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Original Research Article

A Cross- Sectional Study on the Association of Vitamin D and D Binding Protein Levels with Clinical Depression

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Abstract:

Introduction: The underlying pathophysiology of depression remains unknown despite multiple theories and several different mechanisms are probably involved. A number of studies have given conflicting results about the association of vitamin D deficiency with depression. Studies have identified the emergent role of D binding protein in the physiological effect of Vitamin D in most of the target tissues. Hence this study was taken up to measure DBP levels along with 25 hydroxy vitamin D levels to look for association with Clinical Depression.

Material and Methods: Participants aged 18-60 years visiting the outpatient department of Psychiatry with clinical depression were assessed consecutively between 2018 and 2020. The blood samples were analyzed for 25 hydroxy vitamin D and DBP levels and were compared to the severity of anxiety and depression as determined by HAM-D and HAM-A scores.

Results: Out of 108 subjects, 53(49.1%) were males and 55(50.9%) females. Half of the participants had vitamin D deficiency and significant had insufficient levels. There was no association between Vitamin D levels and the severity of Depression and anxiety. Vitamin DBP was found to be associated with HAM-A and not with HAM-D. There was no correlation between vitamin D, Vitamin DBP with depression and anxiety.

Conclusion: Vitamin D deficiency can be seen in clinical depression. However, there is no association between vitamin D and the severity of depression. Vitamin DBP is found to be associated with anxiety symptoms of depression due to particular assessment method. The current study suggests RCT for a better understanding of vitamin DBP's role in Clinical depression.

Keywords: Vitamin D, Vitamin D binding Protein, Depression, Anxiety.

Introduction

Depression is strongly associated with disability, loss of relationships, increased risk of self-harm, and rising health care expenses. The underlying pathophysiology of Depression is unknown despite the advancement of psychological, biological, and environmental theories, and several distinct mechanisms are likely involved [1]. Some studies have found an association between serum vitamin D concentrations and Depression [1–3]. A study showed that Vitamin D concentrations were low in mood disorder patients and associated with cognitive functions [4].

Vitamin D plays an essential role not only in bone health but also contributes to the synthesis of norepinephrine and dopamine through the regulation of gene expression. Both neurotransmitters are involved in mood disorders and Depression [5]. To explore the biological plausibility of vitamin D concerning Psychiatric disorders, mapping the distribution of the Vitamin D receptor (VDR) in the human brain was done, and it was noted that the receptor has a widespread expression in the adult brain, particularly in the dopaminergic neuron-rich substantia nigra. Vitamin D receptors are present in neurons and glia in many areas of the brain, including the frontal cortex and hippocampus, which may play an essential role in the pathophysiology of Depression [6,7]. Vitamin D has been associated with Depression in a few studies, but one pointed out that supplementation might play an essential role in treatment [1]. 25-

hydroxyvitamin D [25(OH)D], the precursor of the active form of vitamin D, is recognized as the optimal indicator of vitamin D status. 25(OH)D and 1,25- dihydroxy vitamin D_3 [$1,25(OH)_2D_3$] circulate bound to vitamin D–binding protein (85% to 90%) and albumin (10% to 15%), with less than 1% of circulating hormone in its free form. The affinity of vitamin D binding protein (DBP) for 25OHD is much higher than for the VDR, thereby keeping this precursor preferentially in the serum pool. As a result, factors affecting DBP alter the interpretation of 25(OH) D levels [8]. The same study suggested that many assays used to measure 25(OH)D depend on the concentration of DBP and levels of 25 hydroxy vitamin D need to be analyzed along with vitamin D binding protein levels to decide on the vitamin D status of an individual [8]. 25(OH)D measurements performed with most immunoassays suffer from DBP concentration-dependent inaccuracies [9,10].

Apart from acting as a carrier protein for vitamin D, DBP has been shown to possess antioxidant and anti-inflammatory properties, without binding to vitamin D, that likely contribute to its role in disease prevention and treatment. DBP has been found to regulate immune function and play a role in removing toxins and pathogens from the bloodstream. [8,11]. Studies of hypovitaminosis D and clinical Depression have yielded conflicting results, as few studies showed no association between vitamin D and Depression [12–14]. However, most of the studies that looked into the association between vitamin D levels and depression did not measure DBP levels. DBP with its strong affinity for vitamin D, enormously influences the bioavailability of vitamin D in various target tissues.

