Pravara Med Rev; June 2026, 18 (02), 39 -48
DOI: 10.36848/PMR/2026/45500.02991

Original article

Neuro- Metabolic Correlates of Diabetic Distress- The role of Vitamin B12 and Urinary 5-HIAA

Rajendra Channa[1], Srivani Singaswamy[1], Sankaranarayana Thumma[1], Vadivel Mani[2*], Sethu Imayan M S[3], Ankamma Sarvepalli[4]

[1]Department of Biochemistry, Rangaraya Medical College, Kakinada-533003, Andhra Pradesh, India.
[2]Department of Biochemistry, Sri Devaraj URS Medical College, SDUAHER, Tamaka, Kolar-563103, Karnataka, India.
[3]Department of Biochemistry, Sree Balaji Medical College and Hospital, Chrompet, Chennai-600044, Tamil Nadu
[4]PhD Scholar, Department of Anatomy, Saveetha Medical College & Hospital, SIMTS, Saveetha University & Assistant Professor, Narayana Medical College, Nellore, Andhra Prades
Corresponding Author: Vadivel Mani

Abstract

Background: Diabetes-related distress is a common yet underrecognized contributor to poor clinical outcomes in Type 2 diabetes mellitus (T2DM). Emerging evidence suggests that micronutrient deficiencies and altered neurotransmitter metabolism may influence psychological well-being. This study aimed to evaluate the relationship between diabetic distress, serum Vitamin B12 levels, and urinary 5-hydroxyindoleacetic acid (5-HIAA), a marker of serotonergic turnover.

Methods: In this cross-sectional study, 300 adults with T2DM were recruited from a tertiary care center. Diabetic distress was assessed using the Diabetes Distress Scale–17 (DDS-17) and categorized into no, moderate, and severe distress groups. Fasting blood glucose, HbA1c, serum Vitamin B12 (CLIA), and 24-hour urinary 5-HIAA (HPLC) were measured. Correlation analysis, multivariable linear regression, and receiver operating characteristic (ROC) analysis were performed.

Results: Vitamin B12 deficiency was observed in 41.3% of participants, while 33.3% exhibited high diabetic distress. Individuals with severe distress had significantly lower Vitamin B12 (182 ± 58 pg/mL) and urinary 5-HIAA levels (3.1 ± 0.8 mg/24 h) compared to those with no distress (468 ± 92 pg/mL and 5.6 ± 1.1 mg/24 h; $p < 0.05$). DDS scores showed strong negative correlations with Vitamin B12 (r up to −0.846) and 5-HIAA (r up to −0.845) across all groups ($p < 0.0001$). Vitamin B12 positively correlated with 5-HIAA (r up to 0.884). Multivariable regression identified emotional burden as an independent predictor of both reduced Vitamin B12 ($\beta = -0.36$, $p < 0.001$) and 5-HIAA levels ($\beta = -0.42$, $p < 0.001$). ROC analysis demonstrated that the combined model (Vitamin B12 + 5-HIAA) had superior discrimination for high distress (AUC = 0.84) compared to individual markers.

Conclusion: Diabetic distress is strongly associated with Vitamin B12 deficiency and reduced serotonergic turnover in T2DM. Emotional burden appears to be a key driver of this neuro-metabolic interaction. Combined assessment of Vitamin B12 and urinary 5-HIAA may provide a useful biomarker-based approach for identifying patients at risk of high diabetic distress, supporting more personalized and integrated diabetes care.

Keywords: Diabetic distress; Vitamin B12 deficiency; 5-hydroxyindoleacetic acid (5-HIAA); Serotonin metabolism; Neuro-metabolic link; Emotional burden; Diabetes Distress Scale (DDS-17).

Introduction:

Diabetes is a chronic condition that affects both physical and mental health and necessitates strict adherence to regimens and ongoing medical care[1]. People with diabetes frequently experience diabetes distress in addition to the usual symptoms, which manifests as emotional tiredness, discontent, anxiety, guilt about the need for medication, and worries about the future[2]. Self-care, medication compliance, and general quality of life are all hampered by this distress. Depending on where you look and how you quantify it, between 18% and 45% of persons with type 2 diabetes worldwide have moderate to severe diabetic distress[3]. The percentage rises even further in low- and middle-income nations like India, where between 25% and more than half of people with type 2 diabetes experience distress[4]. What actually makes matters more challenging is that people usually have to pay for their own medical care.

Even though diabetes-related misery is increasingly acknowledged as a factor in clinical outcomes, there is still little access to organized diabetes education, and societal perceptions of chronic illness are frequently unfavorable. Particularly in environments with limited resources, educational resources are often dispersed or non-existent. As a result, despite its great incidence, diabetes-related discomfort is still poorly understood and handled in standard clinical treatment.

A commonly used and validated tool for measuring diabetes-related distress is the Diabetes Distress Scale–17 (DDS-17). Emotional load, physician-related distress, regimen-related distress, and interpersonal distress are its four key components[5]. Poorer glycemic control, fewer self-care practices, inadequate treatment adherence, and a higher risk of complications from diabetes have all been repeatedly linked to higher DDS-17 scores. The DDS-17 offers a solid framework for assessing the psychological aspects of diabetes and their interaction with metabolic outcomes in both clinical and research settings because of its good psychometric qualities and clinical relevance.

