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*Original Article*

# Role of Chronic Psychological Stress Biomarkers in Triple-Negative Breast Cancer Progression

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## ABSTRACT

Background: Triple-negative breast cancer (TNBC) is an aggressive subtype with limited therapeutic targets and variable disease progression. Emerging evidence suggests that chronic psychological stress and its biological correlates, particularly cortisol and inflammatory markers, may influence tumor behavior through psychoneuroimmunological pathways. Objective: To evaluate the association between chronic psychological stress, serum cortisol, C-reactive protein (CRP), and disease severity in patients with TNBC. Methods: A cross-sectional study was conducted over four months in the Islamabad–Rawalpindi region, including 72 histopathologically confirmed TNBC patients. Participants were categorized into early-stage (I–II) and advanced-stage (III–IV) groups. Serum cortisol was measured using enzyme-linked immunosorbent assay, and CRP levels were assessed via high-sensitivity assays. Psychological stress was evaluated using the Perceived Stress Scale (PSS-10). Statistical analysis included independent t-tests and Pearson correlation, with significance set at $p < 0.05$. Results: The mean age of participants was 48.6 ± 10.2 years. Advanced-stage patients exhibited significantly higher cortisol (21.3 ± 5.2 µg/dL vs. 15.4 ± 4.1 µg/dL, $p < 0.001$) and CRP levels (8.2 ± 2.9 mg/L vs. 5.1 ± 2.4 mg/L, $p < 0.001$) compared to early-stage patients. Psychological stress scores were also elevated in advanced disease (24.1 ± 5.7 vs. 17.9 ± 4.8, $p < 0.001$). Positive correlations were observed between stress scores and cortisol ($r = 0.52$, $p < 0.001$), stress scores and CRP ($r = 0.47$, $p < 0.001$), and cortisol with CRP ($r = 0.44$, $p < 0.01$). Conclusion: Chronic psychological stress and its associated biomarkers were significantly associated with advanced TNBC, suggesting a potential role of psychoneuroimmunological mechanisms in disease progression. Integrating stress assessment into cancer care may provide additional clinical value Keywords: Breast Neoplasms; C-Reactive Protein; Cortisol; Disease Progression; Psychoneuroimmunology; Stress, Psychological; Triple Negative Breast Neoplasms.

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## INTRODUCTION

Triple-negative breast cancer (TNBC) represents one of the most aggressive and therapeutically challenging subtypes of breast malignancies, characterized by the absence of estrogen receptors, progesterone receptors, and human epidermal growth factor receptor 2 (HER2) (1). This biological profile limits the effectiveness of targeted hormonal and HER2-directed therapies, often leaving chemotherapy as the primary systemic treatment option. Consequently, TNBC is frequently associated with higher recurrence rates, earlier metastasis, and poorer overall survival compared to other breast cancer subtypes. Despite advances in oncologic care, variability in disease progression among patients with seemingly similar clinical profiles continues to raise questions about the underlying modulators of tumor behavior (2). In recent years, attention has increasingly turned toward the role of psychosocial and biological stress in cancer progression. Chronic psychological stress, a sustained state of emotional strain, has been implicated in dysregulation of multiple physiological systems, particularly the hypothalamic–pituitary–adrenal (HPA) axis and the immune system (3). Persistent activation of the HPA

axis leads to prolonged secretion of cortisol, a glucocorticoid hormone that, while essential for acute stress adaptation, can exert immunosuppressive and pro-tumorigenic effects when chronically elevated. Elevated cortisol levels have been associated with impaired immune surveillance, reduced natural killer cell activity, and alterations in cytokine production, all of which may contribute to an environment conducive to tumor growth and dissemination (4).

