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Evaluation of Vitamin D Status in Patients with Epilepsy

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Abstract

Background: Patients with epilepsy face an even greater risk of vitamin D deficiency than healthy population, as seizures may interfere with their ability to move outdoors and remain active which limits the exposure to sunlight necessary for producing vitamin D.

Objective: Correction of vitamin D deficiency is an issue of concern and attention in patients with epilepsy. The present study was undertaken to understand and analyze the effect of vitamin D deficiency in epileptic patients.

Methods: Epileptic subjects with an age range of 3 to ≤ 80 years of both gender, below poverty line category, physically active patients on anti-epileptic drug treatment regularly, attending epilepsy clinic of the Super Specialty Hospital Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati were included in the study. Blood samples were collected from selected subjects after informed consent and their willingness to be a part of the study and samples were analysed for parathormone and 25- (OH)₂ D₃ levels.

Results: The present study was undertaken to evaluate the effect of vitamin D deficiency in epileptic patients. The mean serum Parathormone levels were found to be higher in epileptic males whereas females showed high prevalence of vitamin D deficiency.

Conclusions: The present study revealed that both epileptics and non- epileptics have vitamin D deficiency. In both the groups the number of subjects with normal vitamin D levels is very less, compared with vitamin D deficiency and insufficiency. In both males and females of epileptic and non-epileptic groups, when categorized based on 25(OH)₂ vitamin D₃ levels, the Parathormone levels were inversely proportional to 25(OH)₂ vitamin D₃ levels. Females have high prevalence of vitamin D deficiency than males.

Keywords: Epilepsy, Vitamin D deficiency, Parathormone levels, 25- (OH)₂ D₃ levels and anti-epileptic drug treatment.

1. Introduction

Vitamin D is unique with essential metabolic roles in the maintenance of calcium and phosphorus homeostasis and absorption, cell differentiation, functional maintenance of membranes etc. (Bonner and Stein, 1995) [3]. Its availability in the body largely depends on its synthesis in the skin when exposed to sunlight and thus, its dietary requirement is usually very small especially in the Indian context. Deficiency of this vitamin leads to abnormal homeostasis resulting in defective mineralization of the growing skeletal system (rickets) or decrease in the mineral content bone matrix (osteomalacia) resulting in weak bones (Mahan and Krause's, 2000; Michael *et al.*, 2003; ICMR, 2010) [21, 22, 20].

Brain requires the use of various neurosteroids viz., thyroid hormones, and glucocorticoids to develop and function efficiently. Current research has shown this vitamin to be a neurosteroid which influences 3,000 genes in human body as well as its receptors and 1- α -hydroxylase which are distributed throughout the body viz., brain, spinal cord and central nervous system (Bouvard *et al.*, 2011) [5]. Studies have revealed a correlation between vitamin D deficiency and neuropsychiatric disorders which include Alzheimer's disease, Parkinson's disease, multiple sclerosis, epilepsy, schizophrenia and cerebrovascular disorders. Adequate vitamin D levels during initial stages of life safeguard normal receptor transcriptional activity vital for brain development and mental functioning (Groff and Gropper, 2000; Eastwood, 2003) [10, 8]. Moreover, this vitamin affects proteins directly involved in learning, memory, motor control, and social behavior (Harms *et al.*, 2011) [11]. Therefore, patients with epilepsy face an even greater risk of vitamin D deficiency than healthy population, as seizures may interfere with

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their ability to move outdoors and remain active which limits the exposure to sunlight necessary for producing vitamin D. Additional threat to epileptic patients is increased risk of bone fractures because of poor nutrition, reduced exposure to sunlight and reduced vitamin D levels (Bouillon *et al.*, 1975; Babayigit *et al.*, 2006; Nettekoven *et al.*, 2008) [4, 2, 24]. The prohormone form of vitamin D₃ is activated by two sequential hydroxylations. The first involves a side chain reaction which occurs in the liver and yields 25-hydroxy (OH) vitamin D₃. Before biological use, this metabolite is further hydrolyzed to 1, 25-hydroxyvitamin D₃ in kidney. Calcium and vitamin D are essential for development and maintenance of skeletal system. Parathyroid hormone (PTH) released by the parathyroid gland, and thyrocalcitonin (TCT) released by the thyroid C cells also play significant roles in bone metabolism. In normal condition, the active metabolite of 25-hydroxy (OH) vitamin D₃ regulates serum calcium levels by increasing intestinal calcium absorption and calcium deposition in the bone. During vitamin D deficiency, PTH increases serum calcium levels by escalating the activity of 1- α -hydroxylase levels in kidney (Carolyn, 1998; Mahan and Krause's, 2000; Christakos *et al.*, 2011) [6, 21, 7]. Another major risk factor for epileptic patients is use of anti-epileptic drugs which interfere with vitamin D metabolism and further aggravate the condition. Therefore, correction of vitamin D deficiency is an issue of concern and attention in patients with epilepsy. The present study was undertaken to understand and analyze the effect of vitamin D deficiency in epilepsy patients.