In recent years, there has been significant interest in exploring Vitamin DBP. However, no study has been done to assess vitamin DBP with vitamin D and the severity of Depression. Hence, the present study is taken up to study the total vitamin D and D binding protein (DBP) levels in newly diagnosed cases of clinical depression and the association between vitamin D levels, vitamin DBP levels, and severity of depression.

Materials and Methods

Participants

Patients aged 18-60 years visiting Psychiatric OPD at Mysuru Medical College & Research Institute during 2018 and 2020 were included in the study. The diagnosis was made using Structural interviews of patients according to DSM V for Major Depressive Disorder. Newly diagnosed patients of Major depressive disorder drug naïve were assessed consecutively. Patients with bipolar disorder currently in depression/ depressive disorder on antidepressants/ pregnancy/ osteoporosis/other bone diseases/ history of severe head trauma or central nervous system disorder/ known history of hepatic or renal disorders/ history of alcohol/substance abuse or dependence spanning the previous six months/ history of prior episodes of depression along with Patients routinely taking Vitamin D or calcium supplements were excluded from the study.

Institutional ethical clearance was taken before the recruitment of patients for the study. Patients were informed about the study, the need for assessment, and blood sample collection, and consented were included. The severity of depression was assessed by administering the Hamilton Rating Scale for Depression and Hamilton Rating Scale for Anxiety questionnaires to the participants**.** At the same time, blood samples were collected at the psychiatry OPD by Medical Research Unit (MRU) technicians, and serum concentrations of 25(OH) vitamin D and vitamin D binding protein were estimated at the Multidisciplinary Research Unit facility of the institution.

Hamilton Rating Scale for Depression (HDRS)

HDRS is the most widely used scale for assessing the severity of Depression. It's a 17-item questionnaire with closed-end questions(15). Scored as normal when it is < 7, mild – 8 to 16, moderate – 17-23, severe > 24 (16).

Hamilton Anxiety Rating Scale (HAM-A)

The HAM-A is used to measure the severity of anxiety symptoms in Depression. The scale consists of 14 items, each defined by a series of symptoms, and measures both psychic anxiety (mental agitation and psychological distress) and somatic anxiety (physical complaints related to anxiety (17). Each item is scored on a scale of 0 (not present) to 4 (severe), with a total score range of 0–56. A score < 7 indicates normal, 8 – 14 indicates mild to moderate anxiety, 15–23 is moderate to severe, and >24 is severe anxiety.

Vitamin D [25(OH) D]

The Vitamin D levels were measured using liquid chromatography-tandem mass spectrometry, and total serum 25(OH) vitamin D was assessed as the additive sum of 25(OH)D2 and 25(OH)D3 components in all the participants**.** Further categorized as vitamin D deficient if values ≤20 ng/mL, insufficient when 21–29 ng/mL, and normal - ≥30 ng/mL according to Endocrinology Society guidelines (1,18,19).

Vitamin D binding protein (DBP)

In the current study, vitamin D– D-binding protein levels were measured by a commercial enzyme-linked immunosorbent assay that uses two monoclonal antibodies in a sandwich format (interassay coefficient of variation, 7.2%) (20). Normal DBP levels in humans were between 200-600 micrograms/ml. As normal DBP level has a

wide range, the values were placed in quartiles for analysis (21). This wide range exists probably due to a lack of standardization of DBP assays and references (22). Most of the studies used polyclonal antibodies, few used monoclonal antibodies, and recently, the mass spectrometry method was also used.

Statistical Analysis

The results were expressed as Median ± Standard deviation. Statistical analysis was performed using SPSS.21.0, and the test used was the Student t-test. Levels of Vitamin D and DBP and severity of depression as per HAM-D & HAM-A scores were correlated with Pearson's correlation coefficient. P < 0.05 was considered statistically significant.

