Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Elevated serum cystatin C and IP-10 levels are associated with the pathophysiology and development of generalized anxiety disorder patients: A case-control study

Zahra Labiba Ahmed
BRAC University
Md. Aminul Haque
BRAC University
Md. Rabiul Islam
robi.ayaan@gmail.com

BRAC University

Article

Keywords: generalized anxiety disorder, cystatin C, IP-10, biomarkers, neuroinflammation, pathophysiology, mental disorder

Posted Date: March 8th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8867830/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Background

Generalized anxiety disorder (GAD) is a long-term mental health disorder often linked with immune system dysregulation. According to recent research, neuroinflammation may be involved in the pathogenesis of GAD. However, specific roles of cystatin C and interferon-gamma inducible protein-10 (IP-10) in GAD remain less explored. This study aims to investigate the association between assessed serum cystatin C and IP-10 in GAD patients.

Methods

This case-control study included 100 GAD patients and 100 healthy controls (HCs). Participants were examined using the GAD-7 scale. Serum cystatin C and IP-10 levels were measured using ELISA. Data were analysed using t-tests, Spearman's correlation, and Receiver Operating Characteristic (ROC) curve analysis to assess diagnostic performance.

Results

GAD patients had significantly elevated serum cystatin C and IP-10 levels compared to HCs (2.54 ± 0.16 vs. 1.62 ± 0.14 mg/L and 126.20 ± 10.18 vs. 88.54 ± 0.94 pg/mL; $p < 0.001$). Both cystatin C and IP-10 levels showed significant positive correlations with GAD-7 scores in patient group (cystatin C: $r = 0.777$, $p < 0.001$; IP-10: $r = 0.279$, $p = 0.005$), while elevated cystatin C and IP-10 levels indicated positive correlation with each other ($r = 0.479$, $p < 0.001$). ROC analysis indicated that cystatin C had higher diagnostic accuracy (AUC = 0.844, sensitivity = 74.0%, specificity = 83.0% at cut-off value of 2.02 mg/L) compared to IP-10 (AUC = 0.768, sensitivity = 71.4%, specificity = 80.5% at cut-off value of 96.40pg/mL).

Conclusion

Elevated serum cystatin C and IP-10 levels are significantly associated with GAD severity, suggesting an immune imbalance. These cytokines may serve as promising diagnostic biomarkers and therapeutic targets for GAD. Further longitudinal studies are recommended to inquire about the causal relationship and underlying mechanisms.

1. Background

Generalized anxiety disorder (GAD) is a prevalent and debilitating mental illness characterized by severe psychological and physical symptoms, including pervasive and uncontrollable anxiety across various life domains for a minimum duration of six months [1]. GAD impacts 3.7% of the global population over a lifetime and 1.8% annually, with variations based on geography and demographics [2]. It is more

prevalent in females and frequently coexists with other anxiety disorders, such as panic disorder or major depressive disorder, which complicates diagnosis and treatment [3]. Muscle tension, trouble sleeping, and being too worn out are some of the signs. Being restless and irritable are signs of emotional problems. Cognitive symptoms include worrying too much and thinking negatively. Symptoms that make it harder to work, socialize, and take care of yourself lead to a lower quality of life and higher healthcare costs. GAD is caused by genetic, psychological, neurological, and immunological factors. Neuroimaging studies indicate that people with GAD have an overactive amygdala, which processes emotions and detects threats [4]. Due to amygdala hyperactivity, prefrontal cortex abnormalities, particularly in the medial and dorsomedial portions, make it harder to control feelings [4, 5]. Disorganized links between these components cause GAD sufferers to worry and feel tense all the time.

Primarily, GAD is associated with irregularities in several neurotransmitter systems, particularly those related to gamma-aminobutyric acid (GABA), serotonin, and norepinephrine [6, 7]. GABA, the primary inhibitory neurotransmitter in the central nervous system, exhibits reduced activity in individuals with GAD, leading to diminished inhibition of hyperactive neural circuits and intensified anxiety symptoms [6]. Serotonergic dysfunction results in mood dysregulation and increased stress reactivity, while dysregulated norepinephrine transmission leads to hypervigilance and the physiological symptoms of anxiety [7]. The hypothalamic-pituitary-adrenal (HPA) axis, the primary stress-response system in the body, malfunctions in GAD [8, 9]. Long-term stress and repeated activation of the HPA axis increase cortisol levels, which alter the structure and function of the brain, particularly in regions responsible for regulating emotions, such as the hippocampus and prefrontal cortex [8]. A feedback loop from HPA axis hyperactivity exacerbates anxiety symptoms and perpetuates the condition. Latest studies [10, 11] show that neuroinflammation significantly affects GAD characterization, therapy prediction or targeting, and maintenance. Previous studies found that persistently activating inflammatory pathways with pro-inflammatory cytokines worsens HPA axis instability by altering neurotransmitter metabolism and neuronal circuitry [10, 12]. Research suggests that anxiety disorders are associated with increased levels of inflammatory markers such as CRP, IL-6, and TNF-α, supporting the neuroimmune hypothesis [11, 13]. Cystatin C (CST3) and interferon-gamma inducible protein-10 (IP-10)/CXCL10 are biomarkers linked to neuroinflammatory processes and mental illnesses [14, 15].

