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Increased serum EGF but not SDF-1 levels are associated with the pathophysiology and development of generalized anxiety disorder

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Generalized anxiety disorder (GAD) is a common and persistent mental illness accompanied by uncontrollable worries for daily staff. Physiological, environmental, genetic, and daily stress from surroundings is involved in developing GAD. Here, we aimed to assess the association of stromal cell-derived factor-1 (SDF-1) and epidermal growth factor (EGF) in the pathophysiology of GAD and their role as diagnostic tools. This case-control study recruited 50 GAD patients from the psychiatry department of a tertiary care teaching hospital in Dhaka city and 38 age-sex-matched healthy controls (HCs) from the surrounding areas. A qualified psychiatrist conducted standardized psychiatric interview to diagnose GAD patients and evaluate HCs by applying the diagnostic and statistical manual for mental disorders, 5th edition (DSM-5) and used the GAD-7 scale to assess the severity of the GAD symptoms. After confirming the inclusion and exclusion criteria, we collected 5 ml blood samples from each participant. We measured serum SDF-1 and EGF levels using ELISA techniques. We observed increased serum levels of EGF in GAD patients compared to HCs (5.91 ± 3.90 ng/ml vs. 1.59 ± 1.08 ng/ml; $p < 0.001$). Also, this increment is positively associated with the severity of GAD in patients ($r = 0.479$, $p = 0.002$). The receiver operating characteristic analysis showed good diagnostic performance (AUC 0.869, $p < 0.001$) with high sensitivity (82.5%), and specificity (81.1%) at cut off value of 2.31 ng/ml. However, we didn't find significant differences in serum SDF-1 levels between GAD patients and HCs (11.96 ± 4.50 ng/ml vs. 12.70 ± 11.76 ng/ml; $p = 0.337$). The present study suggests that GAD patients have increased serum EGF levels but not SDF-1 levels compared to HCs and that increased EGF levels are associated with increased GAD symptoms as well. Moreover, EGF levels could serve as a biomarker for GAD However, further interventional studies with larger and homogeneous samples are suggested to confirm and establish these findings.

Keywords Generalized anxiety disorder, Epidermal growth factor, EGF, Stromal cell-derived factor-1, SDF-1, Protein, Hormone, Case-control study

Generalized anxiety disorder (GAD) is a prevalent and persistent psychiatric disorder accompanied by excessive and unmanageable worries about various domains of life, including health, work, finances, relationships, and overall well-being regarding almost every routine life activity[1]. Individuals diagnosed with GAD frequently exhibit prolonged and heightened levels of anxiety that surpass the appropriate magnitude concerning the perceived threat or difficulty encountered[2]. These anxieties are typically challenging to manage and can interfere with daily activities, interpersonal relationships, and overall life quality[3,4]. The etiology and pathogenesis of GAD are multifaceted and encompass a confluence of genetic susceptibility, neurochemical dysregulation within the central nervous system (CNS), and environmental stressors, including traumatic experiences and chronic stress[5,6]. Several personality traits, such as perfectionism and heightened sensitivity to threats, are also influential factors in this phenomenon[7]. GAD is a condition that affects individuals across all age groups, although it typically manifests during adolescence[2]. Globally, the prevalence of GAD is 3–6%, making it one of the most prevalent anxiety disorders, and 33.7% of the population suffers from anxiety at any stage of their lifespan[8]. The

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global prevalence of anxiety disorders rose from 298 million to 374 million people, increasing by 25.6% during the COVID-19 pandemic in 2020[9].

