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Evaluation of serum GDNF, TIMP4, and tPA levels in generalized anxiety disorder: a case–control study

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Abstract

Background Generalized anxiety disorder (GAD) is a psychological condition marked by persistent, overwhelming concern and intensified anxiety reactions that surpass situational stimuli, profoundly impacting social, vocational, and daily activities. Emerging evidence indicates that altered levels of cytokines and other biomarkers may contribute to mental disorders; however, knowledge on GDNF, TIMP4, and tPA with GAD remains ambiguous, limited, and inconsistent. The objective of this study was to evaluate their association with the pathophysiology of GAD.

Methods This case–control study enrolled 101 GAD patients from the National Institute of Mental Health Hospital in Dhaka and 101 healthy controls from several sites across Dhaka city. The participants were assessed by a qualified psychiatrist according to DSM-5 criteria. To determine the severity of GAD symptoms, the GAD-7 scale was employed. Serum levels of GDNF, TIMP4, and tPA were quantified by ELISA methods.

Results We found elevated serum GDNF levels in patients compared to control subjects. This increment demonstrated a significantly positive correlation with the patients' GAD severity. The ROC analysis of serum GDNF levels revealed diagnostic efficacy, with a sensitivity of 79.2% and a specificity of 74.7%. Furthermore, patients with GAD showed reduced serum TIMP4 levels than controls. This reduction exhibited a significantly negative correlation with the severity of GAD in patients. The ROC analysis of serum TIMP4 levels indicated diagnostic performance, with sensitivity and specificity of 75.2% and 78.2%, respectively. Additionally, a significant negative correlation was observed between serum GDNF and TIMP4 levels. However, we noted no such changes in serum tPA levels in GAD.

Conclusions The current study demonstrates the altered serum GDNF and TIMP4 levels may be associated with the pathophysiology of GAD. The findings emphasize the potential of GDNF and TIMP4 for risk evaluation and therapeutic response for GAD.

Keywords Generalized anxiety disorder, Glial cell line-derived neurotrophic factor, Tissue inhibitor of metalloproteinase 4, Tissue plasminogen activator, Mental health, Psychiatry

1 Introduction

Anxiety, characterized by excessive and uncontrollable worry for various subjects without appropriate triggers or in an extent disproportionate to actual threat, serves as the primary diagnostic criterion for generalized anxiety disorder (GAD) [1]. While simultaneously inflicting an enormous economic strain on communities, this mental disorder profoundly impacts a person's quality of life

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and social engagement [2]. The defining characteristics of GAD include pervasive anxiety, nervous and motor tension, dysfunctional concerns, autonomic hyperactivity, and hyperarousal [3]. Individuals diagnosed with anxiety disorders exhibit a heightened mortality risk in comparison with the general population or those without mental problems [4]. Globally, anxiety disorders affect an estimated 359 million people in 2021 which is 4.4% of the global population, with age-standardized prevalence, incidence, and disability-adjusted life year (DALY) rates increasing by 18–20%. Women continue to experience a disproportionately higher burden particularly between ages 20 and 44. Although effective treatments are available, only 27.6% of affected individuals receive care largely due to limited mental health resources, inadequate awareness, and persistent stigma [5, 6]. As the global burden continues to rise, understanding the biological underlying mechanism of anxiety has become increasingly important for improving early detection and targeted intervention. Traditional symptom-based diagnostic approaches often fail to capture the heterogeneity of anxiety presentations particularly in diverse clinical populations.

Beyond this growing epidemiological burden, the neurobiological mechanism of anxiety has also become increasingly well-defined with studies indicating that the lateral habenula (LHb), amygdala, and bed nucleus of the stria terminalis (BNST) regions all contribute distinctively to specific aspects of anxiety-like behaviors [2]. GAD is frequently comorbid with other psychological disorders, including major depressive disorder (MDD), resulting in diagnostic overlap and complicating the diagnosis of GAD [7]. Experiences such as bullying can deepen emotional vulnerability and are strongly linked to higher anxiety levels. Anxiety also often overlaps with low self-esteem and non-typical depressive symptoms, including suicidality-related features which can further complicate diagnosis. Together, these psychosocial factors highlight the broader vulnerability context in which GAD develops [8, 9]. According to research, up to 50% of people with anxiety disorders go undiagnosed, based on commonly used questionnaires [10]. Given these diagnostic challenges, there is a growing need for biological indicators that can assist in distinguishing GAD from overlapping psychiatric conditions. Different biological indicators also known as biomarkers have received growing attention for their ability to distinguish the biological pathways of disorders which improve the diagnostic precision [10, 11]. Therefore, the study of these biomarkers may offer a more coherent understanding of GAD and help to provide more effective treatment regimen.