There are increasing indications that the distress experienced by individuals with diabetes is directly related to inflammation and nerve disorders[6]. Long-term stress can disrupt serotonin levels in the brain and lead to inflammation[7]. Your metabolism and emotional state are linked to these disorders. Serotonin, also known as 5-hydroxytryptamine or 5-HT, is crucial for managing stress, overcoming adversity, and controlling our emotions.

The primary metabolite of serotonin, 5-hydroxyindoleacetic acid (5-HIAA), is eliminated in urine, where its concentration acts as a peripheral measure of serotonergic turnover[8]. Affective diseases, such as depression, anxiety, and stress-related ailments, have been linked to changes in serotonin metabolism[9]. Chronic hyperglycemia, low-grade inflammation, and oxidative stress can all interfere with serotonergic pathways in the context of diabetes[10], which could make it more difficult to control mood. Increased emotional susceptibility and diabetes-related sadness may be caused by such neurochemical imbalance. However, the relationship between serotonin metabolism and emotional stress in individuals with diabetes in particular is still poorly understood.

Simultaneously, vitamin B12 deficiency in T2DM patients especially those receiving long-term metformin therapy is becoming more widely acknowledged[11]. A lack of vitamin B12 has been connected to peripheral neuropathy, depression symptoms, and cognitive decline. Vitamin B12 is essential for brain function, myelin production, and neurotransmitter metabolism[12]. Despite this, little research has been done on the possible relationship between vitamin B12 level and psychological suffering associated with diabetes.

Vitamin B12 is also relevant in this situation. Your body has trouble with methylation, homocysteine levels rise, and the route from tryptophan to serotonin becomes convoluted when you're low on B12[13]. Serotonin turnover is disrupted when your body lacks sufficient methyl donors to produce or metabolize monoamines. Changes in the levels of 5-HIAA in urine are a sign of this. When combined with other metabolic problems, these changes in brain chemistry can exacerbate emotional discomfort in individuals with type 2 diabetes. Strangely, no studies have been conducted on the connection between diabetic distress, urine 5-HIAA, and vitamin B12. This is therapeutically significant since poor treatment compliance and insufficient glycemic control are linked to diabetes-related distress, and undiagnosed vitamin B12 insufficiency can exacerbate neuropsychiatric symptoms and neuropathic consequences. In people with type 2 diabetes mellitus, the possible presence of psychological distress and biochemical insufficiency may consequently increase the illness burden, highlighting the need to investigate their interaction. In order to better understand the psycho-neurobiological pathways impacting diabetes outcomes, the current study examines the relationship between urinary 5-hydroxyindoleacetic acid, blood vitamin B12 levels, and diabetes-related distress. Because urinary 5-HIAA is easy to assess and non-invasive, it can offer a window into the nervous system's activity when a diabetic experiences emotional overload. Medical practitioners can identify patients who are at danger and offer nutritional or psychological support before things worsen by detecting these biological indicators early.

So, this study sets out to look at the links between vitamin B12 deficiency, urine 5-HIAA levels, and diabetic distress in people with type 2 diabetes. By untangling these relationships, we hope to move toward a more personalized way of managing diabetes one that doesn’t just look at blood sugar, but also takes nutrition and mental health into account.

MATERIALS AND METHODS

Subjects

Researchers brought in adults between 20 and 60 years old from the General Medicine OPD at Rangaraya Medical College and Hospital, Kakinada, Andhra Pradesh. The ethics committee at Rangaraya Medical College and Hospital checked and approved the study, following ICMR guidelines (Ref. No. IEC/RMC/2026/3F).

They included healthy control subjects with fasting blood sugar under 100 mg/dL and HbA1c below 6.0%. For the diabetes group, they only included people diagnosed with type 2 diabetes mellitus based on ADA criteria: fasting blood sugar of 126 mg/dL or higher, 2-hour plasma glucose of 200 mg/dL or more, or HbA1c at least 6.5%[14]. All diabetic participants were treated with a standard antidiabetic drug.

Those who had previously participated in a comparable pilot project, were pregnant or nursing, or had any endocrine or hormonal conditions other than type 2 diabetes were excluded. Additionally, we excluded individuals who were unable to give informed consent, had a serious or life-threatening illness, had chronic liver or kidney disease (such as those receiving hemodialysis or managing azotemia), had a history of mental illness or communication difficulties, had obsessive-compulsive disorder, had chronic inflammatory or autoimmune conditions like rheumatoid arthritis, or had an acute infection at the time of enrollment. Participants with secondary causes of hyperglycemia, allergies to any study drugs, those already receiving hormone therapy or herbal supplements, or those who had trouble adhering to the research protocol or taking their prescribed prescriptions were also eliminated.

