

ORIGINAL RESEARCH

Clinical Depression; Are Biochemicals the Culprit Behind?

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ABSTRACT

Background: Depression is a medical condition, illness of the brain in which an individual is conquered by the feeling of sadness, hopelessness, guilt and so on. Depression is one of the commonest human blight, mental illness causing more 'years lost to disability' (YLD) than any other illness and the sufferer learns to live with it, adapts it both mentally and physically. WHO fact sheets states that depression affects nearly 350 million people; yet, difficult to recognise initially, then diagnose and ultimately treat. Since the disease creates disinterest in day-to-day activities, it also affects dietary pattern of the person, leading to inadequate, imbalanced diet essential for maintaining mental health. B Complex vitamins; pyridoxal phosphate, folate and cobalamin plays an important role in maintaining good physical and mental health as these act as co-enzymes and co-factors in metabolic processes. Biosynthesis of GABA, the inhibitory neurotransmitter of the brain requires PLP. **Aim and Objective:** Estimateserum levels of vitamin B6, B9, B12along with GABA, TSH and total cholesterol in controls and the cases. To evaluate the association of study biochemical variables with disease severity.

Materials and Methods: Present study is an experimental case control study performedon 204 people (102 controls and 102 patients) including both males and females between 18-65 years of age. Participants demoFigureic factors were recorded and biochemical variables were assayed. Hamilton Depression Rating Scale-17 items(HDRS) was used to rate the severity of depression.

Results: Most patients 47.1% enrolled in the study had mild form of depression. Females, married individuals, less educated and those belonging to middle income group of individuals of the study were more depressed. Serum levels of vitamin B6, B9, B12 were much lower in depressed subjects. Whereas, GABA levels were elevated in depressive patients.

Conclusion: Findings of the resultant study shows low levels of pyridoxal phosphate, folate, cobalamin and total cholesterol and higher GABA levels in newly diagnosed depression patients.

Keywords: Depression and biochemical variables, B complex vitamins- Pyridoxal phosphate, folate, cobalamin, GABA, total cholesterol, HDRS.

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INTRODUCTION

Depression is the most common type of mental illness. It negatively affects the feeling, thinking and actions of the individual. It requires the understanding of the disease in addition to the medical care.^[1]Hallmark of depression is dangerousness to self - that is suicide. Depression is notorious as it can express itself in disguise, with a number of ailments, it may

also co-occur with many other disease conditions.^[2] It is a leading cause of disability worldwide with a major share of overall global burden of disease. WHO has stated a global prevalence of 5% and in India about 4.5% of adults suffer from depression,^[3,4] affecting nearly 1 in 15 adults in a given year. Prevalence of the sickness is more in women, nearly 50% higher as per WHO 2008 in old people and additionally in those living under adverse socio-economic circumstances.^[5] It is forecasted that by the year 2030, depression will account for lifetime prevalence of 6.2% with a leading cause of disease burden of life.^[6]

Characteristic features of depression are feeling of severe despondency, dejection, sadness, hopelessness, guilt, agitated, worthlessness, sadness, tiredness, poor concentration, lack of interest in previously enjoyable activities, disturbed sleep and appetite and at times going to the extent of suicide ideation. As depression can captivate anybody rich or poor, young or old, men or women it has a profound effect on various aspects of life; relationships with family and friends, society, performance at school/work. The disease is long lasting and can be recurrent, affecting normal day to day functioning of the person with a rewarding life.^[7]

Contributing factors for developing depression could be heredity, adverse life events like loss of near and dear one, bereavement, trauma at younger age which might alter the responding ability of the brain to stressful, fearful situations; interpersonal events may act as catalysts - parental disharmony, violence, finances, marital upheavals, in some medical illness like hypothyroidism, Diabetes Mellitus, attention-deficit hyperactivity disorder (ADHD) etc. Depression is observed in nearly 30% of drug and alcohol abusers.^[8] Diagnosis of depression is based on minimum of 3-5 persistent symptoms for more than two weeks. Types of Depression three main types of depression are -Unipolar depression (Major depressive disorder), Bipolar disorder (manic depressive illness) and dysthymic disorders/dysthymia. Other types of depression can be post-partum, teen-age depression, seasonal depression etc.

Diet is an important modifiable factor concerning the physical and mental health, especially the vitamins; though required in minor quantities, yet vital for normal body functions via various metabolic processes. It is proven that some of the B complex vitamins like B9, vitamin B12 facilitate the synthesis of monoamine neurotransmitter, suggestive of being involved in developing depressive symptoms. Most of B complex vitamins can neither be synthesised nor stored in the body for a long time so need to be ingested in diet on regular basis, especially vitamin B12 can be acquired from animal source or dairy products. Vitamin B6 is a cofactor in tryptophan-serotonin pathway and serotonin, being an important neurotransmitter in brain functioning, therefore, if pyridoxal phosphate (PLP) is reduced there of chances of developing depression. Various population studies have demonstrated the importance of B complex vitamins in easing depression to some extent.^[9,10]

Theory of Neuro-chemical's functioning in the brain is well established and any kind of imbalance in their levels might disturb the neural circuits responsible for regulating sleep, thinking process, mood, behaviour, appetite, emotions etc. Gamma amino butyric acid (GABA) plays a pivotal role as an inhibitory neurotransmitter; it reduces/blocks/discourages the empowered action of other excitatory neurotransmitters hence aids to execute smooth functioning of the brain. If GABA levels are out of range it might either lead to over control of other brain chemicals or when less might allow the hyperactive neurotransmitters over stimulated to create havoc in an individuals.^[11,12]

Thyroid stimulating hormone (TSH/thyrotropin) secreted by the thyrotrope cells of the anterior pituitary gland, stimulates the thyroid gland to produce tri-iodothyronine (T3) and thyroxine (T4), thus regulating the endocrine function of the thyroid gland. Thyroid hormones play an important role in the pathogenesis of depression by affecting the non-adrenergic and serotonergic neurotransmission.

Cholesterol is the paramount constituent of plasma membranes in higher eukaryotes and is important in regulating the functioning of the cell membrane, organisation, cell sorting in

addition the magnanimous dynamic role it plays in neurotransmission in the CNS. Studies over the years have revealed that a relationship does exist between various psychiatric illnesses including depression and serum cholesterol levels.^[13] In Adults having type II diabetes mellitus, about 1 in 4 had significant symptoms of depression as well as per Peyrot M et al. Symptoms of depression tend to persist for a long time in diabetics.^[14]

The present study was undertaken as there is a limited knowledge and source of information on the role of dietary B complex vitamins; vitamin B6, vitamin B9 and vitamin B12 in association with clinical depression and its severity especially in the Indian population.