2. Materials and methods

2.1 Selection of subjects – Inclusion and exclusion criteria

Epileptic subjects (n= 459) with an age range of 3 to \leq 80 years of both sexes (Males –270 and Females –189), below poverty line category, physically active patients on anti-epileptic drug treatment regularly, attending epilepsy clinic of the Super Specialty Hospital Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati were included in the study. Patients with comorbid illness such as gastrointestinal illness and chronic liver and kidney diseases were excluded from the study. Remaining subjects were (n=243; males –106 and females –137) non-epileptics also belonged to below poverty line category and who were attending other outpatient clinics other than epilepsy were included in the study to compare their general health conditions with epilepsy patients with reference to certain parameters used for the study. None of the patients were on any supplementation tablets of calcium or vitamin D. The medical information relating to epileptic and non-epileptic patients was collected from Medical records of SVIMS, Tirupati. The collected information was recorded and kept safe for future reference.

2.2 Location of the study conducted

The selected area of study had an average cloud free sunshine per day of 8-10 hrs throughout the year with the solar zenith angle of 9.92° and 38.2° in summer and winter, respectively. The ultra violet index (UV index) at this latitude was 7-12. There was little variation in the intensity of sunlight and seasonal variation. This information is needed to understand the synthesis of the vitamin D through the exposure to sunlight (Menon *et al.*, 2010) [23].

2.3 Biochemical analysis

2.3.1 Collection of blood samples

Blood samples were collected from selected subjects after informed consent and their willingness to be a part of the

study. Blood was collected from the subjects under the supervision of a trained doctor and technician from SVIMS, Tirupati. The collected blood samples were stored in cold conditions at the site of collection, processed and stored at 4° C till analysis of parathormone and 25- (OH)₂ D₃ using standard commercial kits available from Diasorin, Stillwater, Minnesota U.S.A.

2.3.2 Estimation of N-tact parathyroid hormone (N-tact PTH)

PTH N-tact assessment was done using N-tact PTH immune radiometric assay (IRMA) kit, supplied by Diasorin, Stillwater, Minnesota U.S.A (Catalogue No: 26100). Reconstitute the lyophilized reagents and allow frozen specimens to thaw completely. Add reagents to the labeled tubes in duplicates as follows: a. Total count tubes - 100 μ l 125I N –tact PTH SP Antibody (red) b. Calibrator 0 - 200 μ l calibrator, 100 μ l 125I N –tact PTH SP Antibody (red) c. Calibrators (1-5) – 200 μ l calibrator, 100 μ l 125I N –tact PTH SP Antibody (red) d. Controls and unknown samples – 200 μ l sample, 100 μ l 125I N–tact PTH SP Antibody (red). Vortex the tubes and dispense one bead into each tube using teflon-coated forceps. Cover the tubes with parafilm and incubate for 22 (\pm 2) hours at 20-25° C. Aspirate the reaction mixture and wash with bead vigorously using 1 ml of wash solution with sufficient force to raise the bead from the bottom of the test tube. Repeat the above step 3 times. Radioactivity was measured using gamma counter and express serum PTH as pg/ml.

2.3.3 Estimation of 25(OH)₂ vitamin D₃

25(OH)₂ vitamin D₃ assessment was done using 25(OH)₂ vitamin D₃ 125I radioimmunoassay (RIA) kit, supplied by Diasorin, Stillwater, Minnesota, U.S.A, (Catalogue No: 68100E A).