The pathophysiological features and etiology of anxiety are being thoroughly studied, particularly in relation to biological and psychogenic factors, which may consistently involve variations in neurotrophic signaling [4]. Neurotrophic factors, including glial cell line-derived neurotrophic factors (GDNF), are crucial regulators of neuroplasticity, highlighting their importance in the development of mental disorders. The role of GDNF in the diagnosis, prognosis, and therapy of brain disorders is the subject of intense research and discussion [12]. Furthermore, the significance of matrix metalloproteinases (MMPs) in inflammation and associated biological processes, which are strictly controlled by endogenous tissue inhibitors of metalloproteinases (TIMPs), is supported by increasing research and evidence linking peripheral inflammatory markers and psychological conditions [13]. Collectively, these pathways, including neurotrophic support (GDNF), extracellular matrix regulation (TIMP4/MMPs), and synaptic plasticity (tPA), work in close connection to maintain healthy brain function. When these systems become disrupted, they increasingly appear to contribute to the biological processes underlying anxiety disorders [14, 15].

GDNF, a vital neuroprotective ligand for midbrain dopaminergic neurons, belongs to the transforming growth factor-β (TGF-β) superfamily that communicates through cell-surface tyrosine kinase receptors [16]. GDNF is linked to essential functions in cognition, including synaptic plasticity, synaptogenesis, neuronal growth, neurogenesis, and the viability and upkeep of neurons, particularly serotonergic and dopaminergic neurons [17, 18]. Additionally, GDNF safeguards glial cells and neurons from oxidative stress [3] and contributes to several. Physiological processes are advantageous for neurological health, encompassing insulin-sensitizing, angiogenic, anti-inflammatory, and vasodilatory effects [19]. Although prior studies have reported altered GDNF levels in mood disorders, the findings remain inconsistent; both decreased [20] and increased [21, 22] levels have been documented. Only a limited number of studies have examined GDNF specifically in GAD population [3, 4], indicating significant knowledge gap.

Compelling evidence underscores the involvement of MMPs in psychiatric diseases, where they govern extracellular matrix (ECM) remodeling, acute and chronic neuroinflammation, neuronal migration, synaptic plasticity, and blood–brain barrier maintenance. These activities are meticulously regulated by TIMPs, which modulate the activity of MMPs [13]. Several substrates, such as signaling molecules, ECM constituents, and inflammatory mediators like cytokines, are cleaved by MMPs. High MMP activity, owing to its numerous proteolytic substrates, can be harmful and is thus stringently controlled by TIMPs [23]. TIMPs encompass TIMP1, TIMP2, TIMP3, and TIMP4, which are crucial for

neuroprotection, neuronal differentiation, and synaptic plasticity within the central nervous system [24]. Previous investigations have established a correlation between TIMP/MMP expression patterns and neurodegenerative conditions including Parkinson's and Alzheimer's illnesses, as well as schizophrenia [25–27]. Thus far, only one recent study has reported an association between plasma levels of TIMP4 and GAD as well as depressive disorders [13], highlighting a need for further research.

Furthermore, the PLAT gene encodes tPA, a 70 kDa serine protease, and reduced serum tPA levels hinder the enzymatic transformation of proBDNF into mature BDNF, thereby contributing to the pathogenesis of depression, which may result in compromised synaptic plasticity, diminished neurogenesis, and heightened neuronal apoptosis [15, 28]. Moreover, tPA and its associated molecular components may serve as promising pharmacological targets for anxiety disorder, as an earlier study demonstrated that tPA is a crucial regulator of neuronal remodeling within the medial amygdala and is indispensable in the progression of stress-related anxiety [29]. Additionally, mice with altered expression of tPA frequently demonstrate changes in stress response, including alterations in anxiety-like behaviors and depressive-like symptoms [28]. Despite its mechanistic relevance, tPA has not been well studied in clinical GAD cohorts.

Numerous etiological factors, including environmental, genetic, neurochemical, psychological, neurobiological, and immunological influences, contribute to the development of GAD [11]. Because these biomolecular mechanisms are objectively measurable, they may serve as promising biomarkers for individualized treatment. Consequently, the integration of novel biomarkers into existing clinical assessment frameworks may facilitate the development of a classification approach for anxiety disorders that correlates with the underlying biological dysfunction [30]. We hypothesized that GDNF, TIMP4, and tPA serum concentration would be significantly altered in individuals with GAD compared to HCs and that these biomarkers would show meaningful correlations with symptom severity. However, no studies have been undertaken to examine the role of GDNF, TIMP4, and tPA in the progression of GAD among patients in Bangladesh. Therefore, the current study sought to assess serum GDNF, TIMP4, and tPA levels in GAD patients relative to healthy participants, to elucidate their association with disease pathophysiology.