The pathophysiology of GAD entails intricate interplays among biological systems, encompassing the brain, neurotransmitters, and the body's stress response[5]. Although the precise mechanisms are not completely comprehended yet, empirical evidence indicates the involvement of various pivotal factors. Neurotransmitters, specifically serotonin, norepinephrine, and gamma-aminobutyric acid (GABA), are associated with the pathogenesis of GAD[10–12]. The amygdala appears hyperactive in GAD patients, leading to heightened fear responses and an augmented sense of perceived threat[5,13]. Genetic factors, along with chemokines and growth factors, are also found involved in GAD[14–17]. Chemokines, which have conventionally been linked with the recruitment of immune cells and inflammation, have been recently identified as potential contributors to anxiety disorders through neuroinflammation, synaptic plasticity, and neurotransmitter modulation[18,19]. Increased levels of chemokines such as CCL2, CCL5, and CXCL8 that have been detected in the cerebrospinal fluid of GAD patients indicate dysregulation of chemokines, affecting the function of various neurotransmitters and neuropeptides that are implicated in the regulation of anxiety[20–22]. Chemokines have been observed to influence the hypothalamic pituitary adrenal (HPA) axis, leading to the overproduction of cortisol, a stress hormone, and exacerbating anxiety symptoms[23]. Growth factors, such as brain-derived neurotrophic factor (BDNF), epidermal growth factor (EGF), and nerve growth factor (NGF), have been linked to the pathogenesis of GAD[24,25]. These growth factors influence synaptic plasticity, cell survival, and neuronal health. Altered expression of these growth factors was found associated with GAD patients, notably significantly decreased levels of BDNF[24–27]. Preclinical models found altered NGF levels and a correlation between anxiety[25,28]. These findings highlight the critical role of chemokines and growth factors in neuronal function and their potential as therapeutic targets for GAD.

The chemokine stromal cell-derived factor-1 (SDF-1), also known as CXCL12, has been studied concerning GAD[18,22]. SDF-1 is an essential mediator in the migration and homing of immune cells and is involved in several neurobiological processes within the CNS. Previous research has indicated a potential correlation between the dysregulation of SDF-1 and the pathophysiology of GAD through neuroinflammatory processes, synaptic plasticity disruption, and neurotransmitter modulation. SDF-1 levels in the cerebrospinal fluid of GAD patients were found elevated in some investigations[18,22,29]. EGF is a pivotal factor in many cellular processes, such as cell proliferation, differentiation, and survival[30]. The precise function of EGF in GAD remains incompletely comprehended. Nonetheless, scientific inquiry indicates that EGF could participate in the neurobiological pathways that underlie anxiety disorders[16]. According to various studies, altered levels and signaling of EGF have been reported in individuals diagnosed with GAD[16,31]. In addition, previous research conducted on animals has demonstrated that the introduction of EGF can regulate behaviors associated with anxiety[32,33]. This indicates that EGF may potentially play a role in regulating anxiety.

While research suggests that associations of genetic, environmental, neurological aspects and neurotransmitter imbalances are involved in the progression of GAD, the precise mechanisms and role of neurochemicals and neurotransmitters in determining the severity of the condition remain unclear[5,34,35]. In addition, the interplay between various brain regions and circuits involved in anxiety regulation requires further study. Knowledge advancement in this area can shed light on potential pharmacological intervention targets and provide a deeper understanding of the neurochemical basis of GAD. Therefore, the present study aimed to evaluate SDF-1 and EGF in GAD patients compared to healthy controls (HCs) to understand the disease pathophysiology, its association with the disease severity, and the capability of early risk assessment as diagnostic tools.

Methods

Study design and study subjects

In this case-control study, 50 GAD patients and 38 HCs participated from January 1, 2023, to March 30, 2023. We enrolled GAD patients from the psychiatry department of tertiary-level teaching hospitals and age-sex-matched HCs from the surroundings of patients aged between 18 and 60. A practicing psychiatrist performed the diagnosis of GAD patients for this study according to the Diagnostic and Statistical Manual for Mental Disorders, 5th edition (DSM-5) criteria. The severity of GAD symptoms were assessed by applying the generalized anxiety disorder 7-item (GAD-7) scale. We briefed participants about the objective and purpose of this study and obtained written informed consent before data collection. We collected the socioeconomic characteristics and clinical history of participants using a pre-structured questionnaire. GAD patients were excluded if they had taken any antidepressant or antipsychotic medication for at least two preceding weeks, and if they had other AXIS I disorders. Other exclusion criteria were applied to both groups. Participants with pregnancy and comorbidity with other diseases, such as inflammatory disease, renal dysfunction, chronic and acute infection, diabetes mellitus, and cognitive impairment, were excluded from the study. Additional exclusion criteria were alcoholism and the use of other substances of abuse.