The CNS and other tissues contain the 13-kDa cysteine protease inhibitor cystatin C. Cystatin C was formerly used as a biomarker for renal function, but its role in neuroinflammation has made it important in neuropsychiatric illnesses [16]. In bipolar disorder and major depressive disorder, serum cystatin C levels are markedly elevated [14, 17]. This suggests that inflammatory biomarkers may affect mental disorder severity. GAD is defined by malfunctioning microglia and HPA axis activity [16]. This situation may be fixed by cystatin C. High cystatin C levels are linked to cognitive and emotional issues in neuropsychiatric diseases. This shows a link between neuroinflammation, protease dysregulation, and mood disorders [14, 17]. Cystatin C is believed to be associated with GAD due to its regulatory influence on cathepsins and other proteases that play a role in inflammatory responses and neuronal remodelling, which could contribute to the anatomical and functional alterations in the brain associated with anxiety disorders [18].

CXCL10, a pro-inflammatory chemokine in the CXC family, is mostly produced by interferon-gamma (IFN-γ). It affects immune cell trafficking and neuroinflammation [15, 18]. IP-10, which weighs around 10 kDa, binds to the CXCR3 receptor on T cells, natural killer cells, and some neuronal populations [15]. IP-10 levels are elevated in neuroinflammation-associated neuropsychiatric disorders, including Parkinson's disease, Alzheimer's disease, and mood disorders [18, 19]. IP-10 regulates neuronal excitability and is associated with anxiety disorders, indicating its influence on amygdala reactivity [15, 20]. Inflammatory chemokines such as IP-10 may induce anxiety-like behaviours by activating neuroinflammatory pathways in animal models [7]. The IP-10-GAD pathway encompasses various processes. GAD is marked by prolonged immune system activation due to psychological stress, potentially elevating IP-10 [13, 19]. Second, neuroinflammation caused by IP-10 throws off the balance of neurotransmitters, which affects the serotonergic and GABAergic systems that control anxiety [10, 12]. Third, IP-10 may influence the HPA axis to elevate cortisol production and neuroendocrine dysregulation associated with GAD [8, 12]. IP-10's synaptic plasticity may influence neural circuit functionality in anxiety-associated brain regions, extending symptoms and fostering treatment resistance [21].

Cystatin C and IP-10 are both possible points of convergence where immune dysregulation, neuroinflammation, and traditional neurotransmitter-based pathophysiology come together. Their measurement in peripheral blood presents practical benefits for clinical application, as they serve as minimally invasive biomarkers that may assist in diagnosis, severity evaluation, and potentially treatment monitoring for GAD. Despite advances in GAD pathophysiology, diagnosis, and therapy, biomarkers remain unexplored and unvalidated. In contrast to major depressive disorders and schizophrenia, GAD is characterized by a scarcity of biomarker studies [21, 22]. GAD is diagnosed solely through professional interviews and self-report tests like the GAD-7 scale. The methodologies are subjective, susceptible to reporting bias and cultural influences, and exhibit overlap with other psychiatric disorders [3]. The absence of objective, quantifiable biomarkers lead to diagnostic delays, misdiagnosis, and inadequate therapy [23]. Genetic and neuroimaging biomarkers are expensive, complex, accessible, and non-specific, limiting their therapeutic value [21, 23]. Neuroinflammatory biomarkers for GAD are novel. Systematic studies link inflammatory indicators like CRP to GAD. However, small sample sizes, different methods, and unpredictable results cause problems [11, 13]. A comprehensive meta-analysis of 14 studies with 1,188 GAD patients highlights research gaps [11]. Researchers prioritize general inflammatory indicators over anxiety-related cytokines and chemokines.

There are significant cystatin C and IP-10 knowledge gaps. Despite their links with mental health issues, blood cystatin C levels in GAD have not been directly studied [14]. Despite research linking IP-10 to melancholy, cognitive issues, and stress, its role in GAD remains unclear. The impact of these biomarkers on GAD severity, treatment efficacy, and clinical subtypes is unknown. Third, integrated research including biomarkers, neuroimaging, genetics, and longitudinal data is needed to determine how these medicines affect GAD aetiology [24].

This study involves examining the variations of these biomarkers, distinguishing GAD from depression, predicting or guiding therapeutic responses, and ensuring validity across populations with diverse

genetic backgrounds and environmental exposures. Biomarker research is challenging due to the interactions between the nervous and immune systems and the diverse range of mental illnesses. Such complexity means that studies need to be large and well-planned and use strict methods and long-term assessments [22, 23]. A systematic investigation of cystatin C and IP-10 in GAD can address knowledge deficiencies and advance the field forward by offering objective diagnostic instruments to enhance clinical risk assessment and biomarker-focused research.

This study aimed to assess serum cystatin C and IP-10 levels in individuals diagnosed with GAD and to investigate their associations with the severity of anxiety symptoms. A case-control study with 202 participants (101 diagnosed with GAD and 101 healthy controls) was conducted to examine the feasibility of these biomarkers as objective indicators for diagnosing GAD and evaluating the severity of the disorder. The primary objective was to evaluate the relationship between elevated serum cystatin C and IP-10 levels and the pathophysiology of GAD, thereby identifying these molecules as potential neuroimmune biomarkers for anxiety disorders and examining their role in the neuroinflammatory processes that characterize the condition.