MATERIALS & METHODS

Study design and participants recruited: Present research study was a single-centric experimental case control study conducted at Santosh Hospitals, Ghaziabad, Delhi, NCR during the year 2017-2018. Total participants enrolled in the project were 204 of whom 102 were cases and 102 controls. Criteria of Selection: Inclusion criteria: Both males and females in the age group of 18-65 years were recruited in the study. Cases group comprised of newly diagnosed clinically depressed patients attending the OPD of Psychiatry of the hospitals. Individuals enrolled in the control group were age and sex matched healthy consenting volunteers of the same place. Exclusion criteria: Same benchmark was followed for both cases and control group subjects. Known cases of depression or any other psychiatric illness under treatment, mental retardation or patients with bipolar disorder with manic episodes were not included in the study. Individuals on vitamin supplements or fluid therapy or with substance abuse disorder (except alcohol, nicotine and caffeine) were exempted from the study.

Institutional ethical clearance was sought for undertaking the research project [F.No.SU/2017/683 (2)]. Patient's performa was filled for the demographic variables and a written informed consent was taken after a brief explanation of the study to all the participants. Identification number of the participants and their results of the biochemical variables were stored in a confidential environment. Standardised protocol was followed for conducting the Laboratory tests. Approximately 5ML of venous blood sample was collected from all the study participants, serum separated and said biochemistry tests were performed.

Methods: Serum Cobalamin (vitamin B12) and Folate (vitamin B9) were estimated by Chemiluminescence (Beckman & Coulter, USA and E COBAS-e 411 respectively). Estimation of Pyridoxal phosphate (PLP/ vitamin B6) and Gamma amino butyric acid (GABA) were done by Sandwich ELISA and Competitive ELISA methods respectively (BIO-RAD 680). TSH analysis by Immunoassay on COBAS-e 411, total cholesterol by CHOD-PAP end point method and Glucose by GOD-POD enzymatic method on an auto analyser (ARK- CKK 200).

To assess the category of depression, Hamilton Depression Rating Scale (HDRS-17 items) was used. HDRS, also known as Ham-D; most widely used, clinician-administered depression assessment scale, measuring severity of depression based on grading scale: No depression 0-7; mild depression 8-13; moderate depression 14-18; severe depression 19-22 and scores >23 very severe depression. The patient's depression was graded on the basis of Psychiatrist's clinical consultation and information provided by family members and nursing staff. The range of alpha reliability coefficients was between 0.766-0.810.^[15]

Statistical Analysis

The data was expressed as mean \pm SD. Independent sample t-test was used to compare age, baseline parameters and specific serum assays of vitamin B6, B9, B12, and GABA levels in both the groups the patients and the healthy controls. The study results were considered

statistically significant when p value was $p < 0.05$, at 95% confidence interval. Probability of type 1 error (α) value of 5% (0.05) and power ($1-\beta$) 90% (0.8). Pearson's correlation coefficient was computed between scores of Hamilton depression rating scale (HDRS) and the baseline levels of biochemical variables.

Univariate analysis was used to compare the exposure and risk factors between cases and the controls group. The proportions for categorical variables in both groups was calculated by Pearson's chi-square test. Further, the normally distributed continuous variables, were summarised with the mean and the standard deviations and student's t-test was used to compare the difference between the two groups. We also used Spearman's rank correlation between HDRS and other continuous variables; and GABA and serum vitamins (vitamin B6, vitamin B9 & vitamin B12) to explore their correlation structure.

In the second model, the HDRS scores were categories into ordered categories that includes no depression (0 – 7), mild to moderate (8 – 18), and severe to very severe depression (≥ 19). We fitted full model by including demographic and biochemical parameters. All statistical analysis was performed on Stata version 14.2 (StataCorp, College Station, TX, USA). A two-tailed p value < 0.05 was considered statistically significant for the analysis.

RESULTS

In the present research study, total subjects enrolled were 204 (102 cases and 102 controls) with 107 males (52.5%) and 97 females (47.5%). While distributing the participants based on age group, 45 were < 25 years of age, 66 people were between 26-35 years, 56 in the age range of 36-49 years and the remaining 37 individuals age was above 50 years [Table 1].

Table 1: Comparison of various demographic variables between cases & controls (t-test)

Variables	Control		Case		Total		p-value
	n	%	n	%	n	%	
Total patients	102		102		204		
Age group (years)							
≤ 25	22	21.6	23	22.5	45	22.1	0.282
26-35	39	38.2	27	26.5	66	32.4	
36-49	26	25.5	30	29.4	56	27.5	
≥ 50	15	14.7	22	21.6	37	18.1	
Mean age \pm SD	35.2 \pm 11.4		37.2 \pm 12.2		36.2 \pm 11.8		0.221
Sex							
Male	60	58.8	47	46.1	107	52.5	0.068
Female	42	41.2	55	53.9	97	47.5	
Marital status							
Married	80	78.4	66	64.7	146	71.6	0.030
Unmarried	22	21.6	36	35.3	58	28.4	
Educational status							
< 12 th Grade	43	42.2	63	61.8	106	52.0	0.005
≥ 12 th Grade	59	57.8	39	38.2	98	48.0	
Variables		Controls		Cases		Total	p- Value
	n	%	n	%	n	%	
Employment status							
Yes	57	55.9	30	29.4	87	42.6	$p < 0.001$
No	45	44.1	72	70.6	117	57.4	
Income group							

Low	40	39.2	44	43.1	84	41.2	0.206
Middle	50	49	53	52	103	50.5	
High	12	11.8	5	4.9	17	8.3	
Area of residence							
Urban	63	61.8	65	63.7	128	62.7	0.772
Rural	39	38.2	37	36.3	76	37.3	
Diet group							
Veg	64	62.7	59	57.8	123	60.3	0.474
Non-veg	38	37.3	43	42.2	81	39.7	

[Table 1] show females 53.9% (n=55) are more prone to depression than men with a less statistically significant result $p=0.068$. On comparing the educational ground, 61.8% of the cases were less than 12th grade qualified than the more educated ones and the results were of statistical significance $p= 0.005$. The unemployed patients were 70.6% in cases group and 44.1% in controls group with a highly significant $p <0.001$. Majority of our study subjects belonged to urban areas. On analysing the dietary pattern of the participants, most of them were on vegetarian diet.