Study design

First, we checked who qualified for the study. Both healthy people and those with diabetes who met the criteria got in. Then, we measured everyone's diabetic distress using the Diabetes Distress Scale–17 (DDS-17), which Polonsky and his team came up with back in 2005[15]. A score under 2.0 meant no distress, 2.0 to 3.0 meant moderate distress, and anything over 3.0 counted as severe distress.

After we got the scores, we split everyone into four groups: Group I was people with type 2 diabetes but no distress (DDS-17 score under 2.0), Group II was people with type 2 diabetes and moderate distress (DDS-17 score between 2.0 and 3.0). Group III was those with type 2 diabetes and severe distress (DDS-17 score over 3.0).

Sample Size Calculation:

The sample size for the present cross-sectional study was calculated to detect a statistically significant association between vitamin B12 levels, urinary 5-hydroxyindoleacetic acid (5-HIAA), and diabetic distress (DDS-17 scores) in patients with type 2 diabetes mellitus.

As previous studies examining the combined association of vitamin B12 deficiency, serotonin metabolism (urinary 5-HIAA), and diabetic distress are limited, the sample size was estimated based on detecting a moderate correlation between biochemical parameters and DDS-17 scores. Assuming a correlation coefficient (r) of 0.30, with a 95% confidence level ($\alpha = 0.05$) and 80% statistical power ($\beta = 0.20$), the required sample size was calculated using the formula for correlation studies:

$$n = \frac{(Z_\alpha + (Z_\beta)2}{(0.5\text{XIn}(1 + r/1 - r))^2}$$

After accounting for an anticipated 10–15% non-response or incomplete data rate, the final sample size was inflated to approximately 100 participants. These were allocated into diabetic distressed and healthy control groups as per the study design.

Study procedure:

Eligible patients attending the outpatient and inpatient departments were identified based on the inclusion and exclusion criteria. After explaining the purpose of the study, written informed consent was obtained from all participants. Diabetes-related distress was assessed using the validated Diabetes Distress Scale-17 (DDS-17) questionnaire. Based on the mean DDS score, participants were categorized into no distress, moderate distress, and severe distress groups. Patients were told not to eat bananas, kiwis, pineapple, walnuts, eggplant, or fruits in general until the study was over.

After an overnight fast of 8–10 hours, venous blood samples were collected from all participants under aseptic conditions using sterile vacutainers. Samples were processed immediately, and serum was separated by

centrifugation at 3,000 rpm for 10 minutes. Aliquoted serum samples were either analyzed on the same day or stored at −20 °C until further analysis. Patient instructed to collect 24 hr urine, urine collection in dark color container with 10-15 ml glacial acetic acid as a preservative. Fasting blood glucose (FBS) levels were measured using the glucose oxidase–peroxidase enzymatic method on an automated biochemistry analyzer. Glycated hemoglobin (HbA1c) was estimated by high-performance liquid chromatography (HPLC) using a National Glycohemoglobin Standardization Program (NGSP)–certified method.

Serum Vitamin B12 concentrations were estimated using an automated chemiluminescent immunoassay (CLIA) based on a competitive binding principle (Architect i1000SR, Abbott Diagnostics). The assay employs intrinsic factor coated paramagnetic microparticles that selectively bind Vitamin B12, with light emission measured in relative light units and converted to concentration using a calibration curve. The analytical sensitivity of the assay was 50 pg/mL, with intra- and inter-assay coefficients of variation <10%. Vitamin B12 deficiency was defined according to laboratory reference ranges (<200 pg/mL), borderline deficiency as 200–300 pg/mL, and normal status as >300 pg/mL. Internal quality control samples at low and high concentrations were run with each batch of analyses.

Urinary 5-hydroxyindoleacetic acid (5-HIAA) concentrations were estimated using high-performance liquid chromatography (HPLC) with electrochemical detection. For HPLC analysis, urine samples were acidified, filtered, and injected into a reversed-phase C18 column, with detection performed at an electrochemical potential optimized for indole compounds. Quantification was achieved by comparison with external standards. The analytical sensitivity of the assay was 0.2 µmol/L, with intra- and inter-assay coefficients of variation <10%. Elevated 5-HIAA levels were defined according to manufacturer-provided or population-specific reference ranges.

Statistical Analysis

Data were analyzed using Graphpad Prism version 10.4.2. Continuous variables were expressed as mean ± SD. Differences in serum Vitamin B12 and 24-h urinary 5-HIAA across distress groups were analyzed using one-way ANOVA with Tukey post-hoc testing. The relationship between diabetic distress severity and Vitamin B12 and 24-h urinary 5-HIAA was assessed using Pearson's coefficients. Multivariable linear regression was used to evaluate independent associations after adjustment for confounders. A p-value < 0.05 was considered statistically significant.

To determine independent associations, multivariable linear regression analysis was conducted with diabetic distress score as the dependent variable and serum Vitamin B12 and urinary 5-HIAA as independent variables, adjusting for potential confounders including age, sex, duration of diabetes, HbA1c, and FBS. Results were reported as β-coefficients with 95% confidence intervals (CI). A two-tailed p-value < 0.05 was considered statistically significant.

In the overall cohort, increasing diabetic distress severity was significantly associated with lower serum Vitamin B12 and altered 5-HIAA levels.