2. Methods

2.1 Study population

This case-control study enrolled 202 participants: 101 GAD patients and 101 age- and sex-matched healthy controls at the National Institute of Mental Health, Dhaka, Bangladesh (June 23, 2025, to August 3, 2025). GAD patients met DSM-5 diagnostic criteria with GAD-7 scores > 4, while controls had GAD-7 scores ≤ 4 and no psychiatric history. Inclusion criteria for GAD patients: (1) confirmed DSM-5 diagnosis; (2) age 18–60 years; and (3) GAD-7 score > 4. Exclusion criteria: cognitive impairment, serious comorbid medical/psychiatric disorders, medications affecting cystatin C or IP-10 levels, infectious diseases, substance abuse, and pregnancy. The institutional ethics committee approved the study in compliance with the Declaration of Helsinki.

2.2 Sample collection and analysis

Five-millilitre venous blood samples were collected between 9:00 and 11:00 AM following overnight fasting from January 1, 2025, to June 30, 2025. Samples were allowed to clot at room temperature for one hour, then centrifuged at 1,000 x g for 15 minutes. Serum was stored at − 80°C until analysis. Serum cystatin C and IP-10 concentrations were measured using ELISA kits (Boster Biological Technology) with detection ranges of 312–20,000 pg/mL and 31.2–2,000 pg/mL, respectively. Analytical sensitivity was < 10 pg/mL and < 1 pg/mL with inter- and intra-assay variability of < 10% and < 12%. Laboratory personnel were blinded to participant clinical status.

2. 3 Statistical analyses

Data were analysed using IBM SPSS Version 25.0. Categorical variables were compared using chi-square tests, and continuous variables using independent t-tests or Mann-Whitney U tests. Spearman's rank correlation assessed relationships between biomarkers and GAD-7 scores. ROC curve analysis determined diagnostic cut-off values and AUC with 95% CI. Statistical significance was set at $p < 0.05$.

3. Results

3.1 General description of study subjects

A total of 202 individuals were recruited, consisting of 101 patients with GAD and 101 healthy controls. The average age of GAD patients was 30.51 ± 0.93 years, while for controls it was 31.22 ± 0.91 years ($p = 0.586$). The gender distribution was comparable between the groups (GAD: 33 females, 67 males; controls: 37 females, 63 males; $p = 0.553$). The body mass index (BMI) of individuals with GAD (23.38 ± 0.34 kg/m²) was markedly elevated compared to the control group (23.32 ± 0.35 kg/m²; $p = 0.907$). A history of smoking was in similar pattern among GAD patients and controls. Table 1 shows in detail the socio-demographic characteristics.

Table 1
Characteristics of the study population.

Parameters	**GAD patients** **n = 100 (%)**	**Healthy controls** **n = 100 (%)**	**p-value**
Age in years			0.742
18–25	27 (27.00%)	35 (35.00%)	
26–35	45 (45.00%)	38 (38.00%)	
36–45	20 (20.00%)	18 (18.00%)	
46 and above	8 (8.00%)	8 (8.00%)	
Sex			0.553
Male	67 (67.00%)	63 (63.00%)	
Female	33 (33.00%)	37 (37.00%)	
BMI (kg/m^2)			0.423
Underweight (< 18.5)	7 (7.00%)	4 (4.00%)	
Normal (18.5–24.9)	59 (59.00%)	67 (67.00%)	
Overweight (≥ 25.0)	34 (34.00%)	29 (29.00%)	
Marital status			0.568
Married	59 (59.00%)	55 (55.00%)	
Unmarried	41 (41.00%)	45 (45.00%)	
Education level			0.334
Illiterate	3 (3.00%)	1 (1.00%)	
Primary	9 (9.00%)	16 (16.00%)	
Secondary	45 (45.00%)	39 (39.00%)	
Graduate and above	43 (43.00%)	44 (44.00%)	
Occupation			0.445
Business	5 (5.00%)	2 (2.00%)	
Service	33 (33.00%)	37 (37.00%)	
Housewife	9 (9.00%)	9 (9.00%)	

Abbreviations: GAD, generalized anxiety disorder; BMI, body mass index. p-value < 0.05 indicates statistically significant (bold).

Parameters	GAD patients n = 100 (%)	Healthy controls n = 100 (%)	p-value
Student	0 (0.00%)	2 (2.00%)	
Unemployed	42 (42.00%)	35 (35.00%)	
Others	1 (0.99)	0 (0.00)	
Economic status			0.284
High	12 (12.00%)	14 (14.00%)	
Medium	42 (42.00%)	51 (51.00%)	
Low	46 (46.00%)	35 (35.00%)	
Residence area			1.000
Rural	18 (18.00%)	18 (18.00%)	
Urban	82 (82.00%)	82 (82.00%)	
Smoking history			0.417
Non-smoker	77 (77.00%)	72 (72.00%)	
Smoker	23 (23.00%)	28 (28.00%)	
Previous history of GAD			0.192
Yes	9 (9.00%)	15 (15.00%)	
No	91 (91.00%)	85 (85.00%)	
Family history of GAD			0.411
Yes	33 (33.00%)	24 (24.00%)	
No	66 (66.00%)	74 (74.00%)	

Abbreviations: GAD, generalized anxiety disorder; BMI, body mass index. p-value < 0.05 indicates statistically significant (bold).