To calibrator, control and patient sample in disposable test tubes add 500 μ l of acetonitrile, vortex and centrifuge at 1200 \times g for 10 minutes at 20 to 25° C. Pipette out the aliquot (25 μ l) from the supernatant in to separate appropriate labeled tubes. Add the reagents to the labelled tubes in duplicates as follows: a. Total count tubes- 50 μ l of 125I 25(OH)₂ vitamin D₃, 1.0 ml of non-specific binding (NSB) buffer/ addition buffer. NSB tubes - 25 μ l of 0 calibrator (extracted) 50 μ l of 125I 25(OH)₂ vitamin D₃, 1.0 ml of NSB/Addition buffer c. Calibrators, controls and unknown samples 25 μ l of calibrator, control, or unknown sample (extracted) 50 μ l of 125I 25(OH)₂ vitamin D₃, 1.0 ml of 25(OH)₂ vitamin D₃ antiserum, vortex the tubes gently without foaming and incubate for 90 (\pm 10) minutes at 20-25 °C. Add 500 μ l of Donkey anti goat precipitating complex [DAG] mix thoroughly before and during use to all tubes except the total count tubes. Mix the contents well and incubate for 25 –25 minutes at 20-25° C. Add 500 μ l of NSB/Addition buffer to all tubes except the total count tubes and vortex gently to mix tubes well. Centrifuge all tubes for 20 minutes at 1800 \times g except the total counts tube. Decant the supernatants, except the total count tubes and inverted carefully onto absorbent paper for 2-3 minutes. Blot the tubes gently to ensure all liquid is removed. Count each tube for a minimum of 1 minute using gamma scintillation counter and express serum 25(OH)₂ vitamin D₃ as ng/ml.

Statistical analysis

Statistical analysis of the data was also performed using a two-way ANOVA calculation with Duncan's pair wise comparisons between groups.

3. Results and discussion

High prevalence of vitamin D deficiency has been reported in South Indian population (Harinarayan, 2005; Harinarayan *et al.*, 2007) [13, 12]. Metabolically, active form of vitamin D is $25(\text{OH})_2\text{D}_3$ which is the major circulating form of vitamin D. It is the best indicator of overall vitamin D status and is used to correlate vitamin D stores with clinical diseases. $1,25(\text{OH})_2\text{D}_3$ is a metabolically active form and is closely regulated by $25(\text{OH})_2\text{D}_3$, PTH, calcium and phosphorus. PTH is one of the most important short-term initiators of defense in reduction of the extracellular calcium concentration. The principle regulator of PTH secretion is ionized calcium concentration. When calcium sensing receptor system senses calcium level below the normal range, increased PTH secretion takes place and mobilize calcium from bones, increase tubular reabsorption of calcium from the kidneys, and increase production of $1,25(\text{OH})_2\text{D}_3$ by the kidneys (Holick, 2008) [18]. Lower concentrations of $25(\text{OH})_2\text{D}_3$ have been found to exhibit reduced effective calcium absorption from the gut (Heaney *et al.*, 2003; Heaney, 2003) [15, 16]. It has been reported that only 10 - 15% of dietary calcium and 60% of dietary phosphorus is absorbed from the intestinal tract in low vitamin D states (Mahan and Krause's, 2000; Groff and Gropper, 2000; Eastwood, 2003) [21, 10, 8]. When $25(\text{OH})_2\text{D}_3$ levels fall below 40 ng/ml, PTH is activated due to decrease in calcium absorption from the intestine. PTH activates osteoblasts that stimulate the formation of osteoclasts which dissolve the calcium: phosphorus collagen matrix in bone. Immediate attention and correction is required otherwise it can lead to osteopenia and rickets in adults and children, respectively. Moreover, patients with epilepsy are more disposed to get vitamin D deficiency along with higher PTH levels.

Data pertaining to parathormone and vitamin D levels of epileptic and non-epileptic males and females is presented in Table 1 and 2. The mean serum PTH was found to be higher in epileptic males and females of all age groups except <14 yrs compared to non-epileptic males and females. In males, the differences were found to be significant at $p < 0.05$ in 19-50 and >50 yrs age groups and at $p < 0.01$ level in <14 and 15-18 yrs age groups. On the other hand, in females the differences observed were significant at $p < 0.05$ and $p < 0.01$ in 15-18, and 19-50 and >50 yrs age groups, respectively. Normal levels for parathormone ranged from 13 - 54 pg/ml and for $25(\text{OH})_2\text{D}_3$ vitamin D_3 is >30 ng/ml. An increase in circulating parathormone level has been found to be associated with antiepileptic drugs (Bouillon *et al.*, 1975; Hahn *et al.*, 1978; Ala-Houhala *et al.*, 1986; Weinstein *et al.*, 1984; Tekgul *et al.*, 2006; Nettekoven *et al.*, 2008) [4, 11, 1, 28, 27, 24].

