Soysal *et al. BMC Oral Health* (2025) 25:1536
https://doi.org/10.1186/s12903-025-06912-4

BMC Oral Health

RESEARCH Open Access

Salivary chromogranin A levels in periodontal health status: a cross-sectional study evaluating clinical and psychological associations

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Abstract

Backgrounds Psychological stress plays a role in the development of periodontal disease by influencing immune function and behavioral responses. Chromogranin A (CgA), a glycoprotein released due to activation of the sympathetic-adrenomedullary system, shows potential as a salivary biomarker for stress-related immune changes. This study aimed to assess salivary CgA levels in individuals with healthy and diseased periodontal conditions while investigating its correlation with clinical periodontal parameters and psychological stress.

Methods This cross-sectional study involved 56 systemically healthy participants classified into groups based on their periodontal condition: periodontal health, gingivitis, Stage II, and Stage III periodontitis. Clinical periodontal parameters were assessed. Psychological stress levels were measured using the Beck Depression Inventory (BDI). Unstimulated saliva samples were collected and analyzed for CgA mRNA expression. Statistical analyses comprised ANOVA, Spearman's correlation, and chi-square tests.

Results Salivary CgA levels were significantly higher in individuals with Stage III periodontitis compared to those with periodontal health, gingivitis, or Stage II periodontitis ($p < 0.01$). No significant correlation was observed between salivary CgA levels and BDI scores.

Discussion Although salivary CgA levels were significantly elevated in patients with advanced periodontal disease, no direct associations were observed between CgA levels and individual clinical or psychological stress measures. These findings suggest that CgA may reflect systemic stress-related changes associated with disease severity rather than individual psychological status.

Keywords Salivary biomarkers, Chromogranin A, Periodontal disease, Psychological stress

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Backgrounds

The accumulation of bacterial dental plaque, along with various environmental and host defense factors, plays a significant role in the development of chronic inflammatory diseases affecting periodontal tissues, collectively referred to as periodontal diseases [1]. Several well-established risk factors contribute to the onset and progression of these conditions, including uncontrolled systemic diseases, smoking, poor oral hygiene, obesity, and certain medications [2]. The bidirectional relationship between periodontal diseases and systemic conditions such as cardiovascular diseases, diabetes, respiratory diseases, and autoimmune disorders has been widely recognized for many years [3]. In addition, psychosocial stress has recently been recognized as a contributory factor in periodontal disease by modulating the host response to bacterial accumulation [4, 5].

Accumulating evidence underscores the association between depression, psychological stress, and both the onset and progression of periodontal disease, as well as their adverse impacts on systemic health. While moderate stress is essential for maintaining homeostasis and adaptive physiological responses, chronic or excessive stress may impair this balance and heighten the risk of inflammatory diseases [6]. Psychological conditions have been shown to disrupt salivary gland function [7], contribute to poor oral hygiene practices [8, 9] and reduce awareness of oral health needs [10], thereby creating a favorable environment for the development and progression of periodontal disease.

Psychological stress also activates the sympathetic-adrenomedullary (SAM) system, resulting in the release of adrenaline and noradrenaline from the adrenal medulla [11]. Chromogranin A (CgA), an acidogenic glycoprotein secreted by the major salivary glands in response to SAM activation, was chosen as the primary biomarker in this study because of its well-established role as an indicator of acute psychological stress and its reliable detection in saliva. As a marker of SAM system activity, CgA reflects noradrenaline secretion levels and plays a crucial role in modulating local immune responses to stress-related stimuli and bacterial toxins in the oral cavity [12]. Molecules released during this stress response can alter the systemic and local immune balance, contributing to the progression of periodontal disease through mechanisms such as inhibition of neutrophil activity, suppression of immunoglobulin secretion, and facilitation of periodontopathogen colonization [4].

Evidence suggests that CgA is not only a marker of systemic stress but may also be produced locally by cells such as polymorphonuclear leukocytes (PMNs), neuroendocrine cells, and other immune cells within inflamed periodontal tissues. Additionally, CgA acts as a prohormone, producing active peptides that modulate immune responses via autocrine or paracrine signaling [13]. This dual origin, indicating both systemic hypothalamic-pituitary-adrenal (HPA) or SAM axis activity and local inflammatory processes, renders salivary CgA a promising biomarker in psychoneuroimmunology for exploring the complex interactions between stress and periodontal disease.