3.2 Clinical and laboratory findings

Serum cystatin C concentrations were markedly increased in GAD patients () relative to healthy controls (2.54 ± 0.16 mg/L vs. 1.62 ± 0.14 mg/L; $p < 0.001$). Serum IP-10 levels were markedly elevated in GAD patients (126.20 ± 10.18 pg/mL) relative to controls (88.54 ± 0.94). pg/mL; $p < 0.001$). Table 2 presents a short summary of the biophysical traits, clinical traits, and lab results. Figure 1 shows the comparison of serum cystatin C and IP-10 levels between the cases and controls.

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

Parameters	GAD patients (n = 100) Mean ± SEM	Healthy controls (n = 100) Mean ± SEM	p-value
Age (in years)	30.51 ± 0.93	31.22 ± 0.91	0.586
BMI (kg/m^2)	23.38 ± 0.34	23.32 ± 0.35	0.907
GAD-7 scores	11.97 ± 0.51	4.88 ± 0.45	**< 0.001**
Serum cystatin C level (mg/L)	2.54 ± 0.16	1.62 ± 0.14	**< 0.001**
Serum IP-10 level (pg/mL)	126.20 ± 10.18	88.54 ± 0.94	**< 0.001**

Abbreviations: BMI, body mass index; GAD, generalized anxiety disorder; IP-10, Interferon-gamma inducible protein-10; SEM, standard error mean. p-value < 0.05 indicates statistically significant (bold).

3.3 Correlation analysis

The Spearman's rank correlation showed a positive correlation between GAD-7 scores and serum cystatin C levels in GAD patients ($r = 0.777$, $p < 0.001$). Serum IP-10 levels also showed positive association with GAD-7 scores in disease group ($r = 0.279$, $p = 0.005$). The levels of cystatin C and IP-10 were positively correlated with each other ($r = 0.479$, $p < 0.001$). However, we didn't observe any such correlations among the parameters in control groups except a positive correlation between GAD-7 sores and serum cystatin C levels. Table 3 shows the correlation parameters among the parameters in study subjects.

Table 3
Correlation study among various research parameters among study participants.

Correlation parameters	GAD patients		Healthy controls	
	r	p-value	r	p-value
Age and GAD-7 score	0.051	0.614	-0.060	0.553
Age and cystatin C	-0.097	0.336	0.084	0.405
Age and IP-10	0.133	0.186	0.047	0.641
BMI and GAD-7 score	0.063	0.617	0.065	0.520
BMI and cystatin-C	0.090	0.375	0.055	0.587
BMI and IP-10	0.145	0.151	0.064	0.526
GAD-7 and cystatin C	0.777	**< 0.001**	0.273	**0.006**
GAD-7 and IP-10	0.279	**0.005**	0.092	0.362
Cystatin C and IP-10	0.479	**< 0.001**	0.078	0.443

Abbreviations: BMI, body mass index; GAD, generalized anxiety disorder; IP-10, Interferon-gamma inducible protein-10. p-value < 0.05 indicates statistically significant (bold).

3.4 Receiver operating characteristic (ROC)

The ROC curve analysis for serum cystatin C produced an AUC of 0.844 (95% CI: 0.788-0.900; $p < 0.001$), with an optimal cutoff value of 2.02 mg/L (sensitivity: 74.0%, specificity: 83.0%). The AUC for serum IP-10 was 0.768 (95% CI: 0.704–0.833; $p < 0.001$), and the value was 96.40 pg/mL (sensitivity: 71.4%, specificity: 80.5%). Figure 2 presents ROC curve analysis of serum cystatin C and IP-10 levels in study participants.

4. Discussion

The present study examined the association between serum cystatin C and IP-10 levels in GAD patients compared to healthy controls. These findings reveal a significant neuroimmune dysregulation in GAD that aligns with and extends the existing understanding of neuroinflammatory pathophysiology in anxiety disorders. Elevated biomarker levels were identified in GAD patients, demonstrating a systemic inflammatory signature characteristic of the disorder. This observation supports the neuroinflammatory hypothesis of GAD, wherein chronic immune activation contributes to the onset and perpetuation of anxiety symptoms [10, 11]. The positive correlation between biomarker levels and anxiety severity substantiates this neuroimmune mechanism, suggesting that inflammatory burden directly corresponds with symptom intensity.

The elevation of cystatin C in GAD patients aligns with findings across psychiatric disorder literature, where cystatin C is increasingly recognized as a prevalent biomarker signifying systemic oxidative stress

and neuroimmune dysregulation [17, 25]. Previous research in major depressive disorder demonstrates that cystatin C levels associate with symptom severity and suicidal ideation [17], indicating a transdiagnostic role for this protease inhibitor in psychiatric pathology. According to a longitudinal cohort study, an elevated rate of serum cystatin C levels was substantially associated with baseline persistent depressive symptoms [26], suggesting that elevated cystatin C may constitute a persistent biomarker of neuropsychiatric vulnerability. The relatively stronger correlation between IP-10 and anxiety intensity compared to cystatin C with anxiety intensity suggests that chemokine-mediated immune responses may have a more prominent role in GAD pathophysiology than systemic oxidative stress markers alone. This finding implies functional significance for the CXCR3 signalling pathway in anxiety pathogenesis. IP-10, through its interaction with CXCR3 receptors on lymphocytes and neurons, facilitates leukocyte trafficking and modulates neuroinflammatory processes [15]. Recent evidence indicates that IP-10-CXCR3 signalling modifies neuronal excitability and emotional processing within key limbic structures [27], mechanisms that directly implicate chemokine signalling in the neurobiology of anxiety.

