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DEPRESSIVE SYMPTOMS;
ASSOCIATION BETWEEN SERUM 25-HYDROXYVITAMIN D IN PAKISTANI PATIENTS

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ABSTRACT… Introduction: Depression is a leading source of disability world-wide with prevalence ranging from 8.8% to 18.3% in people aged 60 years or older. Depression is a not a simple term but has been used to describe a variety of infirmities, ranging from minor to debilitating. It is a multi-symptom disease and can even lead to suicidal attempt. Major depressive disorder (MDD), although one of the most common psychiatric illnesses, has an unknown etiology. Decreased serum levels of vitamin D have been implicated in the pathogenesis of MDD. Case control studies have supported a relationship between low vitamin D as possible predictors of depression. We designed a study to determine relationship, if any, between low vitamin D deficiency with depression and with severity of disease in our local population. **Objective:** The aim of this study to see any association of vitamin D deficiency in depressed Pakistani patients. **Study Design:** Cross-sectional study. **Setting:** Fatima Memorial Hospital, Shadman, Lahore. Period: 2013-2015. **Methods:** Total of 150 diagnosed patients of 18-75 years of age was enrolled for this study, after a written consent. Depressed patients were further categorized into three groups depending on severity of disease i.e.; mild, moderate and severely depressed patients. Blood measures included serum vitamin D levels and data was recorded on structured data collection form. **Results:** In this study, we investigated the association between vitamin D status and depression in our local Pakistani population. Out of 150 depressed patients, 84.7% were vitamin D deficient. In our study, Out of 44 male depressed patients 9 (20.5%) had mild depression, 8 (18.2%) had moderate depression and 27 (61.4%) had severe depression. Out of 106 depressed female patients 42 (39.6%) had mild depression, 26 (24.5%) had moderate depression and 38 (35.8%) had severe depression symptoms. A negative correlation of vitamin D with severity of disease was also found in female depressed patients in our study.A significant negative correlation of vitamin D in all 44 male subjects with severity of disease was observed in our study. Higher prevalence of vitamin D deficiency was found in both males and females in our study. **Conclusions:** Vitamin D deficiency is associated with an increased risk of developing depression, but more prospective observational studies may be needed. Efficacy of vitamin D supplementation for preventing onset of depressive disorder is unknown. Efficacy of vitamin D supplementation for reducing depressive symptoms is largely unstudied. The study suggests dietary intervention with vitamin D would boost brain serotonin concentrations and help prevent and possibly relieve some of the symptoms associated with depression without side effects.

Key words: Vitamin D, major depressive disorder, neurotransmitter deficiency

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INTRODUCTION

Low vitamin D status is a widespread problem in the United State.[1,2] It has been documented in many researches that serum vitamin D concentrations previously considered in the normal range are not sufficient for optimal health.[3-5] Vitamin D may have an important role in the development of depression. Vitamin D receptors are present in many areas of the brain including the cingulate cortex and hippocampus, neurons and glia of the brain, which have been implicated in the pathophysiology of depression.[6-9]

How vitamin D causes depression is not fully understood? It has been documented in many studies that vitamin D is involved in numerous brain processes including regulation of neurotrophic factors, neuroprotection, neuroplasticity and brain development. It is therefore, plausible that this vitamin might be associated with depres-

sion and that its supplementation might play an important part in the treatment of depression.[10-13]

Recently, it has been documented in various studies that vitamin D has arole in cognitive function and mental health.[13,14] Vitamin D concentrations have been shown to be low in patients suffering from depression/ mood disorder, anxiety and have been associated with cognitive function.[14-16]

Exactly how vitamin D works in our brain isn't fully understood. It has been documented in some studies, that vitamin D disturbs the production of serotonin and dopamine in brain.[15,16] According to the Kimlin,a Cancer Council Queensland Professor of Cancer Prevention Research and other researchers."Evidence exists that low levels of serotonin and dopamine are linked to depressive symptoms, therefore it is logical that there may be a relationship between low levels of vitamin D and depressive symptoms".