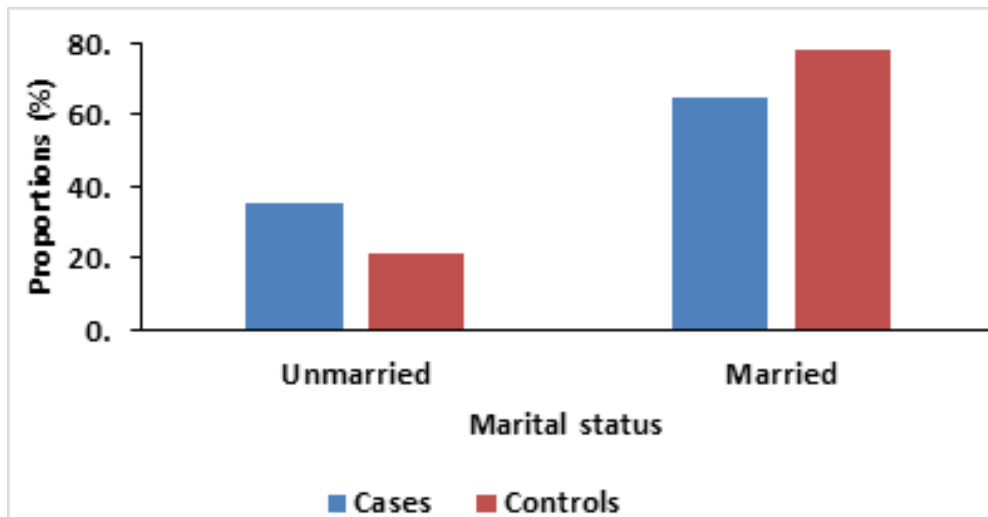


Figure 1: Bar diagram for comparing the marital status in both cases and controls group

[Figure 1] depicts that general study participants were married 71.6% (n=146) but married people were more depressed as well 64.7% (n=66) and the results showed a significance of $p=0.030$ [Table1].

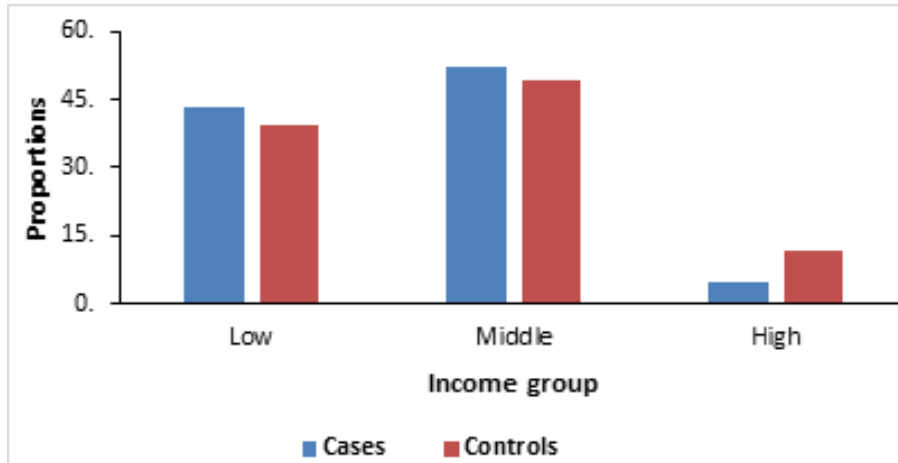


Figure 2: Bar diagram for comparing the mean socio-economic status in both groups [Figure2] shows that the individuals belonging to middle-income group alone comprised half the number of cases with a higher proportion 52% of depression (n=53) than the low 43.1% (n=44) or high income 4.9% (n=5) groups [Table1] with statistically non-significant results.

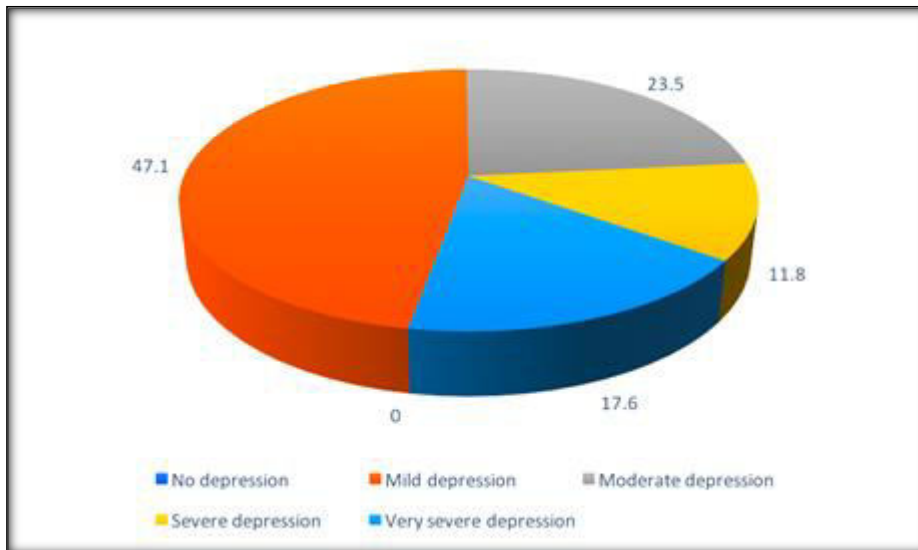


Figure3: Pie diagram depicting the distribution of depression among cases in %

[Figure 3] represents the grades of depression based on Ham-D scale. Of the total number of cases, Majority, 47.1% belonged to the mild category of depression, 23.5% in moderate type of the disease, followed by 11.8% in severe depression category and the remaining 17.6% individuals suffered severe type of depression.

Table 2: Comparison of biochemical variables between two groups (t-test)

Tests in Serum	Control					Case					p-value
	Mean	SD	Percentiles			Mean	SD	Percentiles			
			25 th	50 th	75 th			25 th	50 th	75 th	
Vit B12 (pg/ml)	393.5	199.6	247.8	336.0	437.5	209.5	262.5	87.8	130.5	200.8	p<0.001
Vit B9 (ng/ml)	5.8	1.4	4.9	5.5	6.6	4.9	4.8	1.4	3.1	6.6	p<0.001
Vit B6 (ng/ml)	28.4	9.6	21.4	28.0	33.9	7.8	5.9	5.1	6.5	9.3	p<0.001
GABA (pg/ml)	1626.6	1394.7	733.0	1250.5	2022	5298.7	3214.7	2770.5	4519	7968.8	p<0.001

TSH (mIU/ml)	2.7	1.3	1.7	2.3	3.2	4.7	20.3	1.2	1.9	3.3	0.006
T.Chol (mg/dl)	202	30.8	189.0	207.0	220.1	97.7	41.0	70.0	82.0	109.6	p<0.001
GLC (mg/dl)	115.4	37.0	83.8	110.9	135.2	113.9	57.9	80.0	91.5	134.4	0.037

Vit B12: Vitamin B12, Vit B9: Vitamin B9, Vit: Vitamin B6, GABA: Gamma amino butyric acid, TSH: Thyroid Stimulating Hormone, T. Chol: Total Cholesterol and GLC: Glucose.