The identification of stress-related biomolecules in biological fluids and their association with periodontal disease has offered objective insights into the complex interplay between psychological factors and oral health [5, 14]. Saliva is frequently utilized in such research due to its noninvasive collection method, which reduces participant stress [15]. In this study, psychological status was assessed using the Beck Depression Inventory (BDI), a widely validated self-report instrument commonly used to evaluate depressive symptoms [16]. In addition to psychological assessment, salivary biomarker analysis focused on CgA, a known indicator of psychological stress. Therefore, the aim of this study was to investigate salivary CgA levels in individuals with healthy periodontal status and those with periodontitis, based on the hypothesis that higher salivary CgA concentrations are associated with worsening periodontal clinical parameters and increased psychological stress levels, as measured by the BDI.

Methods

This cross-sectional study was conducted at the Department of Periodontology, Ankara Medipol University. The ethical protocol received approval from the Ankara Medipol University ethics committee, with the approval number (E-85859696-604.01.01-2756). All clinical procedures were conducted in full compliance with the revised Declaration of Helsinki (2013). Participant recruitment took place at the Department of Periodontology, Ankara Medipol University Faculty of Dentistry, from April 2024 to January 2025. The study's objectives were clearly explained to all participants, and written informed consent was obtained prior to enrollment. Additionally, each participant received a detailed information form outlining the study procedures before their inclusion.

Sample size calculation

GraphPad Prism version 10 was used to calculate the sample size. Data from a previous original study with a similar methodology were referenced [17]. A 95% confidence interval was chosen to provide a balance between statistical precision and generalizability of the findings, while a test power of 80% was deemed appropriate to detect a clinically relevant difference with a reasonable degree of certainty, the minimum required sample size was determined to be six subjects per group. To

compensate for possible data loss in biochemical analysis, a total of 56 participants were included in the study.

Study design

A total of 56 systemically healthy individuals (19 females and 37 males) were included in the study. The inclusion criteria were age ≥ 18, presence of at least 20 teeth (excluding third molars), and free of any systemic diseases.

The exclusion criteria comprised recent use of antipsychotic, anticonvulsant, or antibiotic medications within the past six months; periodontal therapy (non-surgical or surgical) within the previous year; Stage I and stage IV periodontitis patients according to guideline [18], and pregnancy or breastfeeding; and acute periodontal or dental pain at the time of examination.

Periodontal clinical examination and saliva sampling

Clinical measurements were performed by two periodontists (FS and ZG). The researchers organized a calibration session to standardize these periodontal measurements. To assess intra- and inter-observer agreement, two researchers conducted repeat measurements on 20 patients who were not part of the study, performing this twice. To confirm consistency, Cohen's kappa statistic was used, resulting in satisfactory intra-examiner reliability ($\kappa = 0.91$).

A Williams-type periodontal probe was used to assess the periodontal clinical status. Plaque index(PI) [19], gingival index (GI) [20], bleeding on probing (BOP), periodontal pocket depth (PD), and clinical attachment levels (CAL) were measured at six surfaces of each existing tooth. Participants were classified into three groups: periodontal health, gingivitis, and periodontitis, according to the 2017 Classification of Periodontal and Peri-Implant Diseases and Conditions [1, 21].

- Periodontal health was defined as < 10% BOP, no CAL, no PD > 3 mm, and absence of clinical signs such as swelling, pus, or no radiographic evidence of bone loss.
- Gingivitis was defined as BOP > 10% without CAL or radiographic evidence of bone loss and with PD ≤ 3 mm.
- Periodontitis (Stage II-III) was diagnosed based on BOP > 10%, PD ≥ 4 mm, CAL ≥ 3 mm, and radiographic evidence of bone loss.

Beck depression inventory (BDI)

Psychological stress was assessed using the Beck Depression Inventory (BDI), developed by T. Aaron Beck [16]. The BDI is a validated self-report inventory comprising 21 multiple-choice questions; each response is scored on a scale from 0 to 3. BDI has become one of the most widely used psychometric assessments for measuring the severity of depression. Total scores range from 0 to 63, with higher scores indicating more severe depressive symptoms. The standard cut-off scores are as follows: 10–18 indicates mild depression, 19–29 indicates moderate depression, and 30–63 indicates severe depression. Higher total scores indicate a greater presence of depressive symptoms. The Turkish version of the BDI, whose validity and reliability have been established [22], was used in this study.