A significant linear trend was observed, with progressive reductions in Vitamin B12 and 5-HIAA levels across increasing distress categories (p-trend < 0.001).

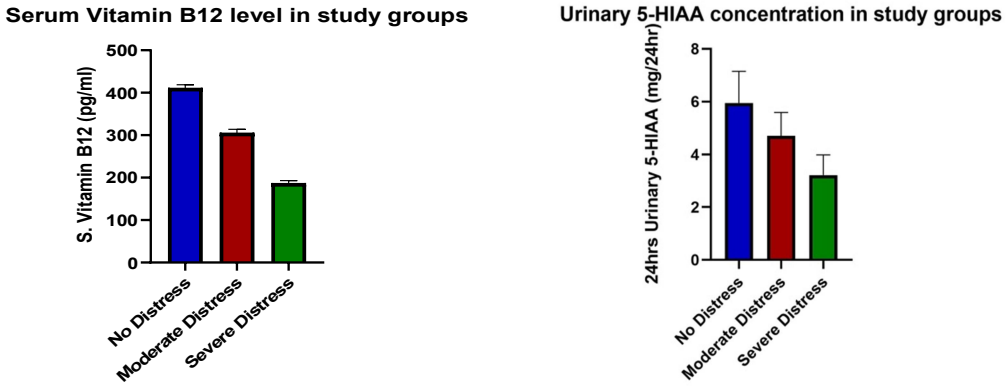
Results

A total of 300 adults with Type-2 diabetes were included in the analysis. The mean age of participants was 54.6 ± 8.9 years, with 56% males. Vitamin B12 deficiency was observed in 41.3% of participants, while 33.3% exhibited high diabetic distress based on the Diabetic Distress Scale (DDS). Participants with high distress had significantly lower serum Vitamin B12 levels compared to those with low distress (182 ± 58 vs. 468 ± 92 pg/mL, $p < 0.05$). Urinary 5-hydroxyindoleacetic acid (5-HIAA) levels were significantly lower among individuals with high diabetic distress (3.1 ± 0.9 vs 5.6 ± 3.1 mg/24hrs, $p < 0.01$).

Table.1-: Illustration of demographic characteristics in control and diabetic experimental subjects.

Total patient	Diabetic No distress	Diabetic Moderate distress	Diabetic Severe distress
Age	36.81±3.3	46.11±8.5	45.61±8.2
Male	60%	33%	39%
Female	40%	67%	61%
Duration of Diabetes	4.2 ± 1.3	8.6 ± 2.1[a]*	12.4 ± 3.2[ab]*
FBS (mg/dl)	231.41±22.29	252.17±29.82[a]*	319.7±39.53[ab]*
HbA1c (%)	7.35±0.29	7.72±0.29[a]*	8.79±0.33[ab]*
DDS-17	1.44±0.3	2.63±0.20[a]*	3.66±0.28[ab]*
Serum Vitamin B12 (pg/ml)	468±92	331±74	182±58
Urinary 5HIAA (mg/24hrs)	5.6± 1.1	4.2± 0.9	3.1 ± 0.8

In the table, the results represent the arithmetic mean with ± predictable error of thirty experimental subjects. The statistical drift of the study was set at p value less than 0.05* or p value less than 0.01**. ANOVA between the experimental groups was performed and mentioned in the bar diagram as a symbol of a-compared with the No distress group mean; and **b**-compared with the moderate distress group.



Correlation Analysis:

Correlation Analysis – No Distress group

Correlation analysis revealed a strong and statistically significant inverse association between DDS-17 scores and serum Vitamin B12 levels (Pearson's r = −0.846, 95% CI: −0.893 to −0.779, p < 0.0001). Similarly, DDS-17 scores were strongly and negatively correlated with urinary 5-hydroxyindoleacetic acid (5-HIAA) levels (r = −0.712, 95% CI: −0.796 to −0.600, p < 0.0001). The coefficients of determination indicated that 71.5% of the variance in diabetic distress severity was explained by Vitamin B12 levels, while 50.6% of the variance was explained by urinary 5-HIAA concentrations, highlighting a robust neuro-metabolic association with diabetic distress. In addition, serum Vitamin B12 levels were significantly negatively correlated with urinary 5-HIAA concentrations (r = -0.1554, p < 0.1207), suggesting altered serotonergic turnover in the presence of Vitamin B12 deficiency.

Table 2: Correlation analysis between DDS score, Serum Vitamin B12 and urinary HIAA using Pearson Correlation Coefficient in the No Distress group.