GAD-7 scale

The GAD-7 scale is a widely used tool for assessing anxiety levels among GAD patients including seven questions, that evaluate symptoms such as nervousness and difficulty relaxing. Participants response about the frequency of experiencing these symptoms over the past two weeks, with options and scores of single questions ranging from "not at all (0)" to "nearly every day (3)". The total score, which can range from 0 to 21, helps evaluate the severity of anxiety: minimal (0–4), mild (5–9), moderate (10–14), and severe (15–21). A study validated this scale among university students in Bangladesh concluding the modified single-factor model of the GAD-7 shows high internal consistency and good convergent validity[36]. We utilized a combination of both the local language and English version GAD-7 scale and all questionnaires. The Bengali version was produced using the back-forward translation method, involving medical and non-medical translators. An independent translator then back-translated it to English to address inconsistencies. A pilot test was conducted to ensure

clarity, and finally, both English and Bengali versions were combined for comprehensive understanding by both psychiatrists and participants.

Collection of blood sample and processing

If all inclusion and exclusion criteria had been fulfilled, 5 ml of blood was taken from the cephalic vein of each patient and the HC for further laboratory testing. After taking a blood sample, we let it sit at room temperature for an hour to allow the blood to clot. The serum sample was then separated from the blood sample by centrifuging at 1000 g for 30 min. The separated serum was then put in an Eppendorf tube and stored at -80 °C for further analysis.

Serum sample analysis

We used the commercially available human SDF-1 and EGF ELISA kits to determine the serum levels of the respective cytokines. We followed the manufacturer's instructions throughout the entire procedure mentioned in our previous study[37–39]. Briefly, 96-well microtiter plates were filled with 100 µl of different serum samples and standard solutions. We covered the plate and incubated it for 120 min at room temperature. The cover was then opened to empty the wells. Each well received 100 µl of biotinylated anti-human antibody and was mixed well. Plate sealer was used again to incubate the plate for 90 min at room temperature. After discarding the liquid from each well, three 300 µl washes were done using wash buffer. Then each well received 100 µl of avidin-biotin-peroxidase complex. After 40 min of incubation at room temperature, upon removing the content from the wells, each well was washed five times using 300 µl of wash buffer. After adding 90 µl of tetramethylbenzidine to each well, the plate was incubated at room temperature in the dark for 30 min. Lastly, 100 µl of stop solution halted the process and measured the absorbance at 450 nm. SDF-1 and EGF serum concentrations were expressed as ng/ml. To avoid variation between assays, all tests were conducted by the same individuals. The investigators who performed the assays were oblivious to the samples' clinical information.

Statistical analysis

We used Microsoft Excel 2019 to process the data and IBM Statistical Package for Social Science (SPSS) version 25.0 (IBM Corporation, Armonk, USA) to do all the statistical studies. We performed descriptive statistics and found our data was normally distributed without having any significant skewness and kurtosis. For numerical variables, we did an independent sample t-test, and for categorical variables, we did a chi-square (χ^2) test. The Pearson correlation coefficient analysis was utilized to examine the association between serum chemokine levels and clinical variables. The serum concentrations of SDF-1 and EGF have been presented as the mean ± standard deviation (SD). Boxplot and scatterplot graphs were employed to compare the levels of chemokines in the serum of patients and HCs. Receiver operating characteristics (ROC) analysis was conducted to evaluate the diagnostic ability of serum EGF levels in distinguishing between GAD patients and HCs. When p-values were less than 0.05, the results were considered statistically significant.

Ethical considerations

This investigation was approved by the Research Ethics Committee of the University of Asia Pacific (Ref: UAP/REC/2023/104). The entire investigation was conducted in accordance with the Helsinki Declaration. Before their participation, we briefed the participants on the aims and objectives of this study. In addition, we obtained informed written consent from each participant prior to beginning this study. When we suspected that a person's mental capacity was impaired, we obtained written permission from the guardians.