Results

Out of 118, 6 were excluded as they had osteoporosis, were on antidepressants, had a history of alcohol dependence/ pregnancy, and 4 did not consent.

A total of 108 subjects' data was available for analysis at the end of the study, collected from patients visiting the outpatient department of psychiatry at Mysore Medical College and Research Institute from October 2018 to October 2020. Due to the COVID pandemic, we could not reach the sample size of 150 as proposed in the study's beginning. Out of the total 108, females (50.9%) were slightly higher than males (49.1%). As shown in Table 1, the participant age group was between 18 and 51 years old. A significant percentage of patients belonged to 21 to 40 years of age.

Table 1: Distribution of age group of clinically depressed patients

Age	Numbers (Percentage %)
<20	8 (7.4%)
21-30	36 (33.3%)
31-40	28 (25.9%)
41-50	27 (25.0%)
>51	9 (8.3%)

HAM – D and HAM-A:

Out of 108 participants, significant patients suffered from moderate (39.8%) and Mild depression (32.4%). Some patients suffer from severe depression (27.8%). As per the HAM-A rating scale, most patients had mild anxiety (47.2%) and No/minimal anxiety (31.5%). Only a few had moderate (14.8%) and severe anxiety (6.5%).

Vitamin D Levels:

The mean vitamin D level was 16.26 for <20 years of age. Similarly, the 21 – 30 age groups was 19.69; the 31-40 age group was 19.94. 41-50 age group for 21.97. >51 were 18.01. The vitamin D levels were higher in males (mean -21.57) than in females (18.35).

However, the gender difference was not statistically significant**.** Further, Vitamin D levels were divided into Normal, insufficiency, and deficiency**.** It was found that half of the participants had vitamin D deficiency (<20ng/ml). Significant patients (39.8%) had insufficient vitamin D levels (20-29ng/ml), and few (10.2%) had Normal levels (>30ng/ml) of Vitamin D. Mean value of Vitamin D among severity of HAM-D rating scale showed that Mild severity had 20.14, Moderate severity had 19.14 and Severe had 20.33. There was no association between Vitamin D levels and the severity of Depression (P=0.78).

Similarly, values of Vitamin D among severity of anxiety (HAM-A) showed a mean of 18.91 in No/minimal anxiety, 20.40 mean in mild anxiety, 22.29 mean in moderate anxiety, and 16.1 mean in severe anxiety.

However, there was no association between vitamin D and HAM-A (P=0.3). Tables 2 and 3 show the association of vitamin D levels (normal, insufficiency, and deficiency) with HAM-D and HAM-A, assessed using the chi-square test. However, Vitamin D was not found to be associated with HAM-D (P=0.9) and HAM-A(P=0.2).

Table 2: Association of vitamin D levels with HAM-D.

Vitamin D level ng/ml	HAM-D				
	Normal(<7)	Mild (8-16)	Moderate (17-23)	Severe (>24)	Total
	Number(%)	Number(%)	Number (%)	Number(%)	Number(%)
Normal (>30ng/ml)	0(0%)	5(15.2%)	3(7.0%)	3(10.0%)	11(10.2%)
Insufficiency (20-29ng/ml)	1(50.0%)	11(33.3%)	17(39.5%)	14(46.7%)	43(39.8%)
Deficiency (<20ng/ml)	1(50.0%)	17(51.5%)	23(53.5%)	13(43.3%)	54(50.0%)
Total	2(100%)	33(100%)	43(100%)	30(100%)	108(100%)

P=0.9, Chi-square test

Table 3: Association of vitamin D with HAM-A.

Vitamin D level ng/ml	HAM-A			
	No/Minimal anxiety(<7)	Mild anxiety(8-16)	Moderate anxiety(17-24)	Severe Anxiety(25-30)
	Number (%)	Number (%)	Number (%)	Number (%)
Normal (>30ng/ml)	3(8.8%)	7(13.7%)	1(6.3%)	0(0%)
Insufficiency (20-29ng/ml)	14(41.2%)	15(29.4%)	11(68.8%)	3(42.9%)
Deficiency(<20ng/ml)	17(50.0%)	29(56.9%)	4(25.0%)	4(5.1%)

P=0.2 Chi-square test

Vitamin D binding Protein:

The mean value of Vitamin D binding protein was 198.70. The mean vitamin D binding protein level associated with HAM-D severity was done using a t-test.