The mean serum $25(\text{OH})_2\text{D}_3$ levels were found to be higher in non-epileptic males of all age groups except <14 yrs age group compared to non-epileptic males. The differences were found to be significant at $p < 0.05$ and $p < 0.01$ levels in >50, and 15-18 and 19-50 yrs age groups, respectively. The $25(\text{OH})_2\text{D}_3$ levels were higher in non-epileptic females of all age groups compared to epileptic females. The differences found were significant at $p < 0.01$ level. In both epileptic and non-epileptic groups, females of all age groups were found to be deficient in vitamin D. However, in epileptic group males of <14 years; in non-epileptic group males of 19-50 and > 50 years were found to be vitamin D insufficient. This exhibited that the prevalence of vitamin D deficiency was higher in females and more prevalent in epileptic females than epileptic males.

Vitamin D deficiency and insufficiency are found frequently in adolescents with severe mental illness. Deficiency of this

vitamin has been linked to an increased risk of developing schizophrenia. Vitamin D deficient adolescents with acute mental health were 3½ times more likely to have psychotic features when compared to vitamin D sufficient patients (Gracious *et al.*, 2010). Vitamin D deficiency has also been associated with depression and seasonal affective disorder. Supplementation with vitamin D in depressed adolescents facilitated to reach serum vitamin D levels >30 ng/ml (Högberg *et al.*, 2012) [17].

Normalized vitamin D levels were observed on administration of vitamin D (37.5 and 125 µg per week) in adults and children on chronic anti-epileptic therapy in mentally retarded individuals for a period of 9 months (Offermann *et al.*, 1979) [26].

A study conducted on ambulatory children on anti-epileptic drugs such as carbamazepine or valproate and on healthy children for a period of three years showed a significant decreasing trend in vitamin D levels and an increase in serum PTH levels in children (Nicolaidou *et al.*, 2006) [25]. Study conducted by Babayigit *et al.*, (2006) [2] on epileptic and control individuals concluded that though the seasonal variation is one of the reasons for vitamin D deficiency, intake of AEDs decreases vitamin D levels.

Supplementation with 1000 to 4000 IU of vitamin D in psychiatric male patients diagnosed with autism and schizophrenia had the lowest levels of vitamin D (<20 ng/ml) from Middle East, South-East Asian, or African ethnicity showed significant clinical improvements (Humble *et al.*, 2010) [19].

Data pertaining to epileptic and non-epileptic males and females grouped based on $25(\text{OH})_2\text{D}_3$ levels is shown in table 3 and 4. In epileptic females and males, PTH levels were high in vitamin D deficient group compared to non-epileptic females. The difference was found to be significant at $p < 0.01$ level. Mean $25(\text{OH})_2\text{D}_3$ levels were high in non-epileptic males and females compared to epileptic group, except in males of normal vitamin D group. Significant difference at $p < 0.01$ and at $p < 0.05$ and $p < 0.01$ were observed in vitamin D deficient males and females, respectively. None of the females of both epileptic and non-epileptic groups were found to have normal vitamin D levels.

Epileptic males (71%) were found to have vitamin D deficiency, 18% were insufficient of vitamin D and only 11% were having normal vitamin D levels. In non-epileptic males, 50.9% were having deficiency, 35.9% were insufficient of vitamin D and only 13.2% had normal vitamin D levels. The deficiency was found to be more severe in epileptics compared to non-epileptics. However, higher percentage (94.6) of deficiency of vitamin D was observed in epileptic females than males, 5.4% were insufficient of vitamin D. In non-epileptic females, 71.5% were having deficiency and 28.5% were insufficient of vitamin D. None of the females in both epileptic and non-epileptic groups were with normal vitamin D levels. This shows higher prevalence of vitamin D deficiency in females over males and epileptic females over non-epileptic females.

4. Conclusions

The present study revealed that both epileptics and non-epileptics have vitamin D deficiency. In both the groups the number of subjects with normal vitamin D levels is very less, compared with vitamin D deficiency and insufficiency. In both males and females of epileptic and non-epileptic groups, when categorized based on $25(\text{OH})_2\text{D}_3$ levels, the PTH levels were inversely proportional to $25(\text{OH})_2\text{D}_3$

levels. Females have high prevalence of vitamin D deficiency than males. This is probably due to hormonal changes, occupation, dress code, and duration of exposure to sunlight.