[Table 2] highlights the comparison of the mean for individual biochemical variables in cases and the controls group. The resultant mean of the three study B complex Vitamins; Vitamin B12, B9 and B6 in control group subjects were 393.5 pg/ml (SD=199.6), 5.8ng/ml (SD=1.4) and 28.4ng/ml (SD=9.6) the values were within the reference range for each. Whereas in cases group, the levels of these vitamins, Vitamin B12, B9 and B6 were reported to be very low; 209.5pg/ml (SD=262.5), 4.9ng/ml (SD=4.8) and 7.8ng/ml (SD=5.9) the results were of high statistical significance, p<0.001. On comparing the levels of the neurotransmitter GABA it revealed an estimate of 1626.6pg/ml (SD=1394.7) in controls but the levels of GABA in the depressed subjects were elevated more than twice the normal subjects 5298.7pg/ml (SD=3214.7) and the results of our study were proven to be statistically significant P< 0.001. TSH levels were noted to be within the biological range in controls 2.7mIU/ml (SD=1.3) and slightly towards the upper limit in depression cases 4.7mIU/ml (SD=20.3). On taking Total cholesterol into consideration, in controls the levels were 202mg/dl (SD=30.8) whereas in cases group of individuals there was a significant decrease in the levels of cholesterol 97.7mg/dl (SD=41.0) with a significant result P< 0.001.

Table 3: Comparison of Demographic variables with HDRS Scores

Variables	HDRS Category						Total (N=204)		p-value
	No depression (n=102)		Mild to moderate (n=72)		Severe to very severe (n=30)		n	%	
	n	%	N	%	n	%			
Age group (years)									
<=25	22	21.6	19	26.4	4	13.3	45	22.1	0.222
26-35	39	38.2	20	27.8	7	23.3	66	32.4	
36-49	26	25.5	21	29.2	9	30.0	56	27.5	
>=50	15	14.7	12	16.7	10	33.3	37	18.1	
Sex									
Female	42	41.2	42	58.3	13	43.3	97	47.5	0.073
Male	60	58.8	30	41.7	17	56.7	107	52.5	
Marital status									
Unmarried	22	21.6	27	37.5	9	30	58	28.4	0.070
Married	80	78.4	45	62.5	21	70	146	71.6	
Educational status									
<12th	43	42.2	46	63.9	17	56.7	106	52	0.016
>=12th	59	57.8	26	36.1	13	43.3	98	48	
Employment status									
No	45	44.1	52	72.2	20	66.7	117	57.4	0.001
Yes	57	55.9	20	27.8	10	33.3	87	42.6	
Income group									
Low	40	39.2	30	41.7	14	46.7	84	41.2	0.480
Middle	50	49	39	54.2	14	46.7	103	50.5	

High	12	11.8	3	4.2	2	6.7	17	8.3	
Area of residence									
Rural	39	38.2	29	40.3	8	26.7	76	37.3	0.414
Urban	63	61.8	43	59.7	22	73.3	128	62.7	
Diet									
Veg	64	62.7	38	52.8	21	70	123	60.3	0.209
Non-veg	38	37.3	34	47.2	9	30	81	39.7	

Above Table gives a comparison of HDRS categories (mild to moderate and severe to very severe) with demographic variables. Severe form of depression was found in the older lot above 50 years of age 33.3% (n=10). Females were more of mild-moderate type 58.3% (n=42) whereas, 56.7% (n=17) men were suffering from severe depression though the results were non-significant (p=0.073).

Regarding marital status, depression was prevalent more in married people with 70% (n=21) and 62.5% (n=45) in severe and moderate category of depression respectively though the statistical significance was not established (p=0.070). Individuals with lower educational qualification (< 12th grade) were 63.9% (n=46) in mild / moderate category and those with \geq 12th grade 56.7% (n=17) having severe type of the disease and the results were of statistical significance (p=0.016). HDRS category when compared to unemployed and employed groups, mild to moderate category were (72.2%), (27.8%) and severe to most severe category were (66.7%), (33.3%) respectively, with a very high statistical significance (p=0.001). Most of the subjects of our study were vegetarian in nature but most 70% (n=21) in severe category and 52.8% (n=38) in mild/moderate category with statistical insignificance (p=0.209).

Table 4: Comparison of biochemical variables based on HDRS categories (Chi square)

Variables	No depression (n=102)		Mild to moderate (n=72)		Severe to very severe (n=30)		p-value
	Mean	SD	Mean	SD	Mean	SD	
Age (years)	35.18	11.37	35.68	11.97	40.87	12.18	0.073
Vit B12 (pg/ml)	393.50	199.56	191.63	254.50	252.27	280.54	p<0.001
Vit B9 (ng/ml)	5.84	1.38	4.78	4.20	5.08	6.01	p<0.001
Vit B6 (ng/ml)	28.36	9.60	8.83	6.76	5.25	1.35	p<0.001
GABA (pg/ml)	1626.57	1394.66	5184.96	3105.22	5571.73	3503.48	p<0.001
TSH (mIU/ml)	2.70	1.31	5.83	24.15	2.00	1.42	0.003
T. Chol (mg/dl)	202.01	30.85	98.46	43.47	95.94	35.15	p<0.001
GLC (mg/dl)	115.41	37.01	114.65	56.59	112.28	61.78	0.080

Vit B: B complex vitamins, Vit B12: cobalamin, Vit B9: folate, Vit B6 pyridoxal phosphate, GABA: Gamma amino butyric acid, TSH: Thyroid Stimulating Hormone, T. Chol: Total Cholesterol and GLC: Glucose.