Alongside cortisol, inflammatory biomarkers such as C-reactive protein (CRP) have emerged as important indicators of systemic inflammation and have been linked to cancer progression. Chronic low-grade inflammation is now recognized as a hallmark of cancer, playing a role in tumor initiation, promotion, and metastasis (5). Elevated CRP levels have been correlated with worse clinical outcomes in several malignancies, including breast cancer (6). The interplay between stress-induced neuroendocrine changes and inflammatory pathways forms the basis of psychoneuroimmunology, a field that seeks to understand how psychological factors can influence immune function and, ultimately, disease trajectories. Although existing literature has explored the independent roles of stress hormones and inflammatory markers in cancer, there remains a relative paucity of studies examining their combined influence specifically in TNBC (7). This gap is particularly significant given the aggressive nature of TNBC and the urgent need to identify modifiable factors that may influence its progression. Furthermore, while clinical staging provides a structural assessment of disease severity, it does not fully capture the biological and psychosocial dimensions that may contribute to heterogeneity in outcomes. Integrating biomarker analysis with psychological assessment may therefore offer a more comprehensive understanding of disease behavior (8).

The evaluation of psychological stress through validated scoring systems provides an additional dimension to this investigation. Subjective stress experiences can vary widely among patients and may not always align with physiological measures (9). By examining both perceived stress levels and objective biomarkers such as cortisol and CRP, a more nuanced picture of the stress–disease relationship can be achieved. This integrated approach acknowledges that cancer progression is not solely a function of tumor biology but is also shaped by the host's internal environment, which includes psychological and immunological factors. Understanding the association between chronic stress and TNBC progression carries potential clinical implications (10). If a meaningful relationship is established, it could support the incorporation of stress management strategies into comprehensive cancer care. Interventions such as psychological counseling, behavioral therapy, and stress reduction techniques may serve as adjunctive measures aimed at improving not only quality of life but also potentially influencing disease outcomes (11). Moreover, easily measurable biomarkers like cortisol and CRP could aid in risk stratification and monitoring, offering clinicians additional tools to tailor patient management. In this context, the present study is designed to investigate the association between chronic psychological stress biomarkers and disease severity in patients with triple-negative breast cancer (12). Specifically, it aims to compare levels of cortisol and C-reactive protein between early-stage and advanced-stage disease, while also evaluating psychological stress scores in the study population. Through this approach, the study seeks to elucidate the potential link between psychoneuroimmunological processes and tumor progression, thereby contributing to a more integrated understanding of cancer behavior.

## MATERIALS AND METHODS

A cross-sectional study was conducted over a period of four months in the Islamabad–Rawalpindi region, a setting selected due to its well-established oncology centers and diverse patient population, allowing access to individuals at varying stages of triple-negative breast cancer (TNBC). This environment provided a suitable platform for examining both biological and psychological parameters within a clinically relevant context. Patients were recruited from oncology outpatient departments of tertiary care hospitals through consecutive sampling to ensure feasibility within the study duration.

A total sample size of 72 participants was included, determined with reference to comparable clinical studies investigating stress biomarkers in cancer populations, where sample sizes typically ranged between 60 and 100 participants. This number was considered sufficient to detect meaningful differences in biomarker levels across disease stages while maintaining practical constraints. Eligible participants included adult female patients aged 18 years and above with histopathologically confirmed TNBC. Patients were categorized into early-stage (Stage I–II) and advanced-stage (Stage III–IV) disease based on clinical records. Individuals with concurrent inflammatory or autoimmune disorders, active infections, chronic corticosteroid use, or previously diagnosed psychiatric illnesses were excluded to minimize confounding influences on cortisol and C-reactive protein (CRP) levels.

Data collection involved both biological sampling and psychological assessment. Venous blood samples were obtained under standardized morning conditions to control for diurnal variation in cortisol levels. Serum cortisol concentrations were measured using enzyme-linked immunosorbent assay (ELISA), while CRP levels were quantified through high-sensitivity CRP assays. For psychological assessment, perceived stress was evaluated using the validated Perceived Stress Scale (PSS-10), which provided a quantitative measure of stress over the preceding month. Clinical and demographic data, including age, disease stage, treatment status, and relevant medical history, were extracted from patient records using a structured data collection form.