It has been documented in many researches that whenever there is deficiency of serotonin in brain, it leads to depression. Many anti-depressant medicines work by increasing the amount of monoamines/serotonin in our brain. Therefore researchers have suggested that vitamin D may also increase the amount of monoamines, which has an effect on depression.[16-18]

Several mechanisms of action have been proposed to explain the association between vitamin D deficiency and depression of mood. It has been documented in many studies that receptors for vitamin D and enzymes which are responsible for its metabolism are present throughout the brain, limbic system, substantianigra, cortex, cerebellum, neurons and glia.[17,18]

The role of calcitriol 1, 25 dihydroxycholecalciferol, the bioactive form of vitamin D, in brain tissue has been confirmed by the presence of vitamin D receptors and the enzymes hydroxylases in various brain regions.[18] One important area of brain, where vitamin D receptors and enzyme hydroxylases have been found is the amygdala, which is the center of the limbic system, where emotions and behavior are regulated.[19] So it can be postulated here, that vitamin D has definitely some link between cognition and mental health.

MATERIALS AND METHODS

Study Population

The present study included 150 diagnosed patients of depression, of 25-65 years of age. Depressed patients were further categorized into three groups depending on severity of disease that is mild, moderate and severely depressed patients. The Patients fulfilling inclusion criteria were enrolled in the study after obtaining his/her written informed consent. The study was approved by the Ethical Committee of FMH College of Medicine and Dentistry and the advanced studies and research board of University of Lahore.

Blood sampling and analysis

Blood samples from patients with depression were obtained after aseptic measures. A total of 3 ml of blood were drawn from each patient and blood was centrifuged at 3,000 rpm for 10-15 minutes to separate serum and aliquoted in two portions and stored at -80º C until analyzed. Serum was separated by centrifugation and serum aliquots were frozen and stored at -80 º C until analysis.

Hormone and vitamin determination

Serum vitamin D levels were determined by ELISA in duplicate using standard procedures with commercially available assay kits (vitamin D) with an automated EIA analyzer (Coda, Bio-Rad Laboratories, Hercules, CA, USA) measured using a high-sensitivity quantitative enzyme immunoassay (ELISA). To determine vitamin deficiency, we used the following cutoffs, which corresponds to the normal ranges of the assays and are described in the literature.[3] Vitamin D deficiency was considered present when the serum vitamin D level was less than 30ng/ml.

STATISTICAL ANALYSIS

The data was entered and analyzed using IBM SPSS (Statistical Package for Social Sciences) version 20.0.

Median with IQR is given for non-normally distributed quantitative variables.
Frequencies and percentages are given for categorical variables.

For non-normally distributed quantitative variables, non-parametric statistics i.e. Mann-Whitney U test and Kruskal-Wallis test were used to compare various variables between two and more than two groups respectively.

Spearman's rho correlation (rho) was used to observe correlation between non-normally distributed quantitative variables.

Chi square test was used to observe association between categorical variables.
p -value of ≤ 0.05 is considered statistically significant for all purposes.

RESULTS

This study included 150 depressed patients with depression. For the purpose of analysis and comparison, 150 depressed patients were further sub-grouped into three groups according to severity of disease: Mild depression, Moderate depression and severely depressed group.

Study sample included 44 male patients and 106 female patients. Median IQR of overall age of depressed group was 27.5 (22.0-35.0), (Table I). Median (IQR) of overall age of mild group was 28.0 (26.0-33.0), median (IQR) of moderate group was 25.0 (22.0-34.0) and of severe group was 33.0 (21.0-42.0), (Table II).

Age (Years)	Depression (n=150)	
	Frequency	Percent
18-25	59	39.3
26-35	61	40.7
36-55	27	18.0
55-75	3	2.0
Total	150	100
Median (IQR) of overall age	27.5 (22.0-35.0)	

Table-I. Frequency distribution of overall age of study groups according to depression.

Frequency distribution of gender in groups according to mild, moderate and severe depression is described in (Fig: 1).