No Distress-Variable 1	No Distress-Variable 2	Correlation coefficient (r)	95% CI	p-value
DDS Score	Vitamin B12	-0.8456	−0.893 to −0.779	p< 0.0001**
DDS Score	U. HIAA	-0.7116	−0.796 to −0.600	P< 0.0001**
Vitamin B12	0.7845	0.7845	0.6958 to 0.8497	P< 0.0001**

Correlation Analysis – Moderate Distress group

In individuals with moderate diabetic distress, Pearson correlation analysis revealed a strong inverse association between DDS-17 scores and serum Vitamin B12 levels ($r = -0.811$, 95% CI: −0.869 to −0.731, $p < 0.0001$), as well as between DDS-17 scores and urinary 5-hydroxyindoleacetic acid (5-HIAA) concentrations ($r = -0.718$, 95% CI: −0.802 to −0.608, $p < 0.0001$; table.3). These findings indicate that lower Vitamin B12 and 5-HIAA levels are associated with greater distress severity, with the two biomarkers accounting for 65.8% and 51.6% of the variance in DDS-17 scores, respectively.

Furthermore, serum Vitamin B12 levels were strongly and positively correlated with urinary 5-HIAA concentrations ($r = 0.884$, 95% CI: 0.832 to 0.921, $p < 0.0001$), with Vitamin B12 explaining 78.1% of the variability in 5-HIAA levels. Together, these results support a closely linked neuro-metabolic pathway, wherein Vitamin B12 status appears to influence serotonergic metabolite turnover and, in turn, diabetic distress severity**.**

Table 3: Correlation analysis between DDS score, Serum Vitamin B12 and urinary HIAA using Pearson Correlation Coefficient in the Moderate Distress group.

Moderate Distress-Variable 1	**Moderate Distress-Variable 2**	**Correlation coefficient (r)**	**95% CI**	**p-value**
DDS Score	Vitamin B12	-0.8110	-0.8690 to -0.7310	p< 0.0001**
DDS Score	U. HIAA	-0.7183	-0.8016 to -0.6076	**P< 0.0001****
Vitamin B12	U. HIAA	0.8838	0.8318 to 0.9205	P<0.0001**

Correlation Analysis – Severe Distress group

In participants with severe diabetic distress, correlation analysis demonstrated strong and statistically significant associations between diabetic distress severity and the studied biochemical markers (table.4). DDS scores were strongly and inversely correlated with serum Vitamin B12 levels ($r = -0.837$, 95% CI: −0.887 to −0.766, $p < 0.0001$), indicating that lower Vitamin B12 concentrations were associated with greater distress severity. Similarly, DDS scores showed a strong negative correlation with urinary 5-hydroxyindoleacetic acid (5-HIAA) levels ($r = -0.845$, 95% CI: −0.893 to −0.777, $p < 0.0001$).

In contrast, serum Vitamin B12 levels were positively correlated with urinary 5-HIAA concentrations ($r = 0.748$, 95% CI: 0.647 to 0.824, $p < 0.0001$), suggesting a close association between Vitamin B12 status and serotonergic metabolite excretion in individuals with severe distress. Collectively, these findings highlight a robust neuro-metabolic link between Vitamin B12 deficiency, altered serotonin turnover, and heightened diabetic distress in the severe distress group.

Table 4: Correlation analysis between DDS score, Serum Vitamin B12 and urinary HIAA using Pearson Correlation Coefficient in the Severe Distress group.

Severe Distress-Variable 1	**Severe Distress-Variable 2**	**Correlation coefficient (r)**	**95% CI**	**p-value**
DDS Score	Vitamin B12	-0.8368	-0.8874 to -0.7664	p< 0.0001**
DDS Score	U. HIAA	-0.8445	-0.8928 to -0.7769	**P< 0.0001****
Vitamin B12	U. HIAA	0.7484	0.6471 to 0.8237	P<0.0001**

Multiple linear regression analysis between DDS score, serum Vitamin B12 and urinary HIAA

In multivariable linear regression analysis adjusted for age, sex, duration of diabetes, HbA1c, fasting blood glucose, BMI, neuropathy score, and other DDS domains, emotional burden emerged as an independent predictor of urinary 5-HIAA levels (standardized $\beta = -0.42$, 95% CI: 0.58 to −0.26, P<0.001). None of the other DDS subdomains showed significant associations (all $p > 0.05$). The overall model explained 48%% of the variance in urinary 5-HIAA concentrations (adjusted $R^2 = 0.48$), supporting a dominant role of emotional burden in serotonergic dysregulation.

When serum Vitamin B12 was modeled as the dependent variable, emotional burden remained independently associated with lower Vitamin B12 concentrations after multivariable adjustment (standardized β = −0.36, 95% CI: −0.52 to −0.19, <0.001). Regimen-related, interpersonal, and physician-related distress scores were not independently associated with Vitamin B12 levels. The fully adjusted model explained 52% of the variability in serum Vitamin B12 (adjusted $R^2 = 0.52$).

Use multivariable linear regression with hierarchical adjustment, where all DDS domains are entered simultaneously, along with key metabolic and clinical confounders. This allows you to demonstrate that emotional burden retains significance independent of shared distress variance and disease severity.