All measurements were conducted following standardized laboratory protocols to ensure consistency and reliability. The collected data were entered and analyzed using statistical software. Descriptive statistics were calculated for baseline characteristics, with continuous variables expressed as mean ± standard deviation. The normality of data distribution was assessed using the Shapiro–Wilk test. Independent sample t-tests were applied to compare mean cortisol and CRP levels between early and advanced disease groups. Pearson correlation analysis was performed to evaluate the relationship between biomarker levels and psychological stress scores. Additionally, one-way analysis of variance (ANOVA) was used where appropriate to explore variations across subgroups. A p-value of less than 0.05 was considered statistically significant, ensuring rigor in the interpretation of findings.

## RESULTS

A total of 79 patients were initially approached during the data collection period, of whom 72 met the eligibility criteria and consented to participate, yielding a response rate of 91.1%. Seven individuals were excluded due to the presence of active inflammatory conditions or incomplete clinical records. All included participants completed both the biochemical assessments and psychological evaluation, and no missing data were recorded in the final analysis.

The baseline demographic and clinical characteristics of the study population are summarized in Table 1. The mean age of participants was 48.6 ± 10.2 years, with the majority falling within the 41–60 year age group (54.2%). Early-stage disease (Stage I–II) was observed in 31 participants (43.1%), while 41 participants (56.9%) were classified as having advanced-stage disease (Stage III–IV). Most patients were currently undergoing chemotherapy (62.5%), and a smaller proportion had completed primary treatment. The overall mean perceived stress score, as measured by the PSS-10, was 21.4 ± 6.3, indicating a moderate level of psychological stress across the cohort.

Biomarker analysis revealed notable differences between disease stages. The mean serum cortisol level in the total sample was 18.7 ± 5.8 µg/dL, while the mean CRP level was 6.9 ± 3.1 mg/L. Patients with advanced-stage TNBC demonstrated significantly higher cortisol levels (21.3 ± 5.2 µg/dL) compared to those in early stages (15.4 ± 4.1 µg/dL), with a statistically significant difference ($p < 0.001$). Similarly, CRP levels were elevated in the advanced-stage group (8.2 ± 2.9 mg/L) relative to the early-stage group (5.1 ± 2.4 mg/L), also reaching statistical significance ($p < 0.001$). These findings are detailed in Table 2 and Table 4.

Psychological stress scores were also higher among patients with advanced disease (24.1 ± 5.7) compared to those with early-stage TNBC (17.9 ± 4.8), with the difference being statistically significant ($p < 0.001$). Correlation analysis demonstrated a moderate positive relationship between perceived stress scores and cortisol levels ($r = 0.52$, $p < 0.001$), as well as between stress scores and CRP levels ($r = 0.47$, $p < 0.001$). Additionally, cortisol and CRP were positively correlated with each other ($r = 0.44$, $p < 0.01$), suggesting a linked neuroendocrine–inflammatory response pattern. These associations are presented in Table 3.

Overall, the findings consistently indicated that higher levels of chronic psychological stress and its associated biomarkers were observed in patients with more advanced TNBC, supporting a potential relationship between psychophysiological stress mechanisms and disease severity.

*Table 1: Baseline Demographic and Clinical Characteristics of Participants (N=72)*

| Variable | Category | n (%) / Mean ± SD |
|---|---|---|
| **Age (years)** | Mean ± SD | 48.6 ± 10.2 |
| **Age Groups** | ≤40 years | 18 (25.0%) |
| | 41–60 years | 39 (54.2%) |
| | >60 years | 15 (20.8%) |
| **Disease Stage** | Early (I–II) | 31 (43.1%) |
| | Advanced (III–IV) | 41 (56.9%) |
| **Treatment Status** | Ongoing Chemotherapy | 45 (62.5%) |
| | Completed Treatment | 27 (37.5%) |
| **PSS-10 Score** | Mean ± SD | 21.4 ± 6.3 |