Saliva collection

Saliva samples were collected prior to the clinical examination to minimize the risk of blood and plaque contamination and to avoid affecting the saliva flow rate. Participants were instructed to avoid eating, drinking, or brushing their teeth for at least one hour before the sample collection. Unstimulated whole saliva samples were obtained between 9:00 AM and 11:00 AM to minimize diurnal variations in salivary composition, under cool and calm standardized clinical conditions, following the protocol previously described by Navazesh et al. [23] Participants were seated in an upright position and instructed to allow saliva to accumulate in the floor of the mouth. They were then directed to expectorate into a sterile tube every 60 s for a duration of 5 min without any external stimulation. The volume of saliva collected was measured, and the flow rate (mL/min) was recorded for each individual.

Salivary chromogranin A mRNA isolation and quantitative real-time PCR

To assess salivary CgA mRNA expression, ACTB (β-actin) was used as the reference gene. Gene-specific primers targeting CgA and ACTB were synthesized with a synthesis scale of 25 nmol and purified using standard desalting purification. The primer sequences were as follows:

- β-actin

  Forward (5′→3′): CCAACCGCGAGAAGATGA.
  Reverse (5′→3′): CCAGAGGCGTACAGGGATAG.

- Chromogranin A (CgA)

  Forward (5′→3′): AAACCGCAGACCAGAGGAC.
  Reverse (5′→3′): CTGGTGGGCCACTTTCTC.

Total RNA was extracted from saliva samples using the TriPure Isolation Kit (Roche, Germany), and the RNA was suspended in diethylpyrocarbonate (DEPC)-treated water. To eliminate genomic DNA contamination, RNA samples were treated with DNase (TurboDNA-free;

Ambion Inc., Austin, TX, USA) and stored at −70 °C until further use. Subsequently, 1 µg of total RNA was used for cDNA synthesis using the First-Strand cDNA Synthesis Kit (Roche Diagnostics Co., Indianapolis, USA), following the manufacturer's instructions. Quantitative PCR was performed using primers designed via LightCycler Probe Design Software (Roche Diagnostics GmbH, Mannheim, Germany). The β-actin gene served as an internal control for both conventional and quantitative PCR assays. PCR amplification conditions were as follows: initial Denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 40 s, and 95 °C for 15 s, followed by 60 °C for 1 min and a final step at 95 °C for 15 s. To confirm the specificity of amplification, melting curve analysis was conducted at the end of each run. Relative gene expression levels were calculated using the $2^{(-\Delta\Delta Ct)}$ method.

Statistical analysis

All statistical analyses were performed using GraphPad Prism, Version 10. The Shapiro–Wilk test was used to assess the normality of the data distributions, and Levene's test was applied to evaluate the homogeneity of variances. One-way ANOVA was used for multivariate comparisons between the groups. For intergroup comparisons of categorical variables, the Chi-square test was employed. Where appropriate, Student's *t*-test was used for post hoc analysis. Spearman's correlation test was applied to assess correlations between groups. A significance level of $\alpha = 0.05$ was used for all statistical tests.

Results

A total of 80 individuals were initially assessed for participation in the study conducted at the Department of Periodontology, Ankara Medipol University Faculty of Dentistry. Of these, five declined to take part, three were excluded due to recent antibiotic use, eight were removed for having undergone recent periodontal treatment, and eight were excluded based on the presence of systemic diseases, resulting in 56 participants being enrolled in the study (Fig. 1**).**

The demographic and clinical periodontal parameters of the participants in the study are summarized in Table 1.

The final participants were categorized as follows: 13 were periodontally healthy, 14 were gingivitis, 13 were diagnosed with Stage II periodontitis, and 16 had Stage III periodontitis. There were no statistically significant differences among the groups in terms of gender distribution or stress assessment questionnaire scores (BDI scores) ($p > 0.05$). Flow rate values did not significantly differ among the groups ($p = 0.7861$).