Notably, few prior studies have examined IP-10 in GAD despite extensive documentation of its involvement in other psychiatric and neurological conditions [18, 19]. The weak positive correlation between cystatin C and IP-10 indicates that both biomarkers are concurrently elevated, suggesting they participate in interconnected rather than distinct inflammatory pathways. This concurrent elevation suggests a shared underlying neuroimmune dysregulation involving both protease-antiprotease imbalance and chemokine-mediated immune activation [21]. The combined assessment of both biomarkers demonstrated superior diagnostic discrimination compared to either biomarker individually, establishing a novel integrated biomarker panel for GAD diagnosis. This combined approach reflects an emerging understanding that psychiatric disorders may require multi-marker biomarker strategies rather than reliance on single indicators [28]. The clinical utility of this panel is substantial given that both biomarkers are measurable in peripheral blood without invasive procedures or expensive neuroimaging, making them accessible for routine clinical use [29].

Furthermore, the association between biomarker elevation and anxiety severity provides preliminary evidence for potential applications in treatment monitoring and prognosis evaluation. Biomarker normalization could serve as an objective measure of therapeutic efficacy, facilitating early detection of treatment-resistant phenotypes and enabling timely treatment modification [30]. This objective monitoring capability addresses a critical limitation of current GAD assessment methods, which rely exclusively on subjective clinical interviews and self-report questionnaires prone to bias [2, 22].

The findings support several potential therapeutic applications in routine psychiatric care. Objective diagnostic adjuncts may assist clinical interviews in determining GAD presence, potentially improving diagnostic accuracy and accelerating diagnosis, particularly in populations at higher risk for underdiagnosis [31]. Additionally, biomarker levels may inform treatment stratification, guiding clinicians to select either standard anxiolytic approaches or immunomodulatory strategies for patients demonstrating elevated inflammatory markers [32]. This precision medicine approach aligns with

emerging evidence that anti-inflammatory agents show promise as adjunctive treatments for inflammation-associated psychiatric conditions [10].

Further research should evaluate these biomarkers longitudinally to determine whether they represent trait characteristics or state-dependent markers responsive to treatment [21]. Investigating biomarker patterns before and after therapeutic intervention in medication-naive patients may better elucidate treatment response predictability. Validation across diverse demographics and geographic regions using multi-site investigations with larger sample sizes would ensure generalizability and demographic specificity [24]. Integrating neuroimaging and cerebrospinal fluid biomarker analysis alongside peripheral markers may clarify how peripheral immune dysregulation translates to central nervous system pathology [32]. Finally, examining these biomarkers across anxiety spectrum disorders could illuminate their transdiagnostic relevance versus disorder-specific involvement [33].

This work is the first to examine blood cystatin C and IP-10 levels in Bangladeshi GAD patients using a rigorously matched case-control approach and standardized ELISA quantification to provide clinically useful diagnostic markers. Through a non-invasive peripheral blood sample, biomarkers can be assessed for their diagnostic significance and link to anxiety severity, which helps traditional psychiatric practice. A small sample size limits generalizability, a cross-sectional design prevents causality assessment, participant restriction to 18–60 years, and a lack of systematic recording of confounding variables like diet, exercise, and medication use. The dearth of evidence on GAD symptoms' duration and chronicity makes it difficult to determine if biomarkers are state-dependent or trait-based. Recruitment from one facility may also influence selection. Comorbid disorders and longitudinal follow-up were not assessed, limiting biomarker specificity and treatment intervention assessment.

5. Conclusions

The findings indicate that individuals with GAD exhibit significantly elevated levels of blood cystatin C and IP-10, which are closely associated with the severity of their anxiety symptoms. The current positioning of these biomarkers serves as objective diagnostic adjuncts to enhance clinical evaluation, due to the improved diagnostic accuracy of combined biomarker assessment. The results presented herein may assist physicians in assessing the severity of an illness and monitoring treatment efficacy. They may also provide support for the neuroimmune theory of GAD. Longitudinal studies are essential for establishing pathways for clinical application by confirming biomarker trajectories across diverse populations and assessing treatment response efficacy.

Abbreviations

CST3
Cystatin C
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
HPA
Hypothalamic-pituitary-adrenal
IP-10
Interferon-gamma inducible protein-10
NIMH
National Institute of Mental Health
ROC
Receiver-operating characteristic
SEM
standard error mean
SPSS
Statistical package for social sciences

Declarations

Clinical trial number

Not applicable.

Ethics statements

The Committee for Ethical Compliance in Research of the Southeast University, Bangladesh, reviewed the study protocol and formally granted ethical clearance for this case-control study, under reference number SEU/Pharm/CECR/023/2024. In compliance with the Helsinki Declaration, the study was carried out.

Human ethics and consent to participate

All participants provided written informed consent prior to enrolment. Informed consent for the publication is not applicable to this study.

Competing interests

The authors declared no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

Author Contributions Statement

Z.L.A. and M.R.I. conceptualization, methodology, data curation, formal analysis, and writing-original draft. M.A.H. investigation, and project administration. M.R.I. conceptualization, methodology, supervision, and writing review & editing.

Funding

The author(s) didn't receive any specific funding for this research.

Author Contribution

Z.L.A. and M.R.I. conceptualization, methodology, data curation, formal analysis, and writing-original draft. M.A.H. investigation, and project administration. M.R.I. conceptualization, methodology, supervision, and writing review & editing.