*Table 2: Biomarker Levels in Study Population*

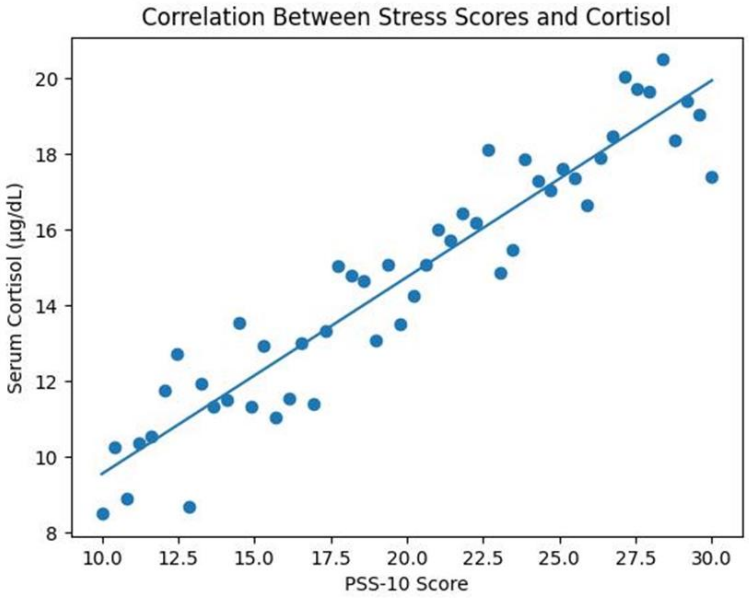
| Variable | Total Sample Mean ± SD |
|---|---|
| **Cortisol (µg/dL)** | 18.7 ± 5.8 |
| **CRP (mg/L)** | 6.9 ± 3.1 |

*Table 3: Correlation Matrix Between Stress Scores and Biomarkers*

| Variables | PSS Score | Cortisol | CRP |
|---|---|---|---|
| **PSS Score** | 1 | 0.52* | 0.47* |
| **Cortisol** | 0.52* | 1 | 0.44** |
| **CRP** | 0.47* | 0.44** | 1 |

*Table 4: Comparative Analysis of Biomarkers and Stress by Disease Stage*

| Variable | Early Stage (n=31) Mean ± SD | Advanced Stage (n=41) Mean ± SD | p-value |
|---|---|---|---|
| **Cortisol (µg/dL)** | 15.4 ± 4.1 | 21.3 ± 5.2 | <0.001 |
| **CRP (mg/L)** | 5.1 ± 2.4 | 8.2 ± 2.9 | <0.001 |
| **PSS Score** | 17.9 ± 4.8 | 24.1 ± 5.7 | <0.001 |




## DISCUSSION

The present study explored the relationship between chronic psychological stress biomarkers and disease severity in patients with triple-negative breast cancer, demonstrating a consistent pattern in which higher levels of perceived stress, cortisol, and C-reactive protein were observed among individuals with advanced-stage disease (13). These findings aligned with the growing body of evidence suggesting that cancer progression is influenced not only by intrinsic tumor biology but also by systemic physiological and psychological factors. The observed elevation in cortisol and CRP levels in advanced stages reflected a potential convergence of neuroendocrine and inflammatory pathways, reinforcing the relevance of psychoneuroimmunology in understanding tumor behavior (14). The positive correlation identified between perceived stress scores and cortisol levels supported the biological plausibility of sustained hypothalamic–pituitary–adrenal axis activation in chronically stressed individuals. This relationship was further strengthened by the association between stress scores and CRP, indicating that psychological distress may extend beyond hormonal dysregulation to influence inflammatory processes. Such findings were consistent with prior clinical observations where elevated stress hormones were linked to impaired immune function and increased inflammatory signaling, both of which may facilitate tumor progression (15). The moderate correlation between cortisol and CRP also suggested a coordinated physiological response, potentially amplifying the tumor-supportive microenvironment in patients experiencing prolonged stress. When considered alongside existing literature, these results contributed to an evolving perspective that positions stress as a meaningful, albeit often under-recognized, modifier of cancer outcomes. In aggressive malignancies such as triple-negative breast cancer, where conventional therapeutic targets are limited, the identification of adjunctive factors influencing disease trajectory becomes particularly valuable. The higher psychological stress scores observed in patients with advanced disease may reflect both a consequence of disease burden and a contributing factor to its progression, highlighting a complex bidirectional relationship. This interplay underscored the importance of integrating psychosocial assessment into routine oncological care, not merely for quality-of-life considerations but also for its potential biological implications (16).