A statistically significant difference was found in smoking status ($p = 0.0106$), indicating a greater percentage of smokers in the periodontitis group. Likewise, the proportion of participants reporting cessation of toothbrushing due to stress was significantly greater in the Stage

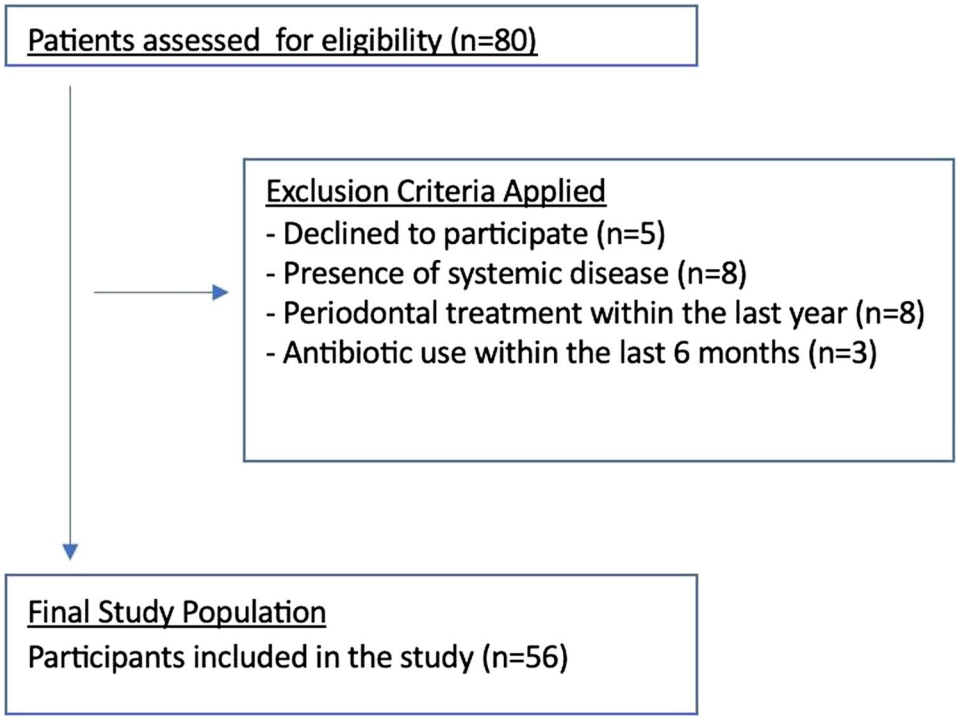


Fig. 1 Flowchart illustrating the process of participant selection

Table 1 Comparison of demographic and clinical periodontal parameters between the groups

Clinical parameters	Periodontally Healthy (*n* = 13)	Gingivitis (*n* = 14)	Stage II Periodontitis (*n* = 13)	Stage III Periodontitis (*n* = 16)	*p*
Gender (n, F/M)	4/9	4/10	3/10	8/8	0.4934
Smoking status (n, non-smoker/smoker)	10/3[d]	13/1[d]	9/4[d]	6/10	**0.0106**
Brushing frequency (n, once/twice/three times/day)	2/10/1	6/7/1	1/12/0	8/7/1	0.0523
Stopping brushing teeth due to stress (n, no/yes)	10/3[d]	6/8[d]	7/6[d]	4/12	**0.0464**
Supportive (n, no/yes)	6/7	10/4	11/2	13/3	0.1343
BDI Score*	17.15 ± 11.13 15.00 (3.00–39.00)	23.50 ± 10.24 23.00 (2.00–44.00)	26.54 ± 17.99 32.00 (0.00–48.00)	23.69 ± 15.26 23.00 (2.00–48.00)	0.3812
Age (year)*	42.23 ± 9.514 42.0 (31.0–59.0)	54.79 ± 10.23 54.0 (33.0–71.0) [a]	54.0 ± 12.58 56.0 (23.0–70.0) [a]	56.31 ± 6.56 55.00 (48.00–69.0) [a]	**0.0015**
PI*	0.58 ± 0.39 0.36 (0.16–1.20)	0.88 ± 0.32 1.0 (0.58–1.5) [a]	1.18 ± 0.62 1.0 (0.90-2.0) [a]	1.38 ± 0.53 1.0 (0.5-2.0) [a, b]	**0.0003**
GI*	0.53 ± 0.5 0.8 (0.0–1.0)	0.66 ± 0.48 1.0 (0.00–1.00)	1.15 ± 0.69 1.0 (0.00–2.00) [a, b]	1.37 ± 0.51 1.0 (0.86-2.0) [a, b]	**0.0003**
PD (mm)*	2.52 ± 0.48 2.7 (1.75-3.0)	2.56 ± 0.58 2.75 (1.5–3.4)	3.34 ± 0.36 3.4 (2.6–3.8) [a, b]	3.92 ± 0.31 3.9 (3.3–4.8) [a, b,c]	**< 0.0001**
BOP (%)*	6.03 ± 2.93 6.0 (1.24-10.00)	19.11 ± 10.99 17.50 (10.00–50.00) [a]	81.31 ± 32.06 100.0 (2.0-100.0) [a, b]	91.19 ± 21.72 100.0 (20.00-100.0) [a, b]	**< 0.0001**
CAL (mm)*	0.92 ± 0.92 1.112 (0.00-2.20)	1.57 ± 1.03 1.9 (0.00-2.98)	2.94 ± 0.58 2.98 (2.00-3.68) [a, b]	4.06 ± 0.49 4.02 (3.30-5.0) [a, b,c]	**< 0.0001**
Flowrate*	0.32 ± 0.11 0.25 (0.20–0.60)	0.35 ± 0.14 0.25 (0.20–0.60)	0.36 ± 0.11 0.40 (0.20–0.60)	0.33 ± 0.13 0.28 (0.20–0.60)	0.7861