Table 5. Multivariable Linear Regression Analysis Showing Independent Associations of Emotional Burden and Other DDS Domains with Biochemical Parameters

Table 5.A. Outcome Variable: Urinary 5-HIAA (mg/24 h)

Predictor Variable	Standardized β	95% CI	p-value
Emotional Burden score	**−0.42**	0.58 to −0.26	**P<0.001****
Regimen-related distress	−0.09	−0.23 to 0.05	0.21
Interpersonal distress	−0.06	−0.19 to 0.08	0.39
Physician-related distress	−0.04	−0.18 to 0.10	0.56
Age (years)	−0.07	−0.15 to 0.02	0.12
Sex (male)	0.05	−0.04 to 0.14	0.28
Duration of diabetes (years)	−0.11	−0.22 to −0.01	0.04
HbA1c (%)	−0.18	−0.30 to −0.07	0.002*
FBS (mg/dL)	−0.10	−0.22 to 0.01	0.07*
Neuropathy score	**−0.21**	**−0.34 to −0.09**	**0.001****

Table 5.B. Outcome Variable: Serum Vitamin B12 (pg/mL)

Predictor Variable	Standardized β	95% CI	p-value
Emotional Burden score	**−0.36**	**−0.52 to −0.19**	**<0.001****
Regimen-related distress	0.08	−0.07 to 0.23	0.29
Interpersonal distress	0.05	−0.10 to 0.20	0.51
Physician-related distress	−0.04	−0.19 to 0.11	0.61
Age (years)	−0.14	−0.25 to −0.03	0.01
Sex (male)	0.06	−0.05 to 0.17	0.26
Duration of diabetes (years)	−0.19	−0.31 to −0.08	0.001*
HbA1c (%)	−0.24	−0.37 to −0.11	<0.001**
FBS (mg/dL)	−0.12	−0.25 to 0.01	0.07*
Neuropathy score	−0.28	−0.41 to −0.15	<0.001**

Values are standardized β coefficients derived from multivariable linear regression models adjusted for age, sex, duration of diabetes, HbA1c, fasting blood glucose, neuropathy score, and other DDS-17 domains. Emotional burden remained the strongest independent predictor of both urinary 5-HIAA and serum Vitamin B12 levels. Statistical significance was set at $p < 0.05$.

ROC Analysis of Serum Vitamin B12 and Urinary 5-HIAA for Discrimination of High Diabetic Distress

Receiver operating characteristic (ROC) analysis demonstrated that the combined model of serum Vitamin B12 and urinary 5-hydroxyindoleacetic acid (5-HIAA) showed good discriminatory ability for identifying individuals with high diabetic distress (AUC = 0.84, 95% CI: 0.79–0.89, $p < 0.001$). This performance was superior to that of serum Vitamin B12 alone (AUC = 0.72, 95% CI: 0.65–0.79, $p < 0.001$) and urinary 5-HIAA alone (AUC = 0.76, 95% CI: 0.69–0.82, $p < 0.001$).

DeLong's test confirmed that the combined model provided significantly greater discrimination compared with Vitamin B12 alone (ΔAUC = 0.12, p = 0.004) and 5-HIAA alone (ΔAUC = 0.08, p = 0.018).

Sensitivity & Specificity for Combined Model (Vitamin B12 + 5-HIAA)

At the optimal cut-off determined by Youden's index, the combined model demonstrated a sensitivity of 81.3% and specificity of 77.8% for identifying high diabetic distress. The corresponding positive predictive value

(PPV) was 79.5%, and negative predictive value (NPV) was 80.0%, yielding an overall diagnostic accuracy of 79.6%.

These findings suggest that integrating neurochemical and micronutrient markers improves identification of patients with clinically significant diabetic distress.

Table 6. ROC Analysis of Serum Vitamin B12 and Urinary 5-HIAA for High Diabetic Distress

Model	AUC	95% CI	p-value
Vitamin B12	0.72	0.65–0.79	<0.001
Urinary 5-HIAA	0.76	0.69–0.82	<0.001
Combined model	**0.84**	**0.79–0.89**	<0.001

Table 7. Diagnostic Performance of Vitamin B12 and 5-HIAA for High Diabetic Distress

Model	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Vitamin B12	≤ 260 pg/mL	68.4	70.2	66.1	72.3	69.3
Urinary 5-HIAA	≤ 3.6 mg/24 h	72.9	73.5	71.8	74.5	73.2
Combined model	Probability ≥ 0.54	**81.3**	**77.8**	**79.5**	**80.0**	**79.6**

Discussion

The present study demonstrates a strong and consistent association between diabetic distress, serum Vitamin B12 levels, and urinary 5-hydroxyindoleacetic acid (5-HIAA), supporting the existence of a neuro-metabolic link in individuals with Type 2 diabetes. The observed high prevalence of Vitamin B12 deficiency (41.3%) is comparable to previous reports in metformin-treated diabetic populations, where impaired absorption of Vitamin B12 has been widely documented [17]. Similarly, the substantial proportion of participants with elevated diabetic distress (33.3%) aligns with earlier studies highlighting the psychological burden associated with chronic glycemic dysregulation[3].