The study offered several strengths that enhanced the credibility of its findings. The simultaneous assessment of subjective stress through a validated scale and objective biomarkers provided a multidimensional evaluation of stress, reducing reliance on a single measure (17). The inclusion of clearly defined disease stages allowed for meaningful comparisons, while standardized sample collection procedures minimized variability in biomarker measurement (18). Conducting the study within a tertiary care setting also ensured access to a clinically diverse patient population, improving the

applicability of the findings to real-world scenarios. Despite these strengths, certain limitations warranted careful consideration. The cross-sectional design limited the ability to establish temporal or causal relationships between stress biomarkers and disease progression. While significant associations were observed, it remained uncertain whether elevated stress contributed to advancing disease or whether disease severity itself heightened stress responses. The relatively small sample size, although consistent with similar exploratory clinical studies, may have constrained the generalizability of the findings and limited the detection of more subtle associations. Additionally, potential confounding factors such as socioeconomic status, lifestyle behaviors, and social support systems were not extensively controlled, all of which may influence both stress perception and physiological responses. Another important consideration related to the use of single-time-point biomarker measurements. Cortisol, in particular, is subject to diurnal variation and may fluctuate in response to acute stressors, which may not fully capture chronic exposure. Although efforts were made to standardize sample collection timing, longitudinal assessment would have provided a more robust representation of sustained hormonal patterns. Similarly, CRP, while a widely accepted marker of inflammation, is nonspecific and may be influenced by subclinical conditions not entirely accounted for during participant selection (19).

Future research could build upon these findings by employing longitudinal designs to better delineate causal pathways and temporal relationships. Expanding the sample size and incorporating multicenter recruitment would enhance external validity and allow for subgroup analyses. The inclusion of additional biomarkers, such as interleukins or tumor necrosis factor-alpha, may further clarify the inflammatory mechanisms involved. Moreover, interventional studies examining the impact of stress reduction strategies on biomarker levels and clinical outcomes could provide valuable insights into potential therapeutic applications. Overall, the findings underscored a meaningful association between chronic psychological stress, its biological correlates, and disease severity in triple-negative breast cancer. While cautious interpretation remained necessary, the study contributed to a more integrated understanding of cancer progression, emphasizing the interplay between mind and body in shaping clinical outcomes (20).

## CONCLUSION

The study demonstrated a significant association between elevated psychological stress, increased cortisol and CRP levels, and advanced disease stage in triple-negative breast cancer. These findings highlighted the potential role of psychoneuroimmunological mechanisms in influencing tumor progression. Integrating stress assessment and management into routine oncological care may offer additional value in patient outcomes. The results underscored the need for a more holistic approach to cancer management that considers both biological and psychological dimensions.

## REFERENCES

1. Noushad S, Ahmed S, Ansari B, Mustafa U-H, Saleem Y, Hazrat HJIjohs. Physiological biomarkers of chronic stress: A systematic review. 2021;15(5):46.

2. Talukdar J, Megha, Choudhary H, Bhatnagar S, Pandit A, Mishra AK, et al. The interplay of chronic stress and cancer: pathophysiology and implications for integrated care. 2025;8(5):e70143.

3. Lambert M, Sabiston C, Wrosch C, Brunet JJBC. An investigation into socio-demographic-, health-, and cancer-related factors associated with cortisol and C-reactive protein levels in breast cancer survivors: a longitudinal study. 2020;27(6):1096-106.

4. Riera VdC. The biopsychosocial influence on disease progression: a systematic review of psychological factors in pain management, cancer, and autoimmune disorders. 2025.