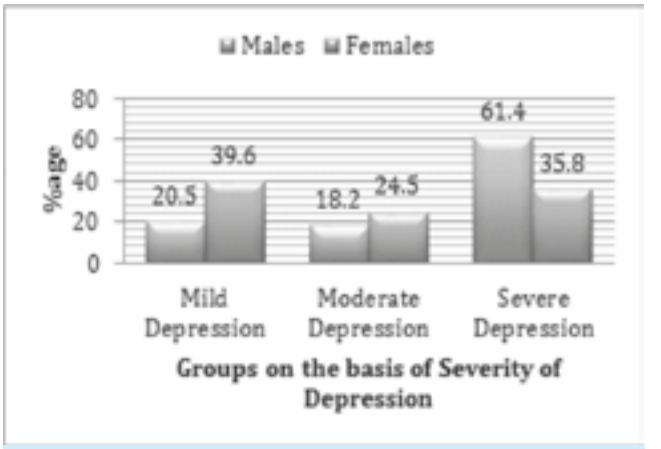

Figure-1. Frequency distribution of gender in groups according to Mild, Moderate and Severe Depression.

Out of 150 depressed patients, 127 (84.7%) had low vitamin D levels (Table III). Median (IQR) of vitamin D level of all depressed patients was 13.0 (7.0-16.0) ng/ml, (Table IV).

Age (Years)	Mild Depression (n=51)		Moderate Depression (n=34)		Severe Depression (n=65)	
	Frequency	Percent	Frequency	Percent	Frequency	Percent
18-25	10	19.6	18	52.9	31	47.7
26-35	35	68.6	10	29.4	16	24.6
36-55	3	5.9	6	17.6	18	27.7
56-75	3	5.9	0	0	0	0
Total	51	100.0	34	100.0	65	100.0
Median (IQR) of overall age	28.0 (26.0-33.0)		25.0 (22.0-34.0)		33.0 (21.0-42.0)	

Table-II. Frequency distribution of overall age of study groups according to Mild, Moderate and Severe Depression.

Vitamin D	Depression (n=150)	
	Frequency	Percent
Low	127	84.7
Normal*	8	5.3
High	15	10.0
Total	150	100.0

Table-III. Frequency distribution of serum Vitamin D levels in depression
**Normal range of Vitamin D is 30 – 100 ng/ml*

There was a significant difference between average vitamin D levels of mild, moderate and severe depression i.e, median (IQR) of vitamin D was 14.0 (8.0-20.0), 7.0 (7.0-13.0), 13.0 (6.0-16.0) ng/ml respectively, (Kruskal-Wallis; p= 0.006, Table Va), normal vitamin D is 30 – 100 ng/ml. Post hoc comparison of vitamin D levels between mild, moderate and severe groups showed that there was a significant difference of vitamin D between mild and moderate depression group (p=≤*0.001**; Mann-Whitney U; Table Vb) but no significant difference between mild and severe and moderate and severe groups (p=0.101; Mann-Whitney U; Table Vb), (p=0.116; Mann-Whitney U; Table Vb) respectively.

Out of 51 Mild Depressed group, 34 (66.7%) of patients had low serum vitamin D levels (Table IV). Median (IQR) vitamin D levels of all mild depressed group was 14.0 (8.0- 20.0) ng/ml (Table Va) and out of 34 moderate depressed group, 34(100%) of patients had low vitamin D levels (Table IV). Median (IQR) vitamin D levels of all moderate depressed group was 7.0 (7.0-13.0) ng/ml (Table Va). Out of 65 severe depressed group, 59 (90%) had low serum vitamin D levels (Table IV). Median (IQR) vitamin D levels of all severe depressed group was 13.0 (6.0-16.0) ng/ml, (Table Va).

There was a significant difference of vitamin D levels between males in individual groups of mild, moderate and severe depression (p= ≤0.001*; Table VIa). Post hoc comparison of vitamin D levels in mild, moderate and severe depression groups between males showed that there was a significant difference between mild and moderate depression (p=≤0.001*, Table VIb) and between mild and severe depression (p=≤0.001*,Table VIb).

Vitamin D	Mild Depression (n=51)		Moderate Depression (n=34)		Severe Depression (n=65)	
	Frequency	Percent	Frequency	Percent	Frequency	Percent
Low	34	66.7	34	100.0	59	90.8
Normal*	8	15.7	0	0	0	0
High	9	17.6	0	0	6	9.2
Total	51	100.0	34	100.0	65	100.0

Table-IV. Frequency distribution of serum Vitamin D levels in study groups according to Mild, Moderate and Severe Depression.
**Normal range of Vitamin D is 20 – 100 ng/ngml*

Sr. No	Parameter	Groups	N	Mean ± SD	Median (IQR)	Distribution
1	Vitamin D (ng/ml)	Mild Depression	51	17.3 ± 12.0	14.0 (8.0-20.0)	Non-normal
		Moderate Depression	34	9.79 ± 3.4	7.0 (7.0-13.0)	Non-normal
		Severe Depression	65	13.8 ± 8.8	13.0 (6.0-16.0)	Non-normal

Table-Va. Average and distribution of biochemical parameter in groups according to Mild, Moderate and Severe Depression.