Statistical difference with the control group, $p < 0.05$

BDI Beck depression inventory, *PI* plaque index, *PD* probing depth, *BOP* bleeding on probing, *CAL* Clinical attachment level

*Values are presented as mean ± standard deviation and median-interquartile range

[a] Statistically significant difference between the Periodontally Healthy Group and the other groups

[b] Statistically significant difference between the Gingivitis Group and the other groups

[c] Statistically significant difference between the Stage II Periodontitis Group and the other groups

[d] Statistically significant difference between the Stage III Periodontitis Group and the other groups

III periodontitis group compared to the healthy group ($p = 0.0464$).

Age significantly increased from the healthy group to the periodontitis groups ($p = 0.0015$). Clinical periodontal parameters, including the PI, GI, PD, Bleeding on BOP, and CAL, were significantly elevated in both periodontitis groups compared to the healthy and gingivitis groups (all $p < 0.0001$). Notably, PI and GI scores were significantly higher in the Stage III periodontitis group ($p = 0.0003$ for both).

In line with disease severity, salivary CgA levels showed a statistically significant increase across the groups. Compared to periodontally healthy individuals, patients with Stage III periodontitis exhibited markedly elevated CgA levels ($p = 0.0035$). CgA concentrations were also significantly higher in Stage III periodontitis compared to both the gingivitis group ($p = 0.0017$) and the Stage II periodontitis group ($p = 0.0023$). These results are visually presented in Fig. 2a.

Spearman correlation analysis revealed that CgA levels were not significantly correlated with any periodontal clinical parameters, including PI ($r = 0.1335$), GI ($r = 0.7825$), PD ($r = 0.8602$), BOP ($r = 0.6981$), or CAL ($r = 0.8457$) ($p > 0.05$ for all). In contrast, strong positive correlations were observed among clinical periodontal indices. GI was strongly correlated with PD internal consistency of periodontal disease severity measures.(Table 2) Fig. 2b shows the distribution patterns of key demographic, clinical, and salivary CgA variables across all 56 participants. The width of each violin plot indicates data density at various values, while the dotted lines in the center mark the median. This visualization emphasizes individual differences and provides a qualitative view of the spread and symmetry of each parameter within the study population.

Discussion

This cross-sectional study evaluates the salivary CgA levels in periodontal health, gingivitis, and Stage II-III periodontitis. Stages I and IV were excluded from the study to minimize confounding factors: Stage I due to its clinical similarity to gingivitis and its mild inflammatory profile, which is unlikely to elicit significant changes in salivary biomarkers such as CgA [24]; and Stage IV due