Results

Socio-demographic profiles of study participants

The socio-demographic profiles of the total study population are presented in Table 1. The preponderance of individuals in both groups were young adults (18–35 years), with no statistically significant difference ($p = 0.960$) between the two groups. The majority of the participants were male. This distribution assures gender parity and reduces the possibility of bias in subsequent analyses. The higher portion of patients were unmarried, and so were HCs. Regarding education level, a greater proportion of participants in both groups were graduates. The distribution of occupations did not differ significantly between patients and HCs, and most of them lived in urban areas. There was no statistically significant difference regarding the body mass index (BMI) of patients and HCs, while the majority of the participants reported within the normal BMI range. There was a prevalence of previous history and a familial history of GAD among patients, however, there was neither a previous nor a familial history among the HCs.

Laboratory findings

We presented laboratory results and clinical profiles in Table 2. The EGF levels in the patient group were significantly lower than those in the HC group ($p < 0.001$): 5.91 ± 3.90 ng/ml versus 1.59 ± 1.08 ng/ml. In the patient group, SDF-1 levels were 11.96 ± 4.50 ng/ml, while among the HCs they were 12.70 ± 1.76 ng/ml. The difference between the two groups' SDF-1 levels was not statistically significant ($p = 0.340$). This alteration in serum SDF-1 and EGF levels has been presented in Fig. 1. Correlation analysis (Fig. 2) showed a significant positive correlation between serum EGF levels and severity scores in GAD patients ($r = 0.48$, $p = 0.002$). Also, female GAD patients with higher severity scores (GAD-7) presented increased serum EGF levels (Fig. 2).

Receiver operating characteristic curve analysis

We conducted ROC curve analysis for serum EGF levels in the study population (Fig. 3), as we only observed a statistically significant change in serum EGF levels in our study. According to the analysis of the ROC curve,

Parameters	GAD patients ($n=50$) mean ± SD	Healthy controls ($n=38$) mean ± SD	p-value
Age in years	31.04 ± 10.80	30.66 ± 12.58	0.983
18–25	20 (40.00%)	14 (36.84%)	
26–35	19 (38.00%)	16 (42.11%)	
36–45	3 (6.00%)	2 (5.26%)	
46–60	8 (16.00%)	6 (15.79%)	
Sex			0.960
Male	30 (60.00%)	23 (60.53%)	
Female	20 (40.00%)	15 (39.47%)	
Marital status			0.416
Married	20 (40.00%)	12 (31.58%)	
Unmarried	30 (60.00%)	26 (68.42%)	
Education level			0.244
Illiterate	4 (8.00%)	0 (0.00%)	
Primary	7 (14.00%)	6 (15.79%)	
Secondary	5 (10.00%)	1 (2.63%)	
Higher Secondary	14 (28.00%)	14 (36.84%)	
Graduate and above	20 (40.00%)	17 (44.74%)	
Occupation			0.861
Business	5 (10.00%)	3 (7.89%)	
Housewife	12 (24.00%)	6 (15.79%)	
Student	12 (24.00%)	11 (28.95%)	
Unemployed	14 (28.00%)	13 (34.21%)	
Others	7 (14.00%)	5 (13.16%)	
Economic status[a]			0.478
High	2 (4.00%)	2 (5.26%)	
Medium	38 (76.00%)	32 (84.21%)	
Low	10 (20.00%)	4 (10.53%)	
Area of residence			0.811
Rural	21 (42.00%)	15 (39.47%)	
Urban	29 (58.00%)	23 (60.53%)	
Smoking history			0.066
Non-smoker	43 (86.00%)	37 (97.37%)	
Smoker	7 (14.00%)	1 (2.63%)	
BMI (kg/m^2)	23.49 ± 4.01	24.70 ± 4.37	0.228
Below 18.5 (CED)	3 (6.00%)	2 (5.27%)	
18.5–25.0 (Normal)	31 (62.00%)	17 (44.73%)	
Above 25.0 (Obese)	16 (32.00%)	19 (50.00%)	
Previous history of GAD			< 0.001
Yes	20 (40.00%)	0 (0.00%)	
No	30 (60.00%)	38 (100.00%)	
Family history of GAD			0.003
Yes	10 (20.00%)	0 (0.00%)	
No	40 (80.00%)	38 (100.00%)	