Table highlights a correlation between various biochemical variables and HDRS categories (mild/ moderate and severe/ very severe). Statistical Mean of serum vitamins in the category of mild to moderate depression was, vitamin B12 =191.63 (SD+254.50), B9 =4.78 (SD+4.20) and B6 =8.83 (SD+6.76) whereas, in severe to very severe categories the mean for all three vitamins; B12 252.27 (SD+280.54), B9 5.08 (SD+6.01) and B6 was found to be 5.25 (SD+1.35) and the results were of very high statistical significance (p<0.001). The neurotransmitter GABA, in mild to moderate and severe to very severe categories showed a mean of 5184.96 (SD+3105.22) and 5571.73 (SD+3503.48) respectively which was of high

significance ($p < 0.001$). Similarly, the mean of Total Cholesterol was 98.46 (SD+43.47) in mild to moderate type and in severe to very severe depressive condition 95.94 (SD+35.15) with statistically significant results ($p < 0.001$). The mean of TSH was found to be statistically significant ($p = 0.003$), with mean 5.83 (SD+24.15) in mild to moderate and 2.00 (SD+1.42) in the other category.

Table 5: Correlation of HDRS scores and biochemical variables (Spearman's Rho)

Parameters	Control (n=102)		Case (n=102)		Total (N=204)	
	Spearman's rho	p-value	Spearman's rho	p-value	Spearman's rho	p-value
Age (years)	0.219	0.027	0.11	0.270	0.148	0.034
Vit B12 (pg/ml)	0.035	0.728	0.091	0.363	-0.526	$p < 0.001$
Vit B9 (ng/ml)	0.17	0.089	-0.128	0.200	-0.328	$p < 0.001$
Vit B6 (ng/ml)	-0.017	0.868	-0.511	$p < 0.001$	-0.783	$p < 0.001$
GABA (pg/ml)	0.143	0.151	0.065	0.517	0.63	$p < 0.001$
TSH (mIU/ml)	0.187	0.060	-0.101	0.313	-0.164	0.019
Total cholesterol (mg/dl)	0.031	0.755	-0.07	0.484	-0.702	$p < 0.001$
GLC (mg/dl)	0.191	0.055	-0.041	0.679	-0.097	0.169

Vit B12: Vitamin B12, Vit B9: Vitamin B9, Vit: Vitamin B6, GABA: Gamma amino butyric acid, TSH: Thyroid Stimulating Hormone, T. Chol: Total Cholesterol and GLC: Glucose.

Above table shows the Spearman's rho correlation between HDRS score and biochemical variables, both positive and negative correlation was observed in the two groups. Vitamin B12 showed a positive correlation in both the controls 0.035 pg/ml ($p = 0.728$) and in cases 0.091 pg/ml ($p = 0.363$). Vitamin B 9 showed a positive correlation 0.17 ng/ml ($p = 0.089$) in controls and a negative correlation -0.128 ng/ml ($p = 0.200$) in cases. Vitamin B6 revealed negative Spearman's rho correlation in both the groups; controls -0.017 ng/ml ($p = 0.868$) and in cases -0.511 ng/ml ($p < 0.001$) with an overall statistically significant negative correlation with stages of depression ($p < 0.001$).

However, GABA revealed an overall positive correlation with HDRS, 0.143 and 0.065 in controls and cases respectively, and the resultant findings were of high statistical significance ($p < 0.001$). Total cholesterol showed a negative correlation with the HDRS in general and a positive correlation (0.031) in controls and a negative correlation in cases (-0.07) with high significance $p < 0.001$. TSH showed a positive correlation with disease severity but in controls group it was positively correlated (0.187) and a negative correlation (-0.101) in cases with less significant results $p = 0.019$.

Table 6: Correlation between GABA & serum vitamins stratified by their group

Serum vitamins	Control (n=102)		Case (n=102)		Total (n=204)	
	Spearman's rho	p-value	Spearman's rho	p-value	Spearman's rho	p-value
Vit B12 (pg/ml)	-0.084	0.400	0.197	0.048	-0.398	$p < 0.001$
Vit B9 (ng/ml)	0.095	0.344	-0.002	0.987	-0.251	$p < 0.001$
Vit B6 (ng/ml)	0.061	0.542	-0.180	0.071	-0.570	$p < 0.001$

Vit B12: Vitamin B12, Vit B9: Vitamin B9, Vit: Vitamin B6, GABA: Gamma amino butyric acid

[Table 6] shows Spearman's correlation coefficients between GABA scores and serum vitamins. Among controls, GABA showed a weak negative correlation with vitamin B12; $\rho = -0.084$ ($p = 0.400$), whereas GABA was positively correlated with vitamin B9; $\rho = 0.095$ ($p = 0.344$) and also with vitamin B6; $\rho = 0.061$ ($p = 0.542$). Whereas in the cases group GABA showed some positive correlation with vitamin B12; $\rho = 0.197$ ($p = 0.048$) whereas it was negatively correlated with vitamin B9; $\rho = -0.002$ ($p = 0.987$) and vitamin B6; $\rho = -0.180$ ($p = 0.071$) which was poorly significant. Overall the GABA scores revealed an inverse correlation with study vitamins exhibiting a negative Spearman's ρ ; vitamin B12 = -0.398 , B9 = -0.251 and B6 = -0.570 and the results were of high statistical significance $p < 0.001$.

Biological reference range for vitamin B12 = 211-911pg/ml, folate = 3-17ng/ml, PLP = 5-50ng/ml, GABA = 30-2000pg/ml, TSH = 0.4-5.5mIU/ml, total cholesterol = 140-250mg/dl and Glucose = 60-200mg/dl (16).

DISCUSSION

Depression is one of the major human blight, an illness of the brain, responsible to cause more 'years lost to disability' (YLD) than any other illness and the sufferer learns to live with it, adapting it both physically and mentally. As per WHO fact sheet nearly 350 million people are affected by it, yet, the disease should be first recognised, then diagnosed and finally treated. Depression is impartial to any demographic, socio economic barrier or any religion all over the world including India. India is home to an estimated 57 million people (18% of the global estimate) affected by depression.^[17] Sandeep Grover et al., submitted that India is witnessing significant demographic and epidemiological transition, leading to a rapid increase in depressive cases which can lead to about 5.7% in the coming years.^[18] Therefore, we chose to record the demographic factors along with the biochemical variables in our study population.