Parameter	N	Groups	Median (IQR)	P- Value
Vitamin D (ng/ml)	9	Mild Depression	17.0 (17.0-43.0)	≤0.001*
	8	Moderate Depression	13.0 (13.0-15.0)	
	27	Severe Depression	13.0 (10.0-14.0)	

Table-VIa. Comparison of biochemical parameter between Males in individual groups of Mild, Moderate and Severe depression by Kruskal-Wallis test.
**p-value ≤ 0.05 is considered statistically significant*

Groups / Parameters	Mild vs Moderate Depression p-value	Mild Vs Severe Depression p-value	Moderate Vs Severe Depression p-value
Vitamin D (ng/ml)	≤0.001*	≤0.001*	0.343

Table-VIb. Posthoc comparison of Vitamin D between Males in individual groups according to Mild, Moderate and severe depression by Mann-Whitney U Test.
**p-value ≤ 0.05 is considered statistically significant*

FEMALES

Comparison of vitamin D between females in individual groups of mild, moderate and severe depression test showed a significant difference of vitamin D *(*Kruskal-Wallis; p=0.011, Table VIIa). Median (IQR) of vitamin D in mild, moderate and severe groups was 9.0 (8.0-20.0) ng/ml, 7.0 (6.8-11.0) ng/ml, 13.0 (5.7-16.7) ng/ml (Kruskal-Wallis test, Table VIIa), respectively.

Post hoc comparison of vitamin D levels in mild, moderate and severe depression groups between females showed that there was a significant difference between mild and moderate depression (p=≤0.001*; Mann-Whitneu U, Table VIIb) but no significance difference between mild and severe depression (p=0.667; Mann-Whitneu U, Table VIIb). There was also a significant difference of vitamin D between moderate and severe depression (p=0.47, Mann-Whitneu U, Table VIIb).

A significant negative correlation of vitamin D in all 44 male subjects with severity of disease was observed in our study (Spearman's rho= - 0.562, p = ≤0.001*; Table VIII).

A negative correlation of vitamin D with severity of disease was also found in all 106 female depressed patients in our study (Spearman's rho= -0.075, p = 0.444; Table IX).

Parameter	N	Groups	Median (IQR)	P- Value
Vitamin D (ng/ml)	42	Mild Depression	9.0 (8.0-20.0)	0.011*
	26	Moderate Depression	7.0 (6.8-11.0)	
	38	Severe Depression	13.0 (5.7-16.7)	

Table-VIIa. Comparison of biochemical parameters between Females in individual groups of Mild, Moderate and Severe depression by *Kruskal-Wallis test.*
**p-value ≤ 0.05 is considered statistically significant*

Groups / Parameters	Mild vs Moderate Depression p-value	Mild Vs Severe Depression p-value	Moderate Vs Severe Depression p-value
Vitamin D (ng/ml)	≤0.001*	0.667	0.047*

Table-VIIb. Posthoc comparison of Vitamin D between females in individual groups according to Mild, Moderate and severe depression by Mann-Whitney U Test.
**p-value ≤ 0.05 is considered statistically significant*

Biochemical Parameters	N	Rho	P=value
Vitamin D (ng/ml)	44 (Male)	-0.562	≤0.001*
Vitamin D (ng/ml)	106 (Female)	-0.075	0.444

Table-VIII. Correlations of Biochemical Parameters with severity of depression in Male & Female subjects by Spearman's Correlation.
**p-value ≤ 0.05 is considered statistically significant*

Depression/ Severity Gender	Mild Depression	Moderate Depression	Severe Depression	P- Value
Males	9 (20.5)	8 (18.2)	27 (61.4)	0.014*
Female	42 (39.6)	26 (24.5)	38 (35.8)	
Chi Square	8.584 (df=2)			

Table-IX. Association of gender with Severity of Depression.
**p-value ≤ 0.05 is considered statistically significant*

DISCUSSION

In this study, we investigated the association between vitamin D status and depression in our local Pakistani patients. Out of 150 depressed patients, 127 patients (84.7%) were vitamin D deficient. In our study, we observed that 60.7% of patients of mild depression had low vitamin D levels and 100% of moderately depressed patients were deficient with vitamin D, whereas 90.8% of severely depressed patients were vitamin D deficient in our study (Table III & IV).