Table 1. Characteristics of study population. GAD, generalized anxiety disorder; SD, standard deviation; BMI, body mass index; CED, chronic energy deficiency. [a]Economic status is categorized based on monthly income: BDT 25,000 and below as 'Low', BDT 25,001–50,000 as 'Medium', above of BDT 50,000 as 'High'.

serum EGF levels demonstrated promising predictive performance. The serum EGF cutoff value was 2.31 ng/ml. The ROC curve revealed a sensitivity of 82.5% and a specificity of 81.1%. The area under the curve (AUC) for EGF was 0.87 ($p < 0.001$).

Discussion

The present study compared the levels of EGF and SDF-1 in GAD patients and HCs. The results revealed that the EGF levels of GAD patients were significantly higher than those of HCs. However, these two groups had no statistically significant difference in SDF-1 levels. In addition, the current study investigated the association between EGF levels and the severity of anxiety symptoms, which demonstrated an increase in EGF levels with

Parameters	GAD patients ($n=50$) Mean ± SD	Healthy controls ($n=38$) Mean ± SD	p-value
Serum SDF-1 levels (ng/ml)	11.96 ± 4.50	12.71 ± 1.76	0.337
Serum EGF levels (ng/ml)	5.91 ± 3.90	1.59 ± 1.08	< 0.001

Table 2. Clinical features and laboratory findings of the study participants. SDF-1, stromal cell-derived factor-1; EGF, epidermal growth factor, SD, standard deviation.

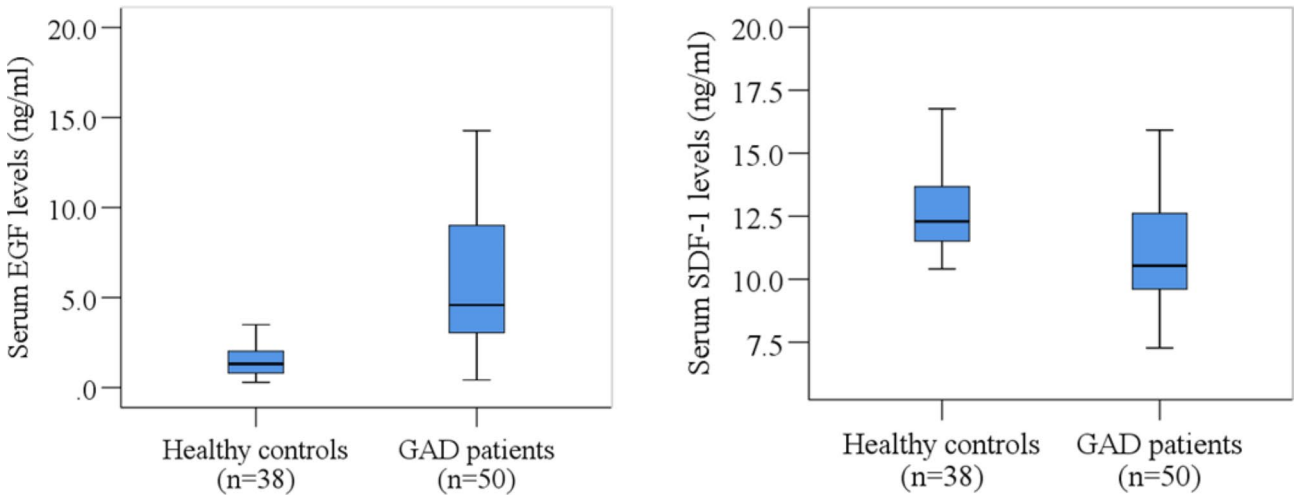

Fig. 1. Distribution of serum EGF and SDF-1 levels in GAD patients and healthy controls. Boxplot graphs showing the median, maximum and minimum value range.