2 Methods

2.1 Study design and participants

From May 1, 2025, to July 30, 2025, this case–control investigation enrolled 101 individuals suffering from GAD and 101 healthy controls (HCs), matched by age, sex, and body mass index (BMI). Patients with GAD were recruited from the National Institute of Mental Health (NIMH) and Hospital, a tertiary psychiatric facility in Dhaka, Bangladesh, whereas HCs were recruited from various community across Dhaka. A qualified neuropsychiatrist assessed all participants and confirmed GAD diagnoses in alignment with the standards listed in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), and symptom severity was evaluated using the Generalized Anxiety Disorder 7-item (GAD-7) scale. Written informed consent was secured from participants before collecting data, following a thorough explanation of the study's purpose and procedure. A pre-structured questionnaire was used to gather the clinical history and sociodemographic data. Individuals aged 18 to 60 and patients with a GAD-7 score of five or more fulfilled the inclusion criteria for the study. GAD patients who had taken any kind of anxiolytic, antidepressant, or antipsychotic drug in the preceding 2 weeks were excluded. Additional exclusion criteria were comorbid with schizophrenia, major depressive disorder (MDD), panic disorder, Parkinson's disease, post-traumatic stress disorder (PTSD), obsessive–compulsive disorder (OCD), Alzheimer's disease, and social phobia. Furthermore, participants were also excluded if they had inflammatory diseases, infectious diseases, cognitive impairments, diabetes mellitus, hepatic and renal disorders, and any other acute or substantial medical conditions fall into this category. Individuals who were pregnant or had comorbidities associated with other AXIS I disorders were also eliminated. Individuals with alcohol dependence or use of other addictive substances were also excluded. These criteria were implemented to ensure a homogeneous study sample and to minimize potential confounding impact on biomarker measurements.

2.2 GAD-7 scale

The GAD-7 scale comprising seven items that measure symptoms like apprehension and relaxation difficulties, is a commonly utilized tool to evaluate anxiety levels in individuals with GAD. The scale was used to evaluate the frequency of core anxiety symptoms over the preceding 2 weeks which yielded a total score ranging from 0 to 21 with higher score indicating more severe symptoms. Standard severity thresholds were applied: < 5 no anxiety, 5–9 mild, 10–14 moderate, and 15–21 severe. The customized one-factor model of the GAD-7 demonstrates strong convergent validity and excellent internal consistency, as evidenced by an investigation validating the scale among a cohort of university students from Bangladesh [31, 32]. Both Bangla and English versions of the GAD-7 were used. With the assistance of both medical

and non-medical translators, the Bengali version was developed utilizing the back-and-forth translation technique. A pilot test was conducted to ensure clarity and comprehension. Participants completed the version most appropriate for their language proficiency.

2.3 Blood sample collection, processing, and storage

Upon verifying eligibility based on all inclusion and exclusion criteria, venipuncture of the cephalic vein was executed to obtain 5 ml of blood from each participant, encompassing both patients and HCs, for subsequent laboratory analysis. The collected blood was permitted to coagulate in Falcon tubes. Serum was separated from blood by centrifuging for 15 min at 3000 rpm. Following centrifugation, the isolated serum samples were preserved for further analysis at −80 °C in Eppendorf tubes.

2.4 Estimation of serum GDNF, TIMP4, and tPA levels by ELISA assays

Serum concentrations of GDNF, TIMP4, and tPA were detected via enzyme-linked immunosorbent assays (ELISA) utilizing the Human GDNF ELISA Kit EZ-Set, Human TIMP4 ELISA Kit EZ-Set, and Human PLAT/TPA ELISA Kit EZ-Set (Boster Bio, USA) following the manufacturer's instructions [33, 34]. Plates were coated with capture antibody and incubated overnight at 4 °C and then blocked and washed before adding standards and serum samples for a 2-h incubation. After washing, biotinylated detection antibody and the avidin–peroxidase complex were applied in successive incubation. Color was developed after adding TMB, and the reaction was then stopped using stop solution and absorbance was read at 450 nm. Standard curves were generated for each biomarker, and then serum concentrations of GDNF, TIMP4, and tPA (pg/ml) were determined from optical density (O.D.) values. To eliminate discrepancies between assays, all tests were performed by the same personnel. Furthermore, the experiments were conducted by researchers without knowing the outcomes, thus ensuring fair interpretation of the data.