However, change of season has a little impact on cutaneous synthesis of vitamin D.

Table 1: Parathormone and vitamin D levels of epileptic and non-epileptic males

Parameters	Age groups (years)							
	<14		15-18		19-50		>50	
	Epileptic (n=13)	Non-epileptic (n=16)	Epileptic (n=19)	Non-epileptic (n=15)	Epileptic (n=60)	Non-epileptic (n=46)	Epileptic (n=10)	Non-epileptic (n=29)
Parathormone (pg/ml)	16.35 ± 1.92	28.09 ± 2.47**	39.37 ± .32**	23.24 ± 1.07	26.59 ± 1.43*	22.42 ± 1.08	31.85 ± 5.6*	23.72 ± 1.17
25(OH) vitamin D (ng/ml)	20.76 ± 3.36	15.53 ± 2.06	13.02 ± 1.2	19.18 ± 1.35**	16.37 ± 1.23	21.92 ± 1.01**	18.45 ± 3.14	25.51 ± 1.59*

All values are Mean ± SE; 95 per cent CIs in parentheses;

P * <0.05 **<0.01 (Significance between the same age group of epileptic and non-epileptic groups)

Table 2: Parathormone and vitamin D levels of epileptic and non-epileptic females

Parameters	Age groups (years)							
	<14		15-18		19-50		>50	
	Epileptic (n=10)	Non-epileptic (n=17)	Epileptic (n=09)	Non-epileptic (n=14)	Epileptic (n=45)	Non-epileptic (n=85)	Epileptic (n=10)	Non-epileptic (n=21)
Parathormone (pg/ml)	21.51 ± 1.42	25.28 ± 1.96	26.16 ± 3.51*	19.86 ± 1.19	33.76 ± 3.77**	23.2 ± 0.69	54.79 ± 9.06**	24 ± 1.72
25(OH) vitamin D (ng/ml)	12.07 ± 2.05	17.34 ± 0.81**	9.00 ± 0.51	18.04 ± 1.67**	12.91 ± 0.82	17.98 ± 0.7**	10.59 ± 1.82	17.29 ± 1.33**

All values are Mean ± SE; 95 per cent CIs in parentheses;

P * <0.05 **<0.01 (Significance between the same age group of epileptic and non-epileptic groups)

Table 3: Epileptic and non-epileptic males grouped based on 25(OH)₂ vitamin D₃ levels

Parameters	Vitamin D deficiency		Vitamin D insufficiency		Normal Vitamin D levels	
	25(OH)D <20 ng/ml		25(OH)D 20.1-30 ng/ml		25(OH)D >30 ng/ml	
	Epileptic (n=71)	Non-epileptic(n=54)	Epileptic (n=18)	Non-epileptic (n=38)	Epileptic (n=11)	Non-epileptic (n=14)
Parathormone (pg/mL)	28.12 ± 1.67	25.18 ± 0.95	26.26 ± 2.88	21.96 ± 0.92	26.35 ± 4.28	23.07 ± 2.96
25(OH) ₂ vitamin D ₃ (ng/mL)	11.44 ± 0.5	15.12 ± 0.57**	24.91 ± 0.74	26 ± 0.47	35.81 ± 1.19	34.29 ± 1.06

All values are Mean ± SE; 95 per cent CIs in parentheses;

P **<0.01 (Significance between the epileptics and non-epileptics within the group)

Table 4: Epileptic and non-epileptic females grouped based on 25(OH)₂ vitamin D₃ levels

Parameters	Vitamin D deficiency		Vitamin D insufficiency		Normal Vitamin D levels	
	25(OH)D <20 ng/ml		25(OH)D 20.1-30 ng/ml		25(OH)D >30 ng/ml	
	Epileptic (n=70)	Non-epileptic(n=98)	Epileptic (n=04)	Non-epileptic (n=39)	Epileptic (n=0)	Non-epileptic (n=0)
Parathormone (pg/mL)	34.35 ± 2.93**	23.22 ± 0.66	23.15 ± 4.85	24.12 ± 1.13	-	-
25(OH) ₂ vitamin D ₃ (ng/mL)	11.47 ± 0.61	14.86 ± 0.38**	21.28 ± 0.59	24.27 ± 0.5*	-	-

All values are Mean ± SE; 95 per cent CIs in parentheses;

P**<0.05, **<0.01 (Significance between the epileptics and non-epileptics within the group)

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