±31.760	26.776±20.002	12.847	<0.0001
AST	112.60±88.78	24.99±14.54	10.884	<0.0001
ALP	130.82±74.66	71.152±32.365	8.177	<0.0001
Total Protein	7.406±1.534	6.464±0.78	6.099	<0.0001
Albumin	4.07±1.028	3.62±0.72	3.987	=0.0001
Globulin	3.33±0.765	2.83±0.82	4.941	<0.0001
Random blood sugar (RBS)	121.54±24.125	160.05±48.01	7.987	<0.0001
HbA1c	5.33±0.497	6.13±1.207	6.836	<0.0001
Vitamin D	21.96±13.06	28.72±23.33	2.791	<0.0001

Table 3: Chi-square test is performed to find out the association between NAFLD & serum levels of liver enzymes and there is strong association between NAFLD and hepatocellular enzymes. Also there is a significant association between random blood sugar levels and HbA1c levels. And Vitamin D levels in both NAFLD and NON-NAFLD group is insufficient which indicates Vitamin D deficiency is quite rampant in Indore region of Madhya Pradesh, India. There are many reasons for it being so common in our country. Increased indoor lifestyle, thereby preventing adequate exposure to sunlight. This is mainly in the urban population due to modernization.

Discussion:

The prevalence of non-alcoholic fatty liver disease (NAFLD) has been rising recently as a result of rising obesity rates. The pathological cause of both type 2 diabetes and non-alcoholic fatty liver disease is insulin resistance (IR). Because pancreatic beta cells express the vitamin D receptor (VDR), vitamin D regulates insulin secretion and has anti-inflammatory properties that help prevent insulin resistance.[18]

Furthermore, vitamin D uses its constitutively expressed VDR receptor to mediate its intracellular signals. Over 200 genes related to inflammation, cellular proliferation and differentiation, apoptosis, and glucose and lipid metabolism are thought to be regulated by VDR. There was a significant correlation between NAFLD and four single nucleotide polymorphisms (SNPs). The GC gene, which codes for the primary carrier protein for vitamin D and is primarily expressed in hepatocytes, was included among these four SNPs. Additionally, we know from animal research that VDR's normal function is essential for liver fibrosis because VDR knockout mice develop spontaneous liver damage and fibrosis.[19]

Globally, non-alcoholic fatty liver disease (NAFLD) is currently the leading cause of chronic liver disease. MicroRNAs (miRNAs) are one of many signalling molecules involved in the complex molecular pathophysiology of non-alcoholic fatty liver disease (NAFLD). Hepatocellular carcinoma, fibrosis, and inflammation are all linked to dysregulation of miRNA expression. The function of vitamin-D-regulated miRNAs in NAFLD pathogenesis has not received much attention, despite the fact that miRNAs are also essential for the cellular response to vitamin D, mediating regulation of the vitamin D receptor and vitamin D's anti-cancer effects.[20] Vitamin D is known to have anti-inflammatory properties and to be a significant modulator of insulin sensitivity by causing the release of insulin by pancreatic β cells and adiponectin by adipocytes.[21] It's deficiency may contribute significantly to the development of non-alcoholic fatty liver disease (NAFLD), in part by inhibiting its anti-

inflammatory attributes. Additionally, vitamin D alleviates FFA-induced insulin resistance in vitro by directly regulating the metabolism of FFAs through its action on the peroxisome proliferator-activated receptor (PPAR-γ). Therefore, elevated FFAs in the bloodstream may encourage hepatocyte fat deposition and the advancement of non-alcoholic fatty liver disease (NAFLD) in the presence of inadequate vitamin D. [22] Furthermore study conducted by ElhamEhrampoush et al. based on the results,745 patients (34.5%) had different degrees of fatty liver. Significant differences in the stiffness of liver tissue were observed between vitamin D categories (285.10±30.56 for severe, 251.82±42.63 for moderate and 201.02±36.08 for mild deficiency). According to the multivariate analysis, age, fasting insulin and vitamin D levels were found as the most significant factors in NAFLD pathogenesis. Vitamin D cut off point was obtained 18nmol/L in women and 21nmol/L in men. And their study concludes that there is significant association between vitamin D level and NAFLD score. Accordingly, increasing the public awareness to maintain a proper level of vitamin D may be a preventative strategy against NAFLD.[23] Similarly another study conducted by Claudia Della Corte, Guido Carpino et.al and showed that DHA plus vitamin D treatment reduced the NAFLD Activity Score (NAS), in the treatment group (5.4 v1.92; $p<0.001$ for baseline versus end of study). There was no change in fibrosis score, but a reduction of the activation of hepatic stellate cells (HSC) and fibrillar collagen content was noted (3.51±1.66 v. 1.59±1.37; $p = 0.003$) in treatment group. Moreover, the triglycerides (174.5 vs. 102.15 mg/dl), ALT (40.25 vs. 24.5 UI/l) and HOMA-IR (4.59 vs. 3.42) were all decreased with treatment. And they concluded that DHA plus vitamin D treatment improved insulin-resistance, lipid profile, ALT and NAS.[24]. Above studies supports our study as vitamin D levels in NAFLD group was (21.96±13.06) and in non-NAFLD group it was (28.72±23.33). Vitamin D levels in both NAFLD and NON-NAFLD group is insufficient which indicates Vitamin D deficiency is quite inadequate in the study population.

Conclusion:

NAFLD and vitamin D deficiency are frequently linked, and the two have even been linked to the severity of the disease. Vitamin D's anti-inflammatory, anti-fibrotic, and metabolic qualities offer tenable ways that it could influence the different stages of disease progression and severity. Together, these findings imply that vitamin D replacement therapy may be useful in the management of non-alcoholic fatty liver disease. According to our findings, there was an inverse relationship between serum vitamin D levels and a higher risk of NAFLD. To lower the risk of NAFLD, patients with hypovitaminosis D may benefit from taking additional vitamin D supplements furthermore higher vitamin D status is protective against various cancers, prediabetes and metabolic syndrome.

Key points:

- Production of vitamin D in the skin is directly proportional to exposure of sunlight and inversely proportional to the pigmentation of the skin. Advance in age and presence of melanin will decrease the formation of Vitamin D3 on skin.
- Nutritional deficiency
- Deficiency is common in obese people, because vitamin is stored in adipose tissue and not released for utilization leading to deficiency.
- Commonly seen in peoples who are not exposed to sunlight properly.
- High phytate content in diet may also reduce the absorption of the vitamin.
- Chronic alcoholics due to nutritional deficiency.
- Prolonged treatment with anticonvulsant drugs.
- Renal and liver disease may retard the hydroxylation reactions to its active form.

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Ethical standard:
1) This material is the authors' own original work, which has not been previously published elsewhere.
2) The paper is not currently being considered for publication elsewhere.
3) The paper reflects the authors' own research and analysis in a truthful and complete manner.
Funding support: Nil
Conflict of Interest: Nil
Ethical Approved: Approved
Informed consent: Informed consent was taken for all the participants who was willing to take part in study.
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