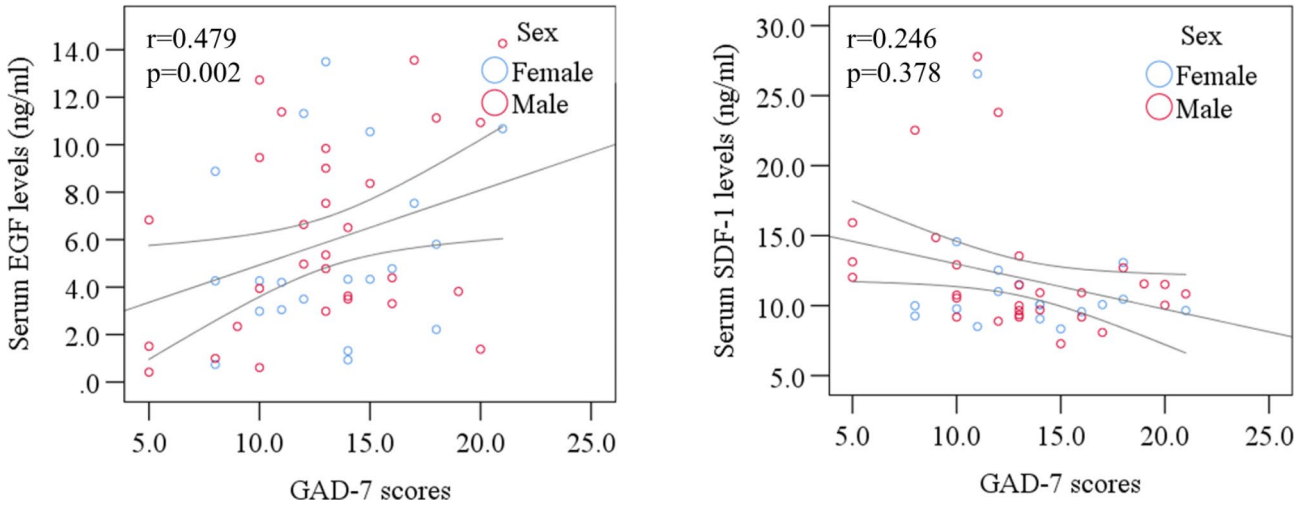

Fig. 2. Association and mean difference of serum EGF and SDF-1 levels with GAD-7 scores of GAD patients.

increasing anxiety severity. Furthermore, sex-based analysis revealed that female patients had lower SDF-1 and EGF levels than male patients. The diagnostic performance evaluation, as per to ROC curve analysis, indicates serum EGF levels can distinguish between individuals with and without the condition with reasonable accuracy. Previous studies had reported elevated levels of a neurotrophic factor in psychiatric disorders[27,38,40,41], which is consistent with the findings regarding EGF levels in GAD patients. Neuroprotection, synaptic plasticity, and neurogenesis are processes implicated in the pathogenesis of psychiatric disorders[42], depending on the EGF[43]. The higher levels of EGF found in the patient group suggest a potential disruption of these neurobiological processes, which may contribute to the onset and severity of anxiety. Fatouros and colleagues observed increased EGF levels in mild inflammation in a study[44], and inflammation is closely associated with GAD. In our study, EGF is significantly correlated with GAD, suggesting that neuroinflammation plays a crucial role in anxiety