In the current multivariate research study conducted on 204 subjects (102 cases + 102 controls) and the mean for all the demographic and the biochemical variables was calculated. When various categories such as mild, moderate, severe and very severe types of depression were analysed, we found that majority of our study patients belonged to the mild category (47.1%) [Figure 3]. As stated by most researches, depression can affect any person of any age groups at any point of time; immaterial of caste, creed, colour or religion, rich or poor, male or female. In the present study, average age of the mildly depressed patients was 37.2 ± 12.2 years and severely depressed patients were above 50 years [Table 3]. A study performed by Seon- Chloe Park et al., specified mean age for the first onset of major depressive symptoms as 37.4 years which was similar to our patients. Whereas, some earlier studies had mentioned 26 years for the development of the sickness.^[19] In a cross-sectional study performed by Barua. A et al., it was said that depression increases with age.^[20]

Demographic factors and depression

On comparing various demographic factors in the two groups of study participants, it was evident that more percentage of women 53.9% were depressed [Table 1]. About 58.3% of females enrolled in the study had mild to moderate category of sickness and men 56.7% of men ($n = 17$) witnessed severe to very severe type of depression with $p = 0.073$ [Table 3]. Our results were in concurrence with a report by Sandeep Grover et al., which stated that the estimates of the Global Burden of Disease for both point prevalence and one year prevalence of unipolar depression was observed more in women than in men.^[18] Another incidence study by Patel. V et al., reported that being women is in itself a risk factor for developing depressive symptoms; additionally poverty, being single, difficult married life could be the reasons.^[21]

On taking marital status into account, married people were more in both the groups [Figure 1]. Present study revealed contraindicating results narrating 64.7% of married people suffering from depression and the results were of appropriate statistical significance $p=0.030$ [Table 1]. A study performed by Ramachandran V et al., had reported that depression often captivates the widowed or divorced or the lonely distressed person.^[22] On comparing the marital status with the HDRS scores, it was found that married people about 70% had severe / very severe type of disease and 62.5% had mild / moderate type with non significant results $p=0.070$ [Table 3].

In the resultant study, people with less education and those who were unemployed majorly fell a prey to the clutches of depression. Depressed patients below 12th grade were 61.8 % and the results were of statistical significance $p=0.005$ [Table 1]. While dividing the less educated depressed subjects in the two categories, it was evident that majority 63.9% had milder form and 56.7% had severe type of depression with poor significant results $p=0.016$ [Table 3]. Nearly 70.6% of the enrolled patients were unemployed proving that they are the major sufferers of the disease and the results were of high statistical significance $p<0.001$ [Table 1]. On distributing the unemployed patients in two categories based on HDRS scores, it was evident that 72.2% had milder form of the disease and 66.7% had severe depression with highly significant results $p=0.001$ [Table 3], thus elaborating the role of socioeconomic factors in contributing to depression in due course of time.

On observing the statistics of the economic condition, depressed people in middle income group were 52% followed by low income people 43.1% [Table 1 & Figure 1]. On analysing the category of depression in different income groups, it was found that in mild-moderate category of depression, there were about 54.2% of the subjects from middle income group followed by 41.7% from low income group. Severely depressed subjects from both middle and low income groups were 46.7% and from high income there were 6.7%, however our findings were not significant $p=0.480$ [Table 3]. Study results are supported by another study conducted by Ramachandran V et al.^[22] Current study was similar to few other studies; Vikram P et al. revealed the same fact that of the many causes of depression, social disadvantages, such as low education played a crucial role in developing depression.^[23]

Zsuzsanna Jakab et al., in a foreword in WHO booklet "Impact of economic crises on mental health" (2011) that the incidences of depression could be because of a rise in unemployment, significant decline in economy, increased number of people living below poverty line etc. An epidemiological study from UP conducted by Sadia et al., showed depression (9.7% prevalence) as most common psychiatric sickness and the socially, educationally and economically weaker section people are more prone to develop this disorder.^[24]

Some recent nutritional studies made emphasis on the importance of diet, which if not taken in adequate amounts, may lead to depression and other diseases. In the cases group, the vegetarian subjects were more (57.8%), than the non-vegetarian population (42.2%) but the results were not statistically significant $p\text{-value}= 0.474$ [Table 1]. There-fore, we included dietary habits as one of the demographic parameters in our study.

Role of B Complex Vitamins in Depression

It was published in Nutritional Corporation that there are a plethora of nutrients responsible for proper functioning of the body including the biomolecules and a vast number of vitamins and minerals, which are required in minor quantities yet inevitable. Most of our study subjects in both groups were vegetarians and the herbivorous diet consists of only adequate amounts of some of these vitamins and the traditional ways of Indian cooking leads to evaporation of most of them, thereby depleting the amounts of B vitamins in our body.

Literature studies propose various reasons for an individual to experience deficiency of B complex vitamins; insufficient dietary intake, malabsorption, genetic diseases, few nutrient

interfering drugs or an unnoticed, underlying medical condition. As age advances, the process of ageing itself may create feelings of loneliness, worthlessness or routine biological processes like absorption, transport and metabolism of these vitamins might be affected. A recent systematic review conducted by Ter Borg, S et al., in a large old community dwellers of the developed Western countries reported high prevalence of low dietary intake of B-vitamins (i.e., below the estimated average requirement, EAR); vitamin B6, folate deficiency which could be a potential public health concern.^[25]

Most B complex vitamins; vitamin B6, B9 and B12 perform vital roles as co-enzymes and co-factors and help in maintaining one-carbon metabolism in the body and they need to be taken in diet regularly. It has been emphasised by various researches that there is an association between depression and low levels of these vitamins, possibly mediated by homocysteine or the synthesis of monoamines in the brain.^[26] Theory does exist showing a link between pyridoxal phosphate, folate and cobalamin vitamins by way of the three interconnecting cycles. Folate cycle gets linked with the methionine cycle and trans-sulphuration cycle, which is PLP dependent.^[27]

Vitamin B6

Vitamin B6, in its active form as PLP (pyridoxal phosphate) performs a very important role in the body in Homocysteine metabolism, as a coenzyme in transamination, decarboxylation reactions, in GABA Shunt pathway and contributes in the synthesis of serotonin and dopamine, from the amino acids tryptophan and tyrosine respectively which in turn are responsible for stimulating the brain activity and hence also called as stimulatory neurotransmitters. If the levels of PLP were determined to be below normal, then these neurotransmitters might not be synthesised optimally or might be blocked, which might lead to low mental state or depression (28). It has been said that in old people, most of the vitamins generally decrease but more often vitamin B6. In a research work by Bernstein AL et al., pyridoxal phosphate plays a vital role on a therapeutic adjunct in cases of neurotransmitter abnormalities based on the fact that PLP is essential for the synthesis of the serotonin and the catecholamine.^[29]