Acknowledgement

We express our gratitude to all the participants and their relatives for their cooperation in this study. We also extend our thanks to all the physicians and administrative staffs of respective medical facilities for their cooperation and support towards this research.

Data Availability

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

References

1. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders [Internet]. Fifth Edition. American Psychiatric Association. [cited 2025 Nov 9]. (2013). https://doi.org/10.1176/appi.books.9780890425596
2. Ruscio, A. M. et al. Cross-sectional Comparison of the Epidemiology of *DSM-5* Generalized Anxiety Disorder Across the Globe. *JAMA Psychiatry*. **74**, 465. https://doi.org/10.1001/jamapsychiatry.2017.0056 (2017).
3. Kessler, R. C. et al. Lifetime Prevalence and Age-of-Onset Distributions of DSM-IV Disorders in the National Comorbidity Survey Replication. *Arch. Gen. Psychiatry*. **62**, 593. https://doi.org/10.1001/archpsyc.62.6.593 (2005).

4. Etkin, A. & Wager, T. D. Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. *Am. J. Psychiatry*. **164**, 1476–1488. https://doi.org/10.1176/appi.ajp.2007.07030504 (2007).
5. Bishop, S., Duncan, J., Brett, M. & Lawrence, A. D. Prefrontal cortical function and anxiety: controlling attention to threat-related stimuli. *Nat. Neurosci.* **7**, 184–188. https://doi.org/10.1038/nn1173 (2004).
6. Nuss, P. Anxiety disorders and GABA neurotransmission: a disturbance of modulation. *Neuropsychiatr Dis. Treat.* **11**, 165–175. https://doi.org/10.2147/NDT.S58841 (2015).
7. Deutch, A. & Charney, D. S. A Functional Neuroanatomy of Anxiety and Fear: Implications for the Pathophysiology and Treatment of Anxiety Disorders. *Crit. Rev. Neurobiol.* **10**, 419–446. https://doi.org/10.1615/CritRevNeurobiol.v10.i3-4.70 (1996).
8. Mikulska, J., Juszczyk, G., Gawrońska-Grzywacz, M. & Herbet, M. HPA Axis in the Pathomechanism of Depression and Schizophrenia: New Therapeutic Strategies Based on Its Participation. *Brain Sci.* **11**, 1298. https://doi.org/10.3390/brainsci11101298 (2021).
9. Gold, P. W. & Chrousos, G. P. Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs low CRH/NE states. *Mol. Psychiatry*. **7**, 254–275. https://doi.org/10.1038/sj.mp.4001032 (2002).
10. Hassamal, S. Chronic stress, neuroinflammation, and depression: an overview of pathophysiological mechanisms and emerging anti-inflammatories. *Front. Psychiatry*. **14**, 1130989. https://doi.org/10.3389/fpsyt.2023.1130989 (2023).
11. Costello, H., Gould, R. L., Abrol, E. & Howard, R. Systematic review and meta-analysis of the association between peripheral inflammatory cytokines and generalised anxiety disorder. *BMJ Open.* **9**, e027925. https://doi.org/10.1136/bmjopen-2018-027925 (2019).
12. Sălcudean, A. et al. Neuroinflammation—A Crucial Factor in the Pathophysiology of Depression—A. *Compr. Rev. Biomolecules*. **15**, 502. https://doi.org/10.3390/biom15040502 (2025).
13. Roknuzzaman, A. S. M., Qusar, M. S., Shahriar, M., Ashraful Islam, S. M. & Islam, M. R. Increased serum EGF but not SDF-1 levels are associated with the pathophysiology and development of generalized anxiety disorder. *Sci. Rep.* **14**, 27409. https://doi.org/10.1038/s41598-024-79166-9 (2024).
14. Zhang, C. et al. Investigation of serum cystatin C levels and their diagnostic value in combination with inflammatory ratios in patients with bipolar disorder. *Front. Psychiatry*. **16**, 1525091. https://doi.org/10.3389/fpsyt.2025.1525091 (2025).
15. Kodangattil, J. N., Möddel, G., Müller, M., Weber, W. & Gorji, A. The Inflammatory Chemokine CXCL10 Modulates Synaptic Plasticity and Neuronal Activity in the Hippocampus. *Eur. J. Inflamm.* **10**, 311–328. https://doi.org/10.1177/1721727X1201000307 (2012).
16. Beynon, R. & Bond, J. S. (eds) *Proteolytic Enzymes: A Practical Approach [Internet]* (Oxford University PressOxford, 2001). [cited 2025 Nov 9]https://doi.org/10.1093/oso/9780199636631.001.0001