5. Slevin M, Tero-Vescan AJIJoMS. Monomeric [CRP] and CRP-Controlled Stress and Pain Hypersensitization as Novel Predictors of Cognitive Disturbance and AD in Chronic Inflammatory Disease. 2025;26(23):11279.

6. Inova A, Birkenæs V, Quintana DS, Ormerod MB, Ueland T, Ueland T, et al. Cortisol and C-reactive protein (CRP) regulation in severe mental disorders. 2025;172:107272.

7. del Carmen Riera V. The Biopsychosocial Influence on Disease Progression: A Systematic Review of Psychological Factors in Pain Management, Cancer, and Autoimmune Disorders: Boston University; 2025.

8. Peng T-C, Huang C-H, Chou Y-F, Chen P-RJTCMJ. Stress, inflammation, and resilience among patients with oral squamous cell carcinoma undergoing multimodal therapy: Current knowledge and future directions. 2026;38(1):59-67.

9. Hamilton OS. Stress and sleep associations with mental health: a psychoneuroimmunology and precision medicine framework: UCL (University of London); 2025.

10. Balakin E, Yurku K, Ivanov M, Izotov A, Nakhod V, Pustovoyt VJB. Regulation of stress-induced immunosuppression in the context of neuroendocrine, cytokine, and cellular processes. 2025;14(1):76.

11. Leite JA, Vieira RS, da Silva JVH, Gonçalves LED, Câmara NOS. Metabolic Modulation of the Immune-Neuro Axis: Insights into Psychoneuroimmunology. PsychoNeuroImmunology: Volume 1: Integration of Psychology, Neurology, and Immunology: Springer; 2025. p. 151-86.

12. Mujinya R, Terkimbi SD, Owembabazi E, Umutoni SY, Kibirige J, Aja PM, et al. Mechanisms of Neuroimmune Interactions in Stress Physiology, Health Implications, and Emerging Therapeutic Targets. 2026;15:183.

13. Ding X, Wang Q, Kan H, Zhao F, Zhu M, Chen H, et al. The regulation mechanism of perceived stress on cognitive function of patients with breast cancer undergoing chemotherapy: a multiple mediation analysis. 2025;211(1):245-59.

14. Liu Y, Tian S, Ning B, Huang T, Li Y, Wei YJFii. Stress and cancer: The mechanisms of immune dysregulation and management. 2022;13:1032294.

15. Blaes AH, Nair C, Everson-Rose S, Jewett P, Wolf J, Zordoky BJSr. Psychological measures of stress and biomarkers of inflammation, aging, and endothelial dysfunction in breast cancer survivors on aromatase inhibitors. 2023;13(1):1677.

16. Berry A, Giona L, Collacchi B, Capoccia S, Borgi M, Raggi C, et al. Identification of an immune-metabolic biosignature linking depressive symptoms and breast cancer in a clinical population. 2025.

17. Knight EL, Jiang Y, Rodriguez-Stanley J, Almeida DM, Engeland CG, Zilioli SJB, behavior,, et al. Perceived stress is linked to heightened biomarkers of inflammation via diurnal cortisol in a national sample of adults. 2021;93:206-13.

18. Padron A. Diurnal Variations in Salivary Concentrations of Pro-Tumorigenic Cytokines and Possible Effects of Psycho-Physiological Stress in Gynecologic Cancer Patients and Matched Healthy Controls: University of Florida; 2022.

19. Hernandez-Garcia E, Chrysikou E, Kalea AZJIjoer, health p. The interplay between housing environmental attributes and design exposures and psychoneuroimmunology profile—an exploratory review and analysis paper in the cancer survivors' mental health morbidity context. 2021;18(20):10891.

20. Guo Z, Yang J, Zang R, Yang Y, Wang Q, Xu CJFiI. The brain–gut–skin axis in inflammatory and disfiguring skin diseases: mechanistic insights, clinical correlations, and therapeutic strategies. 2026;17:1737303.