Mild severity had a mean value of 208.95. Moderate had 194.78 mean, and Severe had 192.36 mean. Vitamin D binding protein is further divided into quartiles. The first quartile (Q1) had 171.60, and 3rd quartile had 216.45. Vitamin D binding protein was associated with HAM-D and HAM-A using the chi-square test, as shown in Tables 4 and 5. An association of vitamin D binding protein with HAM-A was found, but there was no association between vitamin D binding protein and HAM-D.

Table 4: Association of vitamin D binding protein with HAM-D

		HAM-D			
		Normal(<7)	Mild(8-16)	Moderate(17-23)	Severe(>24)
		Number (%)	Number (%)	Number (%)	Number (%)
Vitamin D binding Protein level µg/ml	Q1	0	8 (22.9%)	11 (25.6%)	8 (26.7%)
	Q2	0	3 (8.6%)	14 (32.6%)	10 (33.3%)
	Q3	0	12 (34.3%)	8 (18.6%)	7 (23.3%)
	Q4	0	12 (34.3%)	10 (23.3%)	5 (16.7%)

P=0.1, Chi-square test

Table 5: Association of vitamin D binding protein with HAM-A

	HAM-A			
D binding protein level µg/ml	Mild anxiety(<7)	Mild moderate anxiety(8-16)	Moderate to severe anxiety(17-24)	Severe Anxiety(25-30)
	Number (%)	Number (%)	Number (%)	Number (%)
Q1	3 (8.8%)	20 (39.2%)	4 (25.0%)	0 (0%)
Q2	7 (20.6%)	12 (23.5%)	4 (25.0%)	4 (57.1%)
Q3	9 (26.5%)	11 (21.6%)	4 (25.0%)	3 (42.9%)
Q4	15 (44.1%)	8 (15.7%)	4 (25.0%)	0 (0%)

P=0.008, Chi-square test, P=0.05 significant.

Table 6: Correlation between vitamin D, vitamin D binding protein, and HAM-D and HAM-A.

		VitaminD_level_ngml	D_Binding_protein_level_microgml
Hamilton depression rating scale	Pearson Correlation	.008	-.127
	P	.934	.192
	N	108	108
Hamilton_anxiety_rating_scale	Pearson Correlation	.043	-.185
	P	.658	.055
	N	108	108
D_Binding_protein_level_microgml	Pearson Correlation	.042	1
	P	.666	
	N	108	108

Table 6 shows no correlation between Vitamin D and Vitamin D binding protein with HAM-D and HAM-A using Pearson's correlation.

Discussion

In the Current study, half of the newly diagnosed depressed patients had vitamin D deficiency, and significant patients had insufficient levels of Vitamin D. However, there was no association of vitamin D with the severity of newly diagnosed depressed patients. This finding is similar to

previous results [12–14]. Schnider et al. did not find an association of vitamin D with Depression, although they found hypovitaminosis. A large study was done on the middle and elderly population and did not show an association between vitamin D and Depression [13]. Similarly, a study looked into vitamin D levels in fibromyalgia patients with depressive symptoms and found no correlation between vitamin D and Depression [14]. However, some studies have found an association between hypovitaminosis and depression [1–4,23]. 7-dehydrocholesterol converts to pre-vitamin D3 in the skin by ultraviolet B radiation, which is rapidly converted to vitamin D3. It is then transported to the liver bound to vitamin D-binding protein (DBP) and metabolized to 25(OH)D through an enzymatic process involving 25-hydroxylase [7]. Vitamin D metabolites are carried by vitamin DBP. Vitamin D levels are dependent on Vitamin DBP [10]. DBP is a polymorphic glycoprotein that is produced by the liver. Vitamin DBP circulates at a high concentration in the serum. DBP is responsible for binding 85% of the circulating 25(OH)D and serves as a carrier protein. Three main functions of DBP concerning vitamin D physiology include protecting vitamin D from degradation, limiting its access to target tissues, and reabsorbing vitamin D [24]. The complex of DBP with 25(OH)D is filtered in the kidney glomerulus and then reabsorbed at the brush border of the tubular epithelial cells. Additional functions for DBP include transporting fatty acids, macrophage activation, protecting complement C5a from degradation, neutrophil chemotaxis, and functioning as an actin scavenger. DBP works in association with other serum proteins to protect the cell from actin-mediated damage [25]. Recently, studies have focused on Vitamin DBP assessment. [26] Petrov et al. found an association of Vitamin DBP with Bipolar disorder in adolescents.