2.5 Statistical analysis

Microsoft Excel 2021 was employed to process the data, and IBM Statistical Package for Social Sciences (SPSS) version 27.0 (IBM Corporation, Armonk, USA) was employed to execute all subsequent statistical analyses. The sociodemographic and clinical features of the study cohort were compiled using descriptive statistics. The disparities between patients and HCs in terms of sociodemographic variables and clinical characteristics were examined employing the independent samples T test for continuous variables and the Chi-square (χ^2) test for categorical variables. An independent samples T test was employed for the comparison of serum GDNF, TIMP4, and tPA levels across GAD and control groups, with values summarized as mean ± standard deviation (SD). Additionally, the association among different demographic and clinical factors, as well as serum GDNF, TIMP4, and tPA levels, was examined using the Pearson correlation analysis. Moreover, serum GDNF and TIMP4 levels were visually compared between the patient and HC groups using box plot graphs. Receiver operating characteristic (ROC) curve analysis was employed to rigorously assess the diagnostic effectiveness of serum GDNF and TIMP4 levels to discriminate between individuals diagnosed with GAD and healthy control participants. All statistical tests were deemed significant at $p < 0.05$.

3 Results

3.1 Characteristics of the study population

Table 1 summarizes the sociodemographic features and clinical attributes of the study cohort. The predominant demographic in both patient and control groups consisted of young adults (ages 18–25 and 26–35), with no statistically significant variation ($p = 0.959$). The proportion of male (GAD patients: 63.37%; HCs: 67.33%) and female (GAD patients: 36.63%; HCs: 32.67%) participants were comparable, and the BMI was also similar with most participants (65.35% of both groups) fell within the normal BMI range. Regarding marital status, the predominant demographic among both patients and controls was married individuals (patients: 55.45%, controls: 59.41%; $p = 0.569$). A notable proportion of the patient population (39.61%) and HCs (46.54%) held a graduate degree or higher. Most of the participants in both groups being unemployed (patients: 37.62%, controls: 45.54%) and predominantly residing in urban regions (patients: 79.21%, controls: 84.16%; $p = 0.363$), with a middle-income status (GAD patients: 50.50%, HCs: 43.57%; $p = 0.613$), and none of these aspects show any statistical significance between groups. A significant proportion of individuals were non-smokers (patients: 68.32%, controls: 91.09%; $p < 0.001$). This study found that the prevalence of a prior history of GAD among patients was 38.61%, while the familial history of GAD was 23.76%. Nonetheless, there was no prior or familial history of GAD among the HCs.

3.2 Biophysical characteristics, clinical profile, and laboratory findings

Table 2 details the biophysical and clinical parameters and laboratory outcomes of the study population, and Fig. 1 illustrates the comparative distribution of serum GDNF and TIMP4 levels between GAD patients and HCs. The average age and BMI did not have any significant difference between GAD patients and HCs (age:

Table 1 Characteristics of the study population

Parameters	GAD patients *n* (%)	Healthy controls *n* (%)	*p* value
Age in years			0.959
18–25	33 (32.67)	31 (30.69)	
26–35	42 (41.59)	41 (40.60)	
36–45	18 (17.82)	21 (20.79)	
46 and above	8 (7.92)	8 (7.92)	
Sex			0.554
Male	64 (63.37)	68 (67.33)	
Female	37 (36.63)	33 (32.67)	
BMI (kg/m^2)			0.323
Underweight (< 18.5)	2 (1.98)	6 (5.94)	
Normal (18.5–24.9)	66 (65.35)	66 (65.35)	
Overweight (≥ 25.0)	33 (32.67)	29 (28.71)	
Marital status			0.569
Married	56 (55.45)	60 (59.41)	
Unmarried	45 (44.55)	41 (40.59)	
Education level			0.128
Illiterate	4 (3.96)	0 (0.00)	
Primary	21 (20.79)	15 (14.85)	
Secondary	36 (35.64)	39 (38.61)	
Graduate and above	40 (39.61)	47 (46.54)	
Occupation			0.601
Business	8 (7.92)	5 (4.95)	
Service	36 (35.64)	34 (33.66)	
Housewife	11 (10.89)	7 (6.93)	
Student	7 (6.93)	9 (8.91)	
Unemployed	38 (37.63)	46 (45.55)	
Others	1 (0.99)	0 (0.00)	
Economic status			0.613
High	12 (11.88)	14 (13.86)	
Medium	51 (50.50)	44 (43.57)	
Low	38 (37.62)	43 (42.57)	
Residence area			0.363
Rural	21 (20.79)	16 (15.84)	
Urban	80 (79.21)	85 (84.16)	
Smoking history			**< 0.001**
Non-smoker	69 (68.32)	92 (91.09)	
Smoker	32 (31.68)	9 (8.91)	
Previous history of GAD			**< 0.001**
Yes	39 (38.61)	0 (0.00)	
No	62 (61.39)	101 (100.00)	
Family history of GAD			**< 0.001**
Yes	24 (23.76)	0 (0.00)	
No	77 (76.24)	101 (100.00)	