Vitamin B12, Serotonergic Metabolism, and Distress Severity

The primary finding of the study is that a gradual decrease in vitamin B12 levels in blood and 5-HIAA levels in urine occurs according to the increase in levels of diabetes stress. Vitamins B12 levels and 5-HIAA levels in comparison to those of people in mild distress were very low in people in extreme distress, indicating a decrease in serotonergic metabolism in people suffering from psychological distress. This finding seems to be supported by science because vitamin B12 is a crucial vitamin, and its deficiency has already shown a link to neuropathy, cognitive impairment, and depression[18], all of which can worsen the distress of diabetics suffering from pain and discomfort in their nerves as well as in their minds.

Changes in serotonergic turnover in problematic people are further supported by decreased urine 5-HIAA, a critical serotonin metabolite. Tryptophan metabolism and serotonin transmission are known to be impacted by chronic psychological stress and inflammation, which are prevalent in poorly controlled diabetes[16]. The simultaneous decrease in 5-HIAA and vitamin B12 points to a common neuro-metabolic route in which micronutrient deficiencies and neurotransmitter dysregulation work together to cause the emotional distress that people with type 2 diabetes endure. This finding supports earlier evidence that chronic stress and metabolic disorders can disrupt serotonin pathways and contribute to neuropsychiatric manifestations[19].

Distress-Stratified Correlation Patterns

Vitamin B12 and urine 5-HIAA levels were found to have consistently substantial inverse correlations with DDS-17 scores throughout stratified correlation studies across no, moderate, and severe distress groups. With coefficients of determination showing that these biochemical markers could account for a significant amount of the variability in distress severity, it is noteworthy that the strength of these correlations remained strong across distress categories. These results demonstrate that neuro-metabolic dysregulation may exist throughout the distress spectrum and worsen as distress increases, rather than being only a result of extreme distress.

Further supporting the idea that vitamin B12 status may affect serotonergic turnover and potentially modulate emotional and behavioral responses in diabetes is the positive correlation between vitamin B12 and urine 5-

HIAA seen in groups with moderate to severe distress. This association supports clinical and experimental data that links altered monoamine metabolism to decreased methylation ability[21].

Emotional Burden as a Central Driver

Importantly, multivariable linear regression with hierarchical adjustment demonstrated that emotional burden emerged as the dominant DDS domain independently associated with both urinary 5-HIAA and serum Vitamin B12, even after controlling for age, sex, duration of diabetes, glycemic indices, neuropathy score, and other distress domains. Other DDS subdomains did not retain independent significance, suggesting that emotional burden captures a unique and biologically relevant dimension of diabetes-related distress.

These findings indicate that emotional burden may act as a central psychological driver linking metabolic stress, micronutrient deficiency, and neurochemical imbalance, rather than merely reflecting disease severity or treatment-related challenges. The relatively high adjusted R^2 values for both regression models further support the robustness of this association.

Diagnostic and Clinical Implications

The clinical relevance of these findings is strengthened by ROC analysis, which demonstrated that a combined model incorporating serum Vitamin B12 and urinary 5-HIAA achieved good discrimination for high diabetic distress (AUC = 0.84). The combined model significantly outperformed either biomarker alone, with favorable sensitivity, specificity, and overall diagnostic accuracy. This suggests that integrated neuro-metabolic biomarkers may offer objective support for identifying patients at high risk of clinically significant diabetic distress, complementing questionnaire-based screening tools such as the DDS.

From a translational perspective, these results open avenues for biologically informed screening and intervention strategies, including targeted nutritional assessment, Vitamin B12 supplementation, and stress-modulating therapies aimed at improving serotonergic function. Similar multimarker approaches have been recommended in recent literature to enhance diagnostic accuracy in complex metabolic and neuropsychiatric conditions[21].

Conclusion

In summary, this study demonstrates a robust neuro-metabolic link between diabetic distress, Vitamin B12 deficiency, and altered serotonergic turnover, with emotional burden emerging as the most biologically salient domain of distress. The integration of biochemical markers with psychological assessment may enhance early identification and personalized management of diabetic distress, ultimately improving both mental health and metabolic outcomes in individuals with T2DM.

Strengths and Limitations

The strengths of this study include a large sample size, distress stratification, comprehensive biochemical assessment, and rigorous multivariable statistical modeling. However, several limitations should be acknowledged. The cross-sectional design precludes causal inference, and residual confounding cannot be entirely excluded. Additionally, dietary intake, antidepressant use, and inflammatory markers were not assessed and may influence serotonin metabolism and Vitamin B12 status. Future longitudinal studies are warranted to clarify causal pathways and evaluate the impact of targeted interventions.

References:

1. Kalra S, Jena BN, Yeravdekar R. Emotional and Psychological Needs of People with Diabetes. Indian J Endocrinol Metab. 2018 ;22(5):696-704. doi: 10.4103/ijem.IJEM_579_17.
2. Morales-Brown LA, Perez Algorta G, Salifu Y. Understanding Experiences of Diabetes Distress: A Systematic Review and Thematic Synthesis. J Diabetes Res. 2024;2024:3946553. doi: 10.1155/2024/3946553.
3. Sinha R, Priya A, Sinha A, Hifz Ur Rahman M. Prevalence of diabetes distress among type 2 diabetes mellitus patients in India: a systematic review and meta-analysis. Health Psychol Behav Med. 2024;12(1):2324091. doi: 10.1080/21642850.
4. Alwani AA, Kaur R, Bairwa M, Misra P, Nongkynrih B. Diabetes distress and associated factors among adults with diabetes mellitus residing in a metropolitan city of India: a community-based study. Clin Diabetes Endocrinol. 2024;10(1):40. doi: 10.1186/s40842-024-00203-7.
5. Melo MISPT, Falcão D, Campos DI, Santos MMD, Hernâni-Eusébio J, Silva AB. Diabetes Distress Scale (DDS-17): European Portuguese Validation. Arch Endocrinol Metab. 2026 ;70(3):e260035. doi: 10.20945/2359-4292-2026-0035.