17. Sun, T., Chen, Q. & Li, Y. Associations of serum cystatin C with depressive symptoms and suicidal ideation in major depressive disorder. *BMC Psychiatry*. **21**, 576. https://doi.org/10.1186/s12888-021-03509-3 (2021).
18. Oktay, M., Asoğlu, M., Taskin, S. & Kirmit, A. Biological Markers in Newly Diagnosed Generalized Anxiety Disorder Patients: 8-OHdG, S100B and Oxidative Stress. *Neuropsychiatr Dis. Treat.* **20**, 19–24. https://doi.org/10.2147/NDT.S444506 (2024).
19. Rocha, N. P. et al. Cognitive Status Correlates with CXCL10/IP-10 Levels in Parkinson's Disease. *Park Dis.* **2014**, 903796. https://doi.org/10.1155/2014/903796 (2014).
20. Maldonado-Bouchard, S. et al. Inflammation is increased with anxiety- and depression-like signs in a rat model of spinal cord injury. *Brain Behav. Immun.* **51**, 176–195. https://doi.org/10.1016/j.bbi.2015.08.009 (2016).
21. Shen, Z. et al. Combining S100B and Cytokines as Neuro-Inflammatory Biomarkers for Diagnosing Generalized Anxiety Disorder: A Proof-of-Concept Study Based on Machine Learning. *Front. Psychiatry*. **13**, 881241. https://doi.org/10.3389/fpsyt.2022.881241 (2022).
22. Sharma, A. & Verbeke, W. J. M. I. Understanding importance of clinical biomarkers for diagnosis of anxiety disorders using machine learning models. Li Z, editor. PLOS ONE. ;16:e0251365. (2021). https://doi.org/10.1371/journal.pone.0251365
23. Lakhan, S. E., Vieira, K. & Hamlat, E. Biomarkers in psychiatry: drawbacks and potential for misuse. *Int. Arch. Med.* **3**, 1. https://doi.org/10.1186/1755-7682-3-1 (2010).
24. Abi-Dargham, A. et al. Candidate biomarkers in psychiatric disorders: state of the field. *World Psychiatry*. **22**, 236–262. https://doi.org/10.1002/wps.21078 (2023).
25. Ghidoni, R. et al. Cystatin C is released in association with exosomes: A new tool of neuronal communication which is unbalanced in Alzheimer's disease. *Neurobiol. Aging*. **32**, 1435–1442. https://doi.org/10.1016/j.neurobiolaging.2009.08.013 (2011).
26. Han, T., Zhang, L., Jiang, W. & Wang, L. Persistent Depressive Symptoms and the Changes in Serum Cystatin C Levels in the Elderly: A Longitudinal Cohort Study. *Front. Psychiatry*. **13**, 917082. https://doi.org/10.3389/fpsyt.2022.917082 (2022).
27. Bufi, A. A. et al. The central role of CXCL10-CXCR3 signaling in neuroinflammation and neuropathology. *Cytokine Growth Factor. Rev.* **84**, 20–34. https://doi.org/10.1016/j.cytogfr.2025.05.003 (2025).
28. García-Gutiérrez, M. S. et al. Biomarkers in Psychiatry: Concept, Definition, Types and Relevance to the Clinical Reality. *Front. Psychiatry*. **11**, 432. https://doi.org/10.3389/fpsyt.2020.00432 (2020).
29. Bahn, S. et al. Testes sanguíneos de biomarcadores para diagnóstico e tratamento de desordens mentais: foco em esquizofrenia. *Arch. Clin. Psychiatry São Paulo*. **40**, 02–9. https://doi.org/10.1590/S0101-60832012005000005 (2012).
30. Strawn, J. R. & Levine, A. Treatment Response Biomarkers in Anxiety Disorders: From Neuroimaging to Neuronally-Derived Extracellular Vesicles and Beyond. *Biomark. Neuropsychiatry*. **3**, 100024. https://doi.org/10.1016/j.bionps.2020.100024 (2020).

31. Khandaker, G. M., Zammit, S., Lewis, G. & Jones, P. B. Association between serum C-reactive protein and DSM-IV generalized anxiety disorder in adolescence: Findings from the ALSPAC cohort. *Neurobiol. Stress*. **4**, 55–61. https://doi.org/10.1016/j.ynstr.2016.02.003 (2016).
32. Herron, J. W., Nerurkar, L. & Cavanagh, J. Neuroimmune Biomarkers in Mental Illness. In: (eds Pratt, J. & Hall, J.) Biomark Psychiatry [Internet]. Cham: Springer International Publishing; (2018). [cited 2025 Nov 10]. 45–78. https://doi.org/10.1007/7854_2018_45.
33. Łoś, K. & Waszkiewicz, N. Biological Markers in Anxiety Disorders. *J. Clin. Med.* **10**, 1744. https://doi.org/10.3390/jcm10081744 (2021).