GAD generalized anxiety disorder, *BMI* body mass index

p value < 0.05 indicates statistically significant (bold)

Table 2 Biophysical characteristics, clinical features, and laboratory findings of the study participants

Parameters	GAD patients (n = 101) Mean ± SD	Healthy controls (n = 101) Mean ± SD	*p* value
Age (in years)	30.74 ± 8.73	30.97 ± 9.59	0.860
BMI (kg/m^2)	23.45 ± 3.11	23.11 ± 3.30	0.453
GAD-7 scores	11.07 ± 5.25	–	**< 0.001**
Serum GDNF level (pg/ml)	356.84 ± 127.43	198.13 ± 119.23	**0.002**
Serum TIMP4 level (pg/ml)	2301.14 ± 1226.72	3608.13 ± 1962.82	**0.004**
Serum tPA level (pg/ml)	2131.82 ± 971.95	2002.74 ± 942.36	0.632

BMI body mass index, *GAD* generalized anxiety disorder, *GDNF* glial cell line-derived neurotrophic factor, *TIMP4* tissue inhibitor of metalloproteinase 4, *tPA* tissue plasminogen activator, *SD* standard deviation

p value < 0.05 indicates statistically significant (bold)

30.74 ± 8.73 years vs, 30.97 ± 9.59 years, $p = 0.860$; BMI: 23.45 ± 3.11 kg/m^2 vs 23.11 ± 3.30 kg/m^2, $p = 0.453$). The average GAD-7 scores for GAD patients were 11.07 ± 5.25.

Patients with GAD showed a significant elevation ($p = 0.002$) of serum GDNF levels (356.84 ± 127.43 pg/ml) in comparison with HCs (198.13 ± 119.23 pg/ml). In contrast, significantly lower ($p = 0.004$) serum levels of TIMP4 were found in GAD patients (2301.14 ± 1226.72 pg/ml) compared to HCs (3608.13 ± 1962.82 pg/ml). The serum tPA levels in the patients were marginally elevated (2131.82 ± 971.95 pg/ml) relative to the HCs (2002.74 ± 942.36 pg/ml), although no statistical significance ($p = 0.632$) was observed.

3.3 Correlation analysis

Table 3 demonstrates the correlation analysis among clinical measures and biomarker levels in GAD patients. The Pearson correlation between GAD-7 scores and serum GDNF levels in GAD patients was moderately positive and statistically significant ($r = 0.468$, $p = 0.001$). Conversely, the Pearson correlation between GAD-7 scores and serum TIMP4 levels in GAD patients was strongly negative and statistically significant ($r = -0.542$, $p = 0.004$). In addition, the Pearson correlation between serum GDNF and TIMP4 levels in individuals with GAD was determined to be moderately negative and statistically significant ($r = -0.321$, $p = 0.032$), demonstrating that an elevation in serum GDNF levels correlates with a reduction in serum TIMP4 levels and vice versa.

3.4 Receiver operating characteristic (ROC) curve analysis

Receiver operating characteristic (ROC) curve analyses were performed within the study sample and require