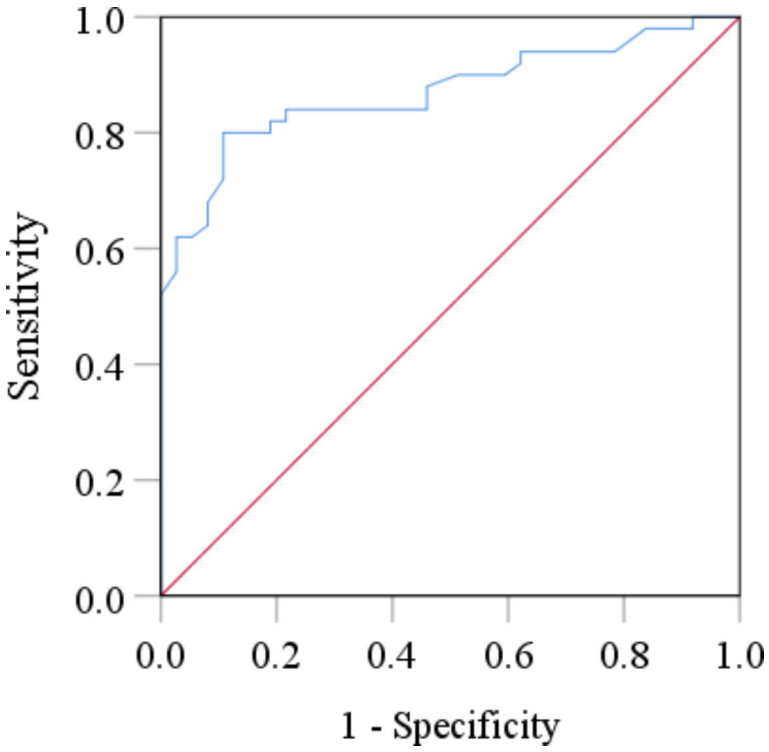

Fig. 3. Receiver operating characteristic (ROC) curves of serum EGF levels among the study population. The serum EGF cutoff value was 2.31 ng/ml, ROC revealed a sensitivity of 82.5% and a specificity of 81.1% and AUC = 0.869 ($p < 0.001$).

disorders. The involvement of EGF in neuroinflammation aligns with elevated levels of inflammatory markers, such as IL-10, IL-6, TNF-α, C-reactive protein (CRP), and oxidative stress indicators, often found in GAD patients[45–48]. These findings suggest that targeting neuroinflammatory pathways could provide new insights into the pathophysiology of GAD. EGF modulates HPA, maintaining stress homeostasis[49]. Galvez-Contreras and colleagues studied EGF in various psychiatric disorders, and they observed elevated EGF levels in bipolar disorder and low levels in schizophrenia and major depressive disorder. However, they did not detect EGF in GAD; however, GAD patients had elevated nerve growth factor (NGF)[25,26]. The study reported overexpression of epidermal growth factor receptor (EGFR) in tumor cells and cancer patients[30]and elevated serum EGF levels in GAD patients, which may be associated with the progression of one another. These EGFRs are resistant to chemotherapy and radiation therapy when they are overexpressed. Therefore, we should consider this correlation while treating tumor cells in GAD patients. In light of the current findings and prior research, it is clear that psychiatric disorders involve complex neurobiological mechanisms[50]. Dysregulation of growth factors, such as EGF, may play a role in the etiology and progression of anxiety disorders. The precise mechanisms underlying these changes in EGF levels and their role in anxiety disorders are still unknown.

According to a previous study, acute and long-term exercise reduces the serum level of EGF. Exercise can alleviate GAD symptoms by lowering serum EGF levels[51]. Mindfulness and cognitive behavioral therapy can also lower serum EGF levels[16]. This finding suggests that EGF may function as a potential biomarker for early risk assessment of the presence and severity of GAD. This biomarker can potentially improve GAD diagnosis, prognosis, and treatment selection. Nevertheless, identifying dependable and clinically applicable biomarkers is still in its infancy, and additional research is required to validate their utility in clinical practice.