Figures

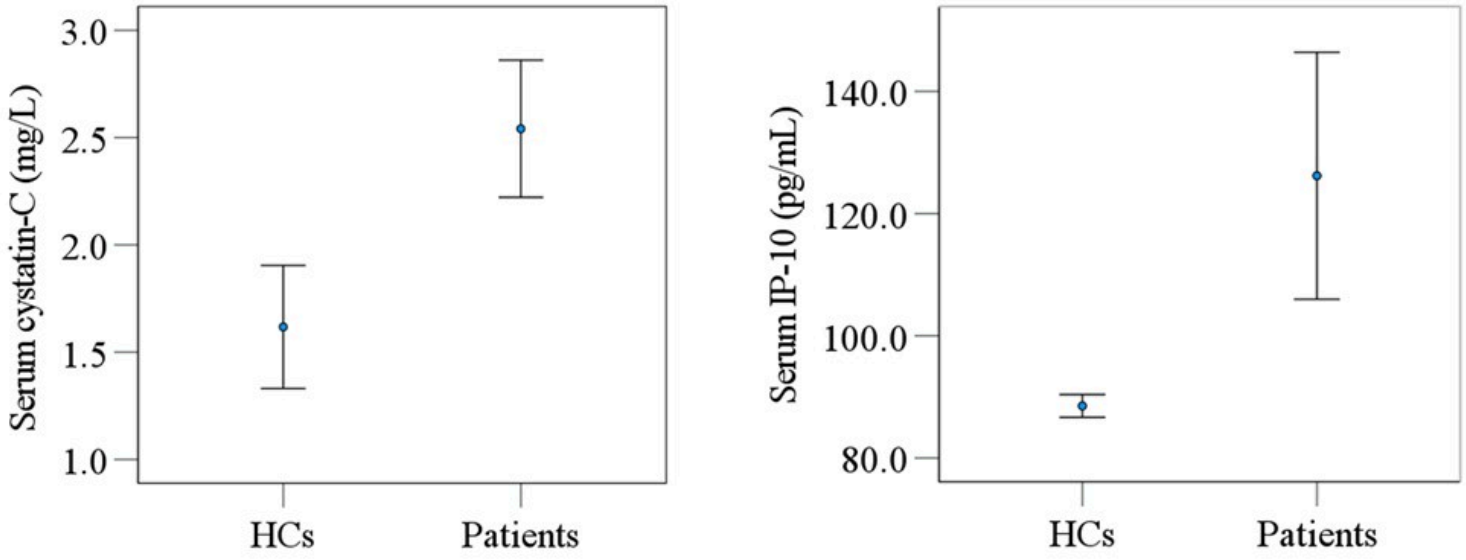


Figure 1

Comparison of serum cystatin C and IP-10 levels between the cases and controls. Error bar graphs showing the median, maximum and minimum value range.

Abbreviations: IP-10, interferon-gamma inducible protein-10; HCs, healthy controls.

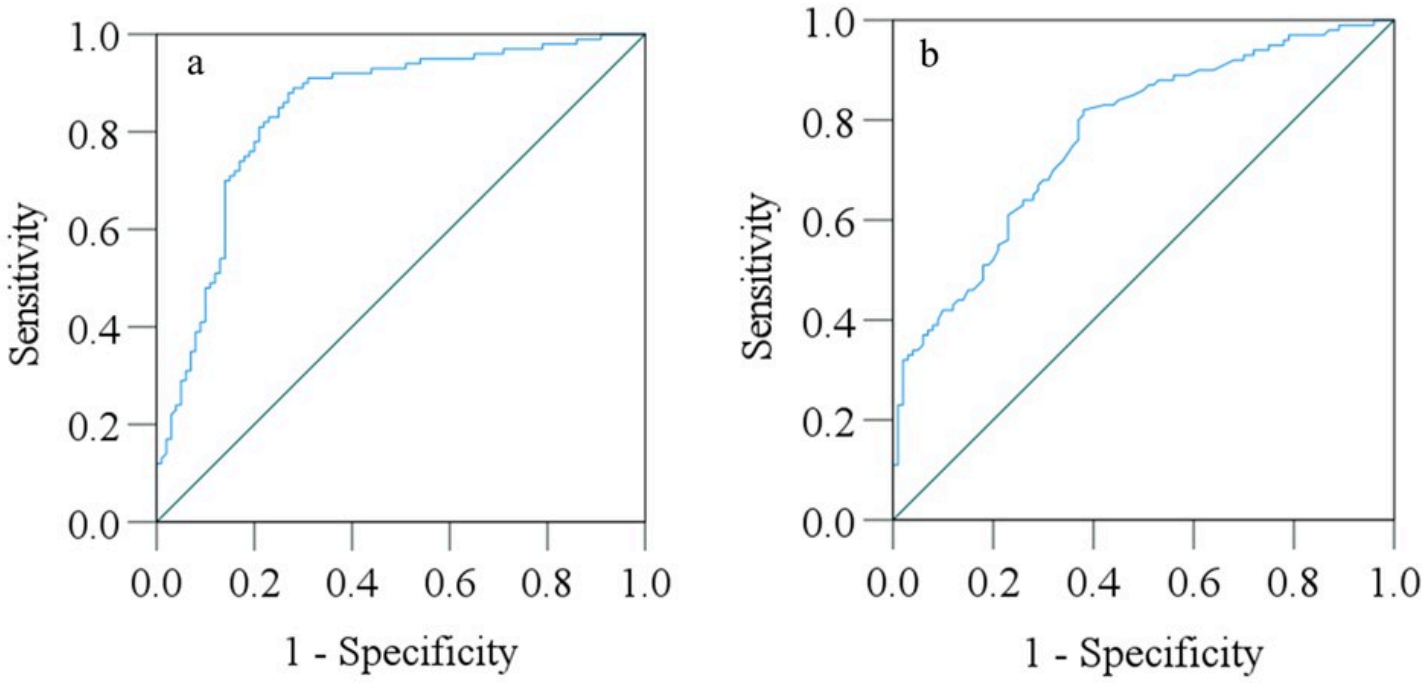


Figure 2

Receiver operating characteristic (ROC) curve analysis of serum cystatin C and IP-10 levels in study participants ($p<0.001$). a) ROC curve of cystatin C reveals area under the curve (AUC), sensitivity, and specificity as 0.844 (95% CI: 0.788-0.900), 74.0%, and 83.0%, respectively, at cut-off value of 2.02 mg/L. b) ROC curve of IP-10 reveals area under the curve (AUC), sensitivity, and specificity as 0.768 (95% CI: 0.704-0.833), 71.4%, and 80.5%, respectively, at cut-off value of 96.40pg/mL.

Abbreviation: IP-10, interferon-gamma inducible protein-10.