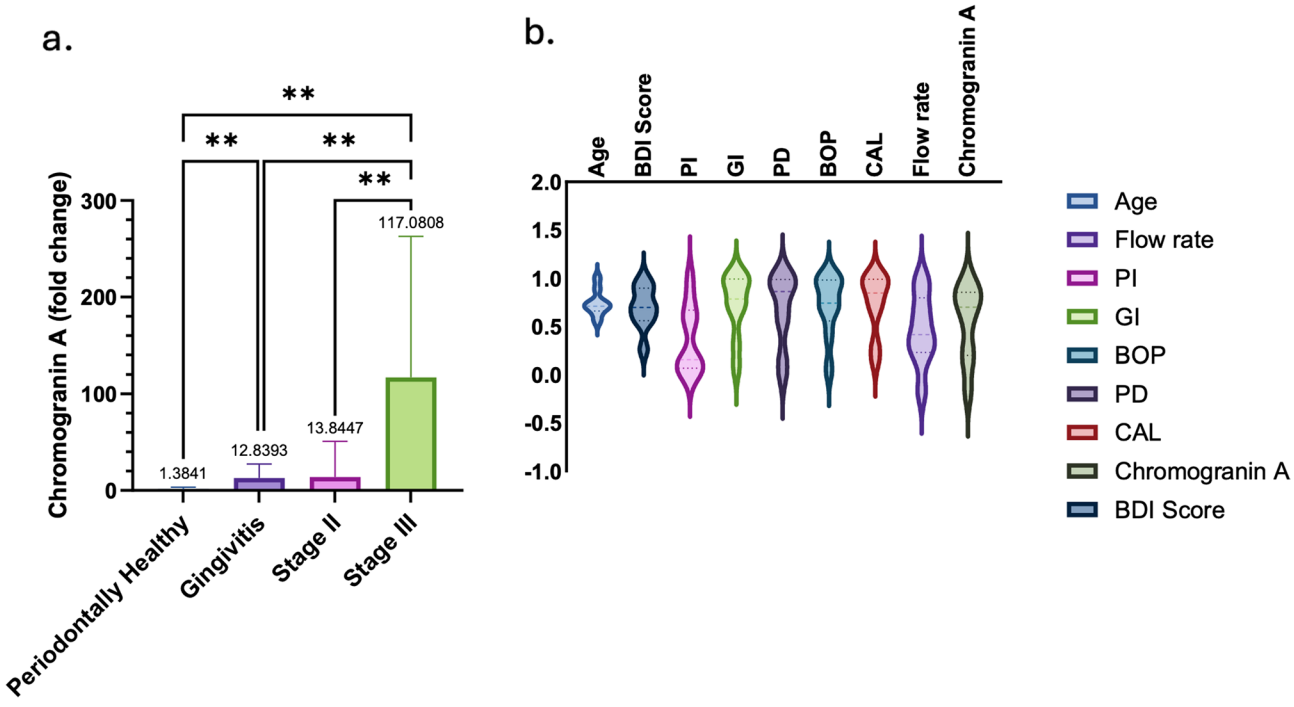


Fig. 2 Comparison of salivary Chromogranin A levels and distribution of clinical variables among study participants Group-wise comparison of salivary Chromogranin A levels among the study groups. The fold change values for each group were: Periodontally Healthy (1.38), Gingivitis (12.84), Stage II Periodontitis (13.84), and Stage III Periodontitis (117.08). Statistically significant differences were observed between Stage III periodontitis and all other groups ($p < 0.01$) Violin plots illustrating the distribution and density of demographic, clinical, and biomarker variables across the total study population ($n = 56$). Each violin plot illustrates the density of the corresponding variable, providing insights into its distribution shape, symmetry, and variability. The width of the plot reflects the data points at different values, and the central dashed line indicates the median variable

Table 2 Correlations between biomarkers and periodontal clinical parameters ($n = 56$, spearman correlation coefficients, r/p values)

Variables	BDI Score	PI	GI	PD (mm)	BOP (%)	CAL (mm)	Flowrate	ChromograninA
Age (year)	0.8884/0.112	0.7550/0.245	0.7086/0.291	0.6265/0.374	0.6976/0.302	0.7457/0.254	0.6909/0.309	0.5508/0.449
BDI Score		0.5556/0.444	0.6956/0.304	0.5640/0.436	0.7424/0.258	0.6736/0.326	0.9024/0.098	0.2617/0.738
PI			0.0803/0.920	−0.0032/0.997	0.0569/0.943	0.1535/0.846	0.5801/0.420	0.1335/0.866
GI				0.9845*/0.015	0.9920**/0.008	0.9918**/0.008	0.3336/0.666	0.7825/0.217
PD (mm)					0.9594*/0.041	0.9864*/0.014	0.1649/0.835	0.8602/0.140
BOP (%)						0.9693*/0.031	0.4127/0.587	0.6981/0.302
CAL (mm)							0.2928/0.707	0.8457/0.154
Flowrate								−0.1709/0.829