In the present study, we applied student's t-test to compare various study vitamins between the two groups, the cases and the controls it was observed that at 50th percentile average vitamin B6 level in the healthy controls was 28.0 ng/ml and in patients it was much reduced 6.5 ng/ml (biological reference range 5-50 ng/ml) [Table 2]. Chi-Square test was used to compare the levels of vitamin B6 with HDRS scores. Very low levels of PLP were seen in severe category of depression 5.25 ng/ml as compared to mild-moderate category 8.83 ng/ml and the results were of statistical significance $p < 0.001$ [Table 4]. For the type of association between vitamin B6 and severity of depression Spearman's Rho correlation was applied and found that vitamin B6 was negatively correlated with depressive scores -0.511 ng/ml with highly significant p value $p < 0.001$ [Table 5]. Another supportive study by Williams A et al.,^[30] on the older adults stated that improving the vitamin B6 intake lowered the chances of depression. However in one of the studies it was said that the symptoms of depression had no relationship with PLP concentration.^[31]

Vitamin B9

Folate plays a lead role in the synthesis of some key proteins & RBCs. Even in the early 1960s, deficiency of folate in individuals was seen to be associated with Depressive symptoms. Results of the present study serum folate levels within the biological reference range (3-17 ng/ml) in the controls group 5.8 ng/ml but a decline in its levels was seen in depressed subjects 4.9 ng/ml (Normal range in males 4.5-32.2 ng/ml and in females 4.8-37.3

ng/ml) a statistically significant $p < 0.001$ [Table 2] establishing a mild negative correlation with the disease. Chi-Square test revealed a statistically significant inverse association of folate with depression [Table 4]. Spearman's Rho correlation showed a negative link between the values of vitamin B9 in cases and the controls group and results were significant $p < 0.001$ [Table 5]. Student's t-test showed folate levels lowered in cases 4.9ng/ml as well as in controls 5.8ng/ml [Table Whenever there is folate deficiency, its quiet but natural that mental symptoms would appear even before anaemia sets in or in other features like apathy, poor memory, withdrawal and reduced basic mental powers which may be due to reduced RNA Synthesis.

Vitamin B 12

Vitamin B12 is synthesised only in bacteria. Therefore an external source of vitamin B12 is required by most of the eukaryotes. The deficiency or inadequacy of vitamin B12 may lead to stunting of brain and development of intellect (33). It is Folate, Vitamin B12 and homocysteine in combination plays a pivotal role in facilitating the synthesis of few indispensable neurotransmitters; the monoamines, serotonin, catecholamines etc., by way of methylation process (one carbon metabolism) (34). If improper synthesis of these neurotransmitters it might lead to mental disorders including Depression. Some researches had found reduced levels of vitamin B12 among depressed patients (35). Similar results were obtained in our study participants with a lowered mean for vitamin B12 in patient group (209.5pg/ml \pm 262.5) in comparison to the control group (393.5pg/ml \pm 199.6) (Biological range 211-911pg/ml)[Table 2] with the results of statistical significance $p < 0.001$. Chi Square test showed a lower level of cobalamin in mild moderate category of depression than in the severe form of the disease 191.63pg/ml and 252.27pg/ml accordingly [Table 4]. Spearman's Rho correlation of cobalamin and HDRS scores were negative thus putting forth inverse correlation with depression and the results were of statistical significance $p < 0.001$ [Table 5].

Our study reveals lower levels of the estimated serum vitamin B6, vitamin B9 & vitamin B12 in depressed cases than in controls group, which was endorsed by a statistically significant p-value $p < 0.001$. The appropriate reason could be the interlinked cycles: folate cycle, the methylation cycle and trans-sulphuration cycle, if any of these three vitamins are absent or decreased, then there is an interrupted methylation metabolism which leads to decreased synthesis of methionine (cofactor cobalamin), N 5methyltetrahydrofolate (cofactor folate) and accumulation of homocysteine (cofactor vitamin PLP).^[27] Thus, depressed people, due to the apathy in their life, feeling of hopelessness might not be consuming healthy, nutritious diet rich in these vitamins thereby reduction in their levels.

Role of Neurotransmitter

In addition to the vitamins, neurotransmitters help to perform a balanced neuron specific functions either regulatory or inhibitory. GABA is being used as the neurotransmitter in at least 40% of the inhibitory synaptic processing in the brain as it is an inhibitory neurotransmitter (36). The enzyme GABA transaminase (GABA-T) is a PLP dependent enzyme which requires two co-substrates- alpha ketoglutarate and GABA.

The inheritable tendency for mood disorders like depression or bipolar disease has been identified with low plasma levels of GABA. If any kind of chemical imbalance arises, communication gets out of control / over stimulated, Petty F et al. postulated that, when there is too little GABA in the brain such as in Bipolar disorder, mania etc and GABA might raise in depressive patients.^[11] When too much GABA is present in the brain the individual is overly relaxed and sedated with impaired normal activities and reactions. GABA acts as a police man neurotransmitter, any change in GABA levels can influence the other

neurotransmitters, especially the nor-epinephrin.^[37] As per our study the mean value of GABA was found to be much higher in depression cases (5298.7pg/ml +/- 3214.7) as compared to the mean value of controls (1626.6pg/ml +/- 1394.7) (reference range 30-2000 pg/ml) [Table2].

Anisman et.al 2008 stated that in stressful conditions, the plasma and CSF levels of GABA were found to increase. Stresser's influence the functioning of GABA receptor.^[38]

Glutamate (Glu) acts as a precursor in the synthesis of GABA, thus any increase in glutamate levels would subsequently lead to an increase in the biosynthesis of GABA, attained by allosteric modification to balance the excitatory and compensatory activity in stressful situations.^[39]

Gos et al. (2009) in their study expressed that the relative density of Glutamic acid decarboxylase (GAD) raise in the hippocampal region of depressed suicidal cases as compared to the healthy people.^[40]

When GABA levels were compared with the HDRS scores, the present study showed raised levels in both categories of depression; mild-moderate 5184.96pg/ml +/- 3503.48 and severe-very severe 5571.73pg/ml +/- 3503.48 and the results were of high statistical significance $p < 0.001$ [Table 4]. On comparing HDRS scores with GABA a direct association was observed between the two thus narrating that GABA levels increased with the severity of depression and the results were statistically proven significant $p < 0.001$ [Table 5].

When correlation between GABA and the study vitamins was analysed by applying Spearman's Rho correlation, GABA showed a negative association with the levels of vitamins, meaning when GABA was seen to increase in patients with depression, vitamins were decreased and the findings were of statistical significance with a $p < 0.001$ [Table 6].