Higher prevalence of vitamin D deficiency was found in both males and females in our study. There was a significantdifference of vitamin D levels between males in individual groups of mild, moderate and severe Depression (p= ≤0.001*; Table VIa). Post hoc comparison of vitamin D levels in mild, moderate and severe depression groups between males showed that there was a significant difference between mild and moderate depression (p=≤0.001*, Table VIb) and between mild and severe depression (p=≤0.001*,Table VIb). Post hoc comparison of vitamin D levels in mild, moderate and severe depression groups between females showed that there was a significant difference between mild and moderate depression (p=≤0.001*, Table VIIb) and between moderate and severe depression (p=0.047*,Table VIIb).

These results are in agreement with the reports documented earlier.[20-22] Additionally, a randomized study conducted by Jorde et al[23] in obese persons also reported that after 1 year of supplementation with vitamin D, subjects had significantly lower Beck Depression Inventory score and lower circulating parathyroid hormone without concomitant increase in calcium compared to those who received placebo. This study suggests a possible causal relationship between vitamin D status and depression.

In contrast to our findings, recently, Zhao et al[24] utilizing the data reported in NHANES 2005-2006 (n = 3916), found no significant association between serum concentrations of vitamin D and the presence of major depression. However, they observed a trend of decreasing depression with increasing quartiles of serum vitamin D concentration in both unadjusted and multivariate adjusted regression models.

Pan et al[25] also reported no significant association between vitamin D status and depression in Chinese adults aged, 50-70 y (n = 3262). It is very difficult to assess whether the differences observed between studies are due to true physiological differences or due to differences in methodology.

The mechanism through which vitamin D plays a role in mental health is not clearly understood. Vitamin Dpromotes neuritogenesis and stimulates the expression of nerve growth factor.[26] Several studies have shown that vitamin D is involved in

brain development and that its deficiency leads to altered behavior in adulthood and also results in altered morphology (enlarged ventricles and reduced cortical thickness as it occurs in schizophrenia).[27]

Active vitamin D promotes antioxidant activities and enhances glutathione metabolism in neurons, therefore protects them, from oxidative degenerative processes.[26-28] Vitamin D also regulates calcium homeostasis, axonal conduction and membrane permeability, it is suggested that it has an indirect role in the regulation of neurotransmission.[29]

In addition, it has been documented in many studies that vitamin D also regulates gene expression of tyrosine hydroxylase, an important enzyme in the synthesis of norepinephrine and dopamine.[30] Both neurotransmitters are involved in mood regulation and depression.[31]

Some methodological issues of the present study need to be considered here. Our study cannot demonstrate whether the observed association with vitamin deficiencies precedes or results from the depression.

CONCLUSIONS

Vitamin D deficiency is associated with an increased risk of developing depression, but more prospective observational studies may be needed. An association between serum vitamin D and cognitive impairment are of considerable public health importance in Pakistani population. The importance of vitamin D to many brain processes including neuro-immunomodulation and neuroplasticity suggests that it might have a role in psychiatric illness such as depression. A plausible hypothesis for this association is that low vitamin D levels cause neurotransmitter deficiency, which causes depression of mood. The study suggests dietary intervention with vitamin D would boost brain serotonin concentrations and help prevent and possibly relieve some of the symptoms associated with depression without side effects. There is little vitamin D present in food and fortification is still inadequate as is the amount in most multivitamin and prenatal supplements. Vitamin D supplements are inexpensive and offer a simple solution to raise vitamin D levels to an adequate status. In addition, vitamin D levels should be routinely measured in everyone and should become a standard procedure in prenatal care.

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"It is difficult to be patient
but to waste the rewards for patience
is worse. "

Hazrat Abu Bakr (R.A)

AUTHORSHIP AND CONTRIBUTION DECLARATION

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