Values in bold are different from 0 with a significance level of alpha < 0.05, Spearman correlation test. Data presented as *r* coefficient/*p* values

BDI Beck depression inventory, *PI* plaque index, *PD* probing depth, *BOP* bleeding on probing, *CAL* Clinical attachment level

* $p < 0.05$

$p < 0.001$

to its complexity, including extensive tooth loss, prosthetic rehabilitation, impaired masticatory function, and a higher prevalence of systemic comorbidities, all of which could independently affect salivary biomarker levels and obscure the relationship between periodontal severity and CgA expression [25].

The primary findings of this study demonstrated that salivary CgA concentrations were significantly higher in individuals with Stage III periodontitis compared to those with periodontal health, gingivitis, or Stage II periodontitis. This observation suggests that elevated CgA levels may reflect increased activity of SAM system in individuals with more severe periodontal inflammation [17, 24, 26]. Previous studies have indicated that CgA is not exclusively a stress-related hormone; it has also been reported to be secreted by polymorphonuclear leukocytes (PMNs) and Merkel cells in response to stress. Furthermore, CgA has been shown to contribute to the upregulation of pro-inflammatory cytokines, thereby providing a mechanistic link between psychological stress and local inflammatory responses [27]. Studies have also suggested that CgA may act as a prohormone,

producing bioactive peptide fragments that exert regulatory effects through autocrine, paracrine, or endocrine pathways [28]. These peptides modulate immune responses, including macrophage and neutrophil activity, and may either promote or suppress inflammation depending on the context. In this light, the increased salivary CgA levels observed in periodontitis may not only reflect systemic sympathetic activation but may also originate from local immune cells in the periodontium as part of the inflammatory and remodeling process [17]. Although there is growing evidence about CgA and its peptides' biological functions, there are few studies examining its specific role in periodontal inflammation. Consequently, further research using longitudinal, interventional, and molecular approaches is necessary to determine whether CgA has a causal role and could serve as a biomarker in periodontal disease pathogenesis.

In the current study, although statistically significant differences in salivary CgA levels were observed across groups, no significant correlations were found between CgA levels and specific periodontal clinical parameters or BDI scores. This finding implies that, while CgA levels may increase with the severity of periodontal disease, they do not display a direct linear association with either clinical measures or psychological stress at the individual level. However, due to the inherent limitations of a cross-sectional study design, a definitive cause-effect relationship cannot be established. The lack of a significant correlation between salivary CgA levels and psychological stress, as measured by the BDI, may result from several factors. First, BDI is a self-reported instrument and may be influenced by individual perceptions and reporting bias, possibly underestimating physiological stress levels. Second, although BDI scores did not show significant differences among the study groups, many participants in the gingivitis and periodontitis groups reported mild to moderate stress levels. This pattern may indicate the presence of subclinical psychological stress in individuals with periodontal inflammation. Although these stress levels are not statistically significant across groups, they may still influence neuroendocrine function, immune regulation, or oral health habits, which could potentially contribute to the development of periodontal disease.

Although the BDI is mainly intended to assess depressive symptoms, it has also been widely used in oral health research to evaluate psychological distress. BDI was chosen for this study due to its validated structure, widespread use, and availability of a culturally adapted Turkish version. The BDI has been used in previous research exploring the link between psychological health and periodontal conditions, allowing for comparisons with existing studies. The findings related to psychological stress levels are consistent with previous studies, which also did not report a significant difference between individuals with periodontitis and healthy controls [29, 30]. However, one study did demonstrate a statistically significant increase in psychological stress levels among patients with aggressive periodontitis, suggesting that the association may vary depending on the type of research and inflammatory disease characteristics [31]. Alternatively, using different inventories from the literature, such as the Perceived Stress Scale (PSS) [32] or the State-Trait Anxiety Inventory (STAI) [33] which measure acute or perceived stress, could produce different