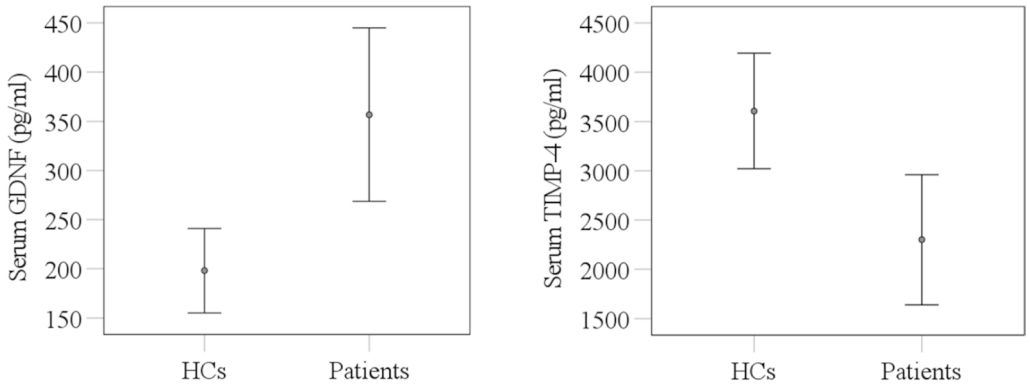

Fig. 1 Comparison of serum GDNF and TIMP4 levels between the cases and controls. Boxplot graphs showing the median, maximum, and minimum value range. *GDNF* glial cell line-derived neurotrophic factor, *TIMP4* tissue inhibitor of metalloproteinase 4, *HCs* healthy controls

Table 3 Correlation study among various research parameters among GAD patients

Correlation parameters	*r*	*p* value
Age and GAD-7 score	−0.088	0.381
Age and GDNF	0.116	0.250
Age and TIMP4	0.109	0.313
Age and tPA	0.183	0.074
BMI and GAD-7 score	−0.003	0.975
BMI and GDNF	0.023	0.820
BMI and TIMP4	−0.070	0.516
BMI and tPA	−0.138	0.180
GAD-7 and GDNF	0.468	**0.001**
GAD-7 and TIMP4	−0.542	**0.004**
GAD-7 and tPA	0.058	0.574
GDNF and TIMP4	−0.321	**0.032**
GDNF and tPA	0.153	0.547
TIMP4 and tPA	0.094	0.735

BMI body mass index, *GAD* generalized anxiety disorder, *GDNF* glial cell line-derived neurotrophic factor, *TIMP4* tissue inhibitor of metalloproteinase 4, *tPA* tissue plasminogen activator, *r* Pearson correlation coefficient

p value < 0.05 indicates statistically significant (bold)

independent validation before clinical interpretation (Fig. 2). ROC analysis demonstrated that serum GDNF levels revealed a descriptive discrimination with an area under the curve (AUC) of 0.829 (95% CI: 0.772–0.886, $p < 0.001$). At a cutoff value of 186.67 pg/ml, serum GDNF levels were detected with 79.2% sensitivity and 74.7% specificity. Moreover, serum TIMP4 levels revealed a strong diagnostic efficacy, evidenced by an elevated AUC value of 0.850 (95% CI: 0.798–0.902, $p < 0.001$). At a threshold of 1828.12 pg/ml, serum TIMP4 levels exhibited a sensitivity of 75.2% and a specificity of 78.2%.

4 Discussion

This study indicated that serum GDNF levels were significantly elevated and serum TIMP4 levels were significantly reduced in individuals with GAD compared with HCs, while serum tPA levels had not have any significant differences between the group. Furthermore, a rise in GDNF levels may be associated with anxiety severity, and a reduction in TIMP4 levels found when the anxiety severity increased. In addition, the GDNF level exhibited a significant inverse correlation with TIMP4 level. The ROC curve analysis revealed that serum levels of GDNF and TIMP4 can effectively differentiate between GAD patients and individuals without the disorder with reasonable accuracy.

In accordance with our findings, a prior investigation identified that GAD patients had higher serum GDNF levels than HCs [4]. Similar elevations have also been noted in MDD and other psychiatric conditions, including schizophrenia, bipolar disorder, and attention-deficit hyperactivity disorder (ADHD) [21, 35–43]. GDNF plays a critical role in neuroprotection by supporting dopaminergic and serotonergic neurons by mitigating oxidative or neuroinflammatory damage and enhancing neurite proliferation in a variety of neuronal types. This neuroprotective properties of GDNF potentially contribute to the emergence and progression of neuropsychiatric illnesses [40]. Dysregulation and imbalance of the neurotransmitters serotonin (5-HT) and dopamine system is well established in the pathophysiology of GAD, and considering the intricate interactions of GDNF with the 5-HT/DA systems, GDNF may significantly influence the pathology and therapeutic response of GAD [3]. However, contrary to our findings, previous investigations have also reported reduced GDNF levels in patients with GAD [3] and MDD [20, 43–45] relative to healthy controls. The contradictory results can be elucidated by several factors,