This study is notable for being the first to investigate SDF-1 and EGF levels in the context of GAD in the Bangladeshi population. We saw a few studies that assessed these pro-inflammatory chemokines and growth factors in GAD patients, which have yielded inconclusive findings. One study found inconsistent significant alterations in MCP-1 and SDF-1 levels in GAD. Another study found IL-10, but not IL-2, was correlated with GAD. Additionally, another study showed that S100B, along with IL-1, IL-1β, IL-2, IL-4, and IL-10, can distinguish GAD from HCs. All of them called for continuous research on the specific role of pro-inflammatory signaling in the maintenance of these unique psychiatric conditions[18,52,53]. Therefore, we hope this study will add knowledge to growth factors and cytokine analysis in GAD patients. Furthermore, a diverse and homogeneous study population would increase the generalizability and dependability of the findings. The sex-specific associations of blood SDF-1 and EGF levels in GAD patients offer a more nuanced understanding of potential sex differences in these biomarkers. Prospective research should prioritize elucidating the intricate mechanisms by which chemokines and growth factors showed their influence on the manifestation of GAD symptoms in a larger population. In addition, the current findings urge additional research to confirm these findings for broad implications in the diagnosis, therapeutic approaches, and overall management of GAD.

GAD is a complicated mental disease that needs multiple management techniques. Since medications alone aren't enough to treat mild to severe anxiety since they cause dependence, a large amount of study data from different parts of the world will help doctors figure out the pathophysiology of GAD and give the proper treatment strategy. Neuroinflammation is crucial in understanding and treating GAD. One study suggested using neuroimaging and neuronally-derived extracellular vesicles (EVs) to predict treatment responses might show usefulness[54]. Functional MRI can identify neuroinflammatory patterns, while EVs reveal individual inflammation, enabling targeted and personalized treatments for GAD. This study will help figure out how likely it is that someone will get anxious. This study will help us learn more about how GAD works and what causes it. Since there are no diagnostic tests for GAD yet, these results may help doctors learn more about the biomarkers of GAD. So, doctors can use these new markers as early risk assessment tools to measure anxiety.

Limitations

There are a few limitations to this study. Although this study has a diverse and homogenous population, it has a small sample size. Similar studies with a larger sample size focusing on the Bangladeshi population would provide a greater insight into understanding the relationship between chemokines and growth factor in GAD. We included patients who did not take any antidepressant or antipsychotic medications prior to two weeks of study; however, a fully drug-naïve sample population would have yielded more generalizable results. The case-control study methodology used in this work has intrinsic limitations, most notably the inability to capture treatment responses and fluctuations in peripheral SDF-1 and EGF levels among GAD patients over time. Furthermore, limiting the investigation to only serum concentrations of SDF-1 and EGF may only cover a portion of the complete pathophysiology of GAD as the lack of cerebrospinal fluid analysis and the potential influence of the blood-brain barrier could affect the precise evaluation of these biomarkers in the CNS. It would be good to include the measurement of additional parameters within the same population and laboratory context to acquire more comprehensive results. Furthermore, we didn't consider the effect of dietary habits in the present study.

Conclusion

The result of this study suggests that serum EGF levels, but not SDF-1, may be associated with the pathophysiology of GAD. Therefore, altered serum EGF levels might contribute to the development of GAD. Also, the positive association between increased serum EGF levels and the severity of GAD can aid in the understanding, and treatment of GAD, opening up more possibilities to research growth factors in the pathophysiology of GAD. Future research should focus on determining the precise mechanisms by which chemokines and growth factors contribute to the manifestation of GAD symptoms. Furthermore, the excellent ability of EGF to distinguish patients from HCs suggests that EGF levels could serve as a potential biomarker for the diagnosis of GAD. It calls for additional research to validate these findings and investigate their broader implications for the risk assessment, treatment, and management of GAD, which could lead to transformative advances in this field.

Data availability

The data supporting the present study findings are obtainable from corresponding authors upon reasonable request.

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Conceptualization: A.S.M.R. and M.R.I.; Data curation; Formal analysis and investigation: A.S.M.R., M.M.A.S.Q. and M.S.; Writing-original draft preparation: A.S.M.R. and S.M.A.I.; Methodology, Supervision, and Writing-review and editing: M.R.I. All authors have read and agreed to the final manuscript and approved the submitted version.

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Declarations

Competing interests
The authors declare no competing interests.

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