At present due to insufficient availability of data it is difficult to confirm that the changed GABA concentrations are the firm biomarkers in cases of depression because there is some evidence with respect to both for & against this opinion. It has been postulated that the abnormalities in L-glutamic acid (glutamate) and GABA signal transmission plays a role in depression but very scanty knowledge is available in relation to the molecular determinants and neural mechanisms underlying the process.^[12] In a study it has been said that in the cerebral cortex of MDD diagnosed patients both these neurotransmitters are reported to be elevated.^[41]

When there is too much GABA, a person is relaxed and sedated often to the extent that the normal reactions are impaired. Petty F et al,^[37] concluded that GABA may increase, causing symptoms of depression or mania, due to environmental factors such as excessive alcohol use and stress. In the study conducted by U Halbreich et al,^[42] it was mentioned that during the luteal phase of the menstrual cycle, GABA increased significantly and it is a well known fact that depression is more frequent in women than men.

Thyroid Stimulating Hormone (TSH)

Thyroid hormones play an inevitable role in the body. As per a study by Trzepacz PT et al., thyroid disorders, including both hypothyroidism and hyperthyroidism may be accompanied by various neuropsychiatric manifestations including depression.^[43] Thyroid hormones play a vital role in the development of the brain by having effect on different processes like dendritic cell proliferation, neurogenesis, glial development, synaptogenesis etc.^[44,45] Clinical features of melancholic depression and hypothyroidism mimic each other and the disease severity is linked with TSH levels, while hyperthyroidism may mimic depression and pseudo-dementia in the elderly patients as per a Hage MP et al.^[46] The higher TSH levels have been found to increase the risk of depressive symptoms. TSH can serve as an additional biomarkers for mental disorders along with cortisol and brain derived neurotrophic factor (BDNF) for improving diagnostic/ therapeutic procedures.

Adam Wysokiński et al, (2014) in their study found that highest TSH levels were observed in patients with bipolar disorder while the lowest level in patients with unipolar depression.^[47] Thus to explore the resultant biochemical abnormalities during the co-occurrence of thyroid and psychiatric diseases we decided to investigate the levels of TSH levels in our subjects. Serum TSH levels were towards upper border 4.7mIU/ml in the patients and in volunteers levels were 2.7mIU/ml with significant results $p=0.006$ [Table 2]. TSH levels were slightly higher 5.83mIU/ml in mild to moderately depressed cases and the results were statistically significant $p=0.003$ [Table 4]. Chueire et al,^[48] study results were coinciding with our findings wherein they had mentioned that subclinical hypothyroidic patients were 4 times more frequently depressed than overt hypothyroidic ones.^[48]

Serum Cholesterol

Total cholesterol plays a key role in neuronal differentiation, serotonergic functioning, synaptogenesis and axonal guidance,^[49] thus raising a possibility that alterations in cholesterol levels might play a pivotal role in neuroprotection and neurogenesis, which in turn is related with stresses and depressive disorder.

Mean value of our study reflects serum total cholesterol levels to be as low as 97.7mg/dl in the patients group and the results were of high statistical significance $p<0.001$ [Table 2]. On comparing the levels by student's t-test, it was evident that total cholesterol levels were much in cases 97.7mg/dl and 202mg/dl in the controls [Table 2]. In the instant study, due to the apathy of the disease, the patients would have neglected healthy diet with irregular eating habits which would have led to decreased total cholesterol levels. In support of our study results with respect to total cholesterol, a Mexican study by Marcela Segoviano-Mendoza et al., (2018), suggested hypocholesterolemia as an independent factor associated with depressive symptoms and attempts of suicide. Altered cholesterol levels at synaptic lipid rafts might cause reduction in serotonin levels thereby leading to mis-communication.^[50]

On viewing the levels of serum total cholesterol levels in relation to severity of depression, it was observed that the variable was drastically reduced in all depressed subjects irrespective of the category of depression, 98.46 mg/dl and 95.94 mg/dl in mild-moderate and severe-severe category respectively with statistically significant result $p<0.001$ [Table 4]. An inverse Spearman's Rho correlation was noted between total cholesterol and depression severity [Table 5]. In an experimental animal study by Shuqin Sun et al., it was reported that when the rats were exposed to mild stress, the total cholesterol was lowered in the medial prefrontal cortex, but not in the hippocampus, of the brain in rats, thus suggestive of the altered levels of cholesterol in specific brain region might play a role in the regulation of mood by downstreaming of specific signal pathways.^[51]

A couple of researches have projected an association of diabetes and depression. Thomas Willis during 17th century described diabetes as a consequence of prolonged sorrow. Studies have reported higher fasting glucose in depressive patients corresponding to depression score. But our results did not match with the existing correlation of being depressed and diabetic as our results were not statistically significant $P=0.037$ [Table 2] and also when HDRS scale was matched with the Glucose levels $p=0.080$ [Table 4].

Limitations

To mention a few limitations of our study only certain B complex vitamins were included leaving a few other relevant ones, such as vitamin B3 (Niacin), vitamin C, D etc which also plays a crucial role in mental health. Secondly, declaring subclinical hypothyroidism in depressive patients based on serum TSH levels would be inappropriate, thus including T3 and T4 tests would have given more accurate interpretation. Future research is warranted on other relevant vitamins, omega fatty acids etc., and life style status such as exercise, yoga,

meditation can be documented in the clinically depressed subjects as the social stigma attached to the psychiatric illness is declining gradually. However, to conclude that these indicators are of very high importance in clinically depressed cases, still larger sample size could be considered.

CONCLUSION

To summarise the findings of the present study performed on newly diagnosed, clinically depressed patients with regard to the biochemical variables, B Complex vitamins; vitamin B6, B9 and B12 showed an inverse, negative association with disease severity. As diet is modifiable, more research is warranted on the probable involvement of dietary factors in depression. Our findings on Indian population are consistent with other studies with respect to the B vitamins, which can influence the public health policies to focus on the role of diet in preventing mental health disorders at early stages.

GABA levels were found to be elevated in all depressed subjects and more so in the severely depressed individuals, thus exhibiting a direct, positive correlation with the disease severity. An inverse relationship was observed between the levels of GABA and the study vitamins in the patients. The present study can help the psychiatrists to take appropriate decision about addition of vitamin B6, folate and cobalamin supplements to prevent the mental illness. Total cholesterol was greatly reduced in the depressed individuals, thus it could act as a predictor for developing depression. Estimation of cholesterol is inexpensive and minimal invasion is required. Therefore, those contemplating public health issues Globally, need to consider a balanced approach and should endeavour to acquire optimal intake of the relevant B-vitamins in all stages of life for all.

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