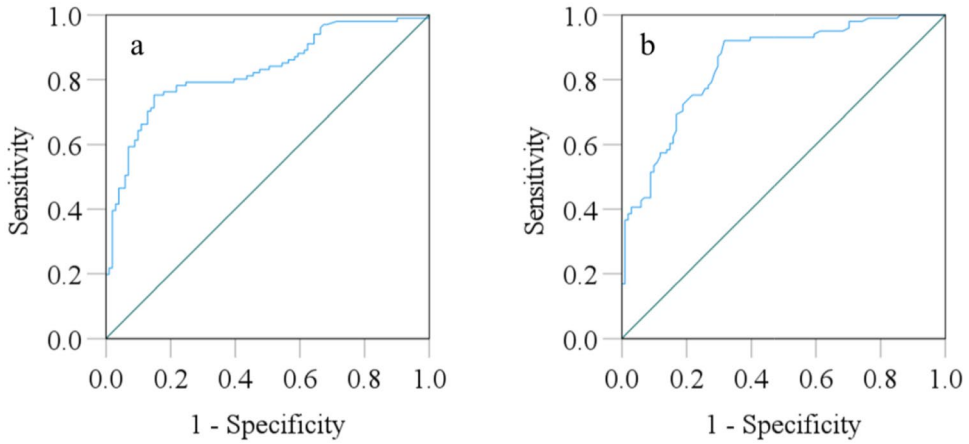

Fig. 2 Receiver operating characteristic (ROC) curve analysis of serum GDNF and TIMP4 levels in study participants ($p < 0.001$). **a** ROC curve of GDNF reveals area under the curve (AUC), sensitivity, and specificity as 0.829 (95% CI: 0.772–0.886), 79.2%, and 74.7%, respectively, at cutoff value of 186.67 pg/ml. **b** ROC curve of TIMP4 reveals area under the curve (AUC), sensitivity, and specificity as 0.850 (95% CI: 0.798–0.902), 75.2%, and 78.2%, respectively, at cutoff value of 1828.12 pg/ml. *GDNF* glial cell line-derived neurotrophic factor, *TIMP4* tissue inhibitor of metalloproteinase 4

including age of individuals, ethnic backgrounds of study participants, distribution of sex, sample types (whole blood, plasma, or serum), GAD severity, methodologies for assessing GDNF levels, or coexisting physical or psychiatric conditions [46]. Such variability highlights the need for standardized biomarker assessment and phenotype-specific analyses in anxiety research.

Unlike GDNF, TIMP4 has been minimally studied in GAD. The only existing study reported increased TIMP4 levels in young adults with GAD and depression compared to HCs, contradicting with the reduced levels in our findings [13]. The conflict results can be because of different detection techniques. Although the concentration was, the precise mechanisms through which TIMP4 levels affect GAD remain incompletely elucidated. The expression mode of TIMP4 differs from other TIMPs, as evidenced by the localization of TIMP4 mRNAs to the brain, skeletal muscle, ovary, and heart, suggesting a more tissue-specific role in extracellular matrix (ECM) regulation [24, 38]. Furthermore, among TIMPs, TIMP4 demonstrates higher specificity for the brain. In alignment with our findings, decreased levels of TIMP4 were found to be directly associated with disorders characterized by neuroinflammation and altered blood–brain barrier (BBB) integrity such as delirium and hydrocephalus [13, 47–49]. These reductions are thought to reflect diminished inhibition of MMPs which led to BBB permeability by tight-junction degradation, which facilitate the infiltration of inflammatory cytokines in the brain parenchyma and participating in neuroinflammatory cascades that can disrupt physiological neurotransmission. This neuroinflammation leads to the manifestation of symptoms of anxiety [50, 51]. TIMPs, conversely, can mitigate these consequences and preserve BBB integrity by blocking MMPs [13]. Our findings support a potential TIPM4–MMP–ECM pathway contributing to anxiety severity and highlight TIPM4 for further mechanistic investigation.

Moreover, tPA promotes tissue regeneration and vascular remodeling by facilitating the degradation of ECM components, activating MMPs, and releasing proangiogenic factors [28]. Although tPA is implicated in the nervous system, its efficacy as a biomarker for GAD is not substantiated by robust evidence. Conversely, individuals suffering from major depressive disorder (MDD) exhibited markedly reduced serum tPA levels in comparison with healthy individuals [52]. Nevertheless, the current study could not identify a correlation between serum tPA levels and GAD.

Anxiety disorders continue to be diagnosed primarily through subjective symptom reports, despite substantial overlap with other psychiatric presentations [53]. The diverse characteristics of GAD patients complicate both diagnosis and the clinical decision-making effort to define fundamental biological mechanisms, while contributing to inconsistent treatment outcomes. The need for objective biomarkers that can complement clinical assessment is therefore increasingly recognized [30]. There are currently no dependable and distinctive proteins that may reliably predict or diagnose anxiety disorders, even though several studies have documented neurological alterations associated with anxiety. Thus far, most research examining protein in anxiety disorders has concentrated on

other neurotrophins, like nerve growth factors (NGF), and brain-derived neurotrophic factors (BDNF) [4]. The present findings suggest that altered serum levels of GDNF and TIMP4 reflect neurobiological process associated with GAD. These biomarkers reflect both neurotrophic signaling and changes in the extracellular matrix of brain offer a clearer link between what we measure in the blood and the deeper biological processes driving anxiety. Although further validation is required, particularly through longitudinal and mechanistic studies.