6. Park, HS., Cho, Y., Seo, D.H. *et al.* Impact of diabetes distress on glycemic control and diabetic complications in type 2 diabetes mellitus. *Sci Rep. 2024;***14:** 5568. https://doi.org/10.1038/s41598-024-55901-0
7. Natarajan R, Northrop NA, Yamamoto BK. Protracted effects of chronic stress on serotonin-dependent thermoregulation. Stress. 2015;18(6):668-76. doi: 10.3109/10253890.2015.1087502.
8. Rossi RE, Lavezzi E, Jaafar S, Cristofolini G, Laffi A, Nappo G, Carrara S, Bertuzzi AF, Uccella S, Repici A, Zerbi A, Lania AGA. Urinary 5-Hydroxyindolacetic Acid Measurements in Patients with Neuroendocrine Tumor-Related Carcinoid Syndrome: State of the Art. Cancers (Basel). 2023;15(16):4065. doi: 10.3390/cancers15164065.
9. Tahiri J, Mian M, Aftan F, Habbal S, Salehi F, Reddy PH, Reddy AP. Serotonin in depression and Alzheimer's disease: Focus on SSRI's beneficial effects. Ageing Res Rev. 2024;101:102537. doi: 10.1016/j.arr.2024.102537.
10. Cai Y, Li X, Zhou H, Zhou J. The serotonergic system dysfunction in diabetes mellitus. Front Cell Neurosci. 2022;16:899069. doi: 10.3389/fncel.2022.899069.
11. Tayong FM, Francis TO, Kj J, Mahato RK, Ullah H, Kamal S, Kamil K. Prevalence of Vitamin B12 Deficiency in Type 2 Diabetic Patients on Long-Term Metformin Therapy. Cureus. 2025;17(7):e88265. doi: 10.7759/cureus.88265.
12. Milind Umekar, Tanvi Premchandani, Amol Tatode, Mohammad Qutub, Neha Raut, Jayshree Taksande, Ujban Md. Hussain. Vitamin B12 deficiency and cognitive impairment: A comprehensive review of neurological impact. Brain Disorders. 2025;18:100220.https://doi.org/10.1016/j.dscb.2025.100220.
13. Harikaran S, Basu S, Mukherjee MP, Kar R, Nair S, Priyadarssini M. Vitamin B12 and homocysteine in patients with major depressive disorder. J Family Med Prim Care. 2024;13(5):2049-2053. doi: 10.4103/jfmpc.jfmpc_1460_23.
14. American Diabetes Association Professional Practice Committee for Diabetes*. 2. Diagnosis and Classification of Diabetes: Standards of Care in Diabetes-2026. Diabetes Care. 2026;49(Supplement_1):S27-S49. doi: 10.2337/dc26-S002.
15. Polonsky WH, Fisher L, Earles J, Dudl RJ, Lees J, Mullan J, Jackson RA. Assessing psychosocial distress in diabetes: development of the diabetes distress scale. Diabetes Care. 2005;28(3):626-31. doi: 10.2337/diacare.28.3.626.
16. Khoshnevisan K, Chehrehgosha M, Sajjadi-Jazi SM, Meftah AM. Tryptophan and serotonin levels as potent biomarkers in diabetes mellitus complications: a new approach of diagnostic role. J Diabetes Metab Disord. 2022;21(2):1923-1934. doi: 10.1007/s40200-022-01096-y.
17. de Jager J, Kooy A, Lehert P, Wulffelé MG, van der Kolk J, Bets D, Verburg J, Donker AJ, Stehouwer CD. Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: randomised placebo controlled trial. BMJ. 2010 ;340:c2181. doi: 10.1136/bmj.c2181.
18. Badar A. Neuropsychiatric Disorders Associated With Vitamin B12 Deficiency: An Autobiographical Case Report. Cureus. 2022 ;14(1):e21476. doi: 10.7759/cureus.21476.
19. Cowen PJ, Browning M. What has serotonin to do with depression? World Psychiatry. 2015 ;14(2):158-60. doi: 10.1002/wps.20229.
20. O'Leary F, Samman S. Vitamin B12 in health and disease. Nutrients. 2010;2(3):299-316. doi: 10.3390/nu2030299. Epub 2010 Mar 5.
21. Hacimusalar Y, Eşel E. Suggested Biomarkers for Major Depressive Disorder. Noro Psikiyatr Ars. 2018;55(3):280-290. doi: 10.5152/npa.2017.19482.