The current study is the first study to compare vitamin DBP with clinical depression in adults. Newly diagnosed patients with Clinical depression were assessed for the association between the severity of depression and anxiety and found no association of vitamin DBP with the severity of depression. Meanwhile, a study showed an association between the severity of anxiety and Vitamin DBP. However, these findings need further exploration as different assessment methods show different results. According to a study by Navinder et al., DBP levels measured by a commercial enzyme-linked immunosorbent assay that uses two monoclonal antibodies in a sandwich may differ from the methods using polyclonal antibodies. These differences may be due to the monoclonal sandwich immunoassay recognizing only one epitope near the polymorphic region of the protein and having different affinities for the different variants [8,20]. This method might have missed other alleles that can be identified by the TMS/polyclonal antibody method [8,27]. Additionally, the current study doesn't have a control sample to compare the vitamin DBP levels.

The current study had no association between vitamin D and vitamin DBP. This finding can be explained as vitamin DBP action doesn't limit in transporting vitamin D but also transports fatty acids, and unsaturated fatty acids compete with vitamin D metabolites, thereby decreasing the apparent affinity of DBP for 25OHD and specifically 1,25(OH)2D. DBP also binds to actin, creating a DPB-Actin complex that avoids actin polymerization of actin in serum after tissue damage. DBP also binds to proteoglycans in the immune cell membrane, thereby enhancing activated neutrophil's complement C5a-stimulated chemotactic activity. However, this role in inflammation is not fully understood [21]. These other actions of Vitamin DBP suggest that vitamin DBP is not dependent on vitamin D levels. This hypothesis is supported by a study in which vitamin D and DBP levels were assessed in chronic kidney failure patients (CKD) and controls. Few chronic kidney failure patients were on hemodialysis (HD). Chronic kidney failure patients with or without hemodialysis (HD) had significantly lower vitamin D than controls. Vitamin DBP levels were found to be similar between patients and controls. The study observed significantly increased levels of Vitamin DBP in CKD patients compared to controls and HD patients. Vitamin D deficiency observed in CKD and HD patients was unrelated to Vitamin DBP serum concentration. The lack of association between vitamin DBP and vitamin D in chronic kidney failure patients suggests some effect on the concentration of vitamin D metabolites [24]. Similarly, the current study found no association between vitamin D and vitamin DBP.

Limitations and Strengths

Unfortunately, because of the pandemic situation, 150 samples were not able to be collected. After completing the analysis of 108 samples, we still could get results without compromising the power of the study. Secondly, this sample consisted of individuals in a tier two city in south India, and our findings may not be generalizable to other geographic areas with changes in dietary habits. This study did not analyze the data regarding seasonality, limiting our ability to determine differences in vitamin D levels by season. Despite these limitations, the current study sample is fairly large and represents the typical tertiary care depression patients in the South Indian community.

Conclusion

Current study suggest that vitamin D deficiency can be seen in Clinical depression. However, the severity of newly diagnosed depression is not

associated with vitamin D levels. Similarly, vitamin DBP is found to be not associated with vitamin D levels and the severity of Depression. Vitamin DBP was associated with anxiety symptoms of depression, which could be because of specific assessment methods warranting assessment of vitamin DBP using other methods. The current study recommends exploring alternative methods for assessing Vitamin DBP and conducting randomized controlled trials (RCTs) for better understanding of Vitamin DBP's role in clinical depression.

Declaration of interest

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