4.1 Strengths and limitations of this study

This study represents the first investigation in Bangladesh assessing the connection between serum GDNF, TIMP4, and tPA levels in the development of GAD, addressing a significant gap in biomarker research within South Asian populations. The robustness of our study stems from the establishment of specific inclusion and exclusion criteria for participant recruitment, which were adhered to acquire both homogeneous and varied population data. The stringent study design significantly aided in mitigating the possible influence of several confounding parameters, which include BMI, sex, age, and other socio-demographic factors, as these were meticulously regulated throughout the groups. ROC analysis demonstrated descriptive discrimination capability of serum GDNF and TIMP4 levels in differentiating GAD patients from healthy controls, indicating their potential as helpful biomarkers for early risk evaluation and monitoring therapy responses.

There are certain limitations to our study that should be noted. The limited sample size, although sufficient for preliminary analysis, limits generalizability. Larger multi-center studies are needed for reproducible conclusions. Secondly, the case–control study cannot establish causality, leaving uncertainty about whether biomarker alterations precede GAD onset or arise because of the disorder. Peripheral serum biomarkers may not fully reflect central nervous system activity. Additionally, unmeasured psychosocial variables such as bullying exposure, self-esteem deficits, trauma history, and dietary habits may influence biomarker levels and were not assessed in this study. Longitudinal studies might mitigate this restriction by monitoring participants over time, enabling researchers to observe variations in biomarker levels in conjunction with the emergence and advancement of symptoms. And finally, we acknowledge smoking as a major confounding factor to this study. Moreover, the lack of adjustment in statistical analysis limits interpretation of biomarker differences between the groups.

5 Conclusion

Elevated serum GDNF and diminished serum TIMP4 levels exhibited a strong correlation with the GAD severity, highlighting a potential link to the disease's pathophysiology and development. The identified associations between altered biomarker levels and GAD highlight their potential role in the disease pathophysiology. These findings may give essential assistance for psychiatrists and healthcare practitioners in improving patient care. The study emphasizes the necessity of incorporating biomarker-based diagnostics into national mental health initiatives, promoting broader execution, professional training, and evidence-based treatments. We consider these findings preliminary and hypothesis generating. Therefore, we recommend further prospective longitudinal studies and external validation cohorts.

Abbreviations

BDNF	Brain-derived neurotrophic factor
BNST	Bed nucleus of the stria terminalis
CAA	Cerebral amyloid angiopathy
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
ELISA	Enzyme-linked immunosorbent assay
GAD	Generalized anxiety disorder
GAD-7	Generalized Anxiety Disorder 7-item
GDNF	Glial cell line-derived neurotrophic factor
HCs	Healthy controls
LHb	Lateral habenula
MDD	Major depressive disorder
MMPs	Matrix metalloproteinases
NGF	Nerve growth factor
NIMH	National Institute of Mental Health
OCD	Obsessive–compulsive disorder
PTSD	Post-traumatic stress disorder
ROC	Receiver operating characteristic
SEM	Standard error mean
SPSS	Statistical Package for Social Sciences
TGF-β	Transforming growth factor-β
TIMP4	Tissue inhibitor of metalloproteinase 4
tPA	Tissue plasminogen activator

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43088-026-00740-3.

Additional file 1 (DOCX 86 kb)

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Authors contribution

P.K. and M.R.I. conceptualization, methodology, data curation, formal analysis, and writing-original draft. P.K. and M.M.A.S.Q. data curation, investigation, and project administration. M.R.I. conceptualization, methodology, supervision, and writing review and editing.

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Data availability
The datasets generated and analyzed during this research are available from the corresponding author upon rational request.

Declarations

Ethics statements
The Research Ethics Committee of the University of Asia Pacific formally granted ethical clearance for this case–control study, under reference number UAP/REC/2025/104. In compliance with the Helsinki declaration, the study was carried out.

Competing Interests
The authors declare no competing interests.

Clinical trial number
Not applicable.

Human ethics and consent to participate
All participants provided written informed consent prior to enrollment. Informed consent for the publication is not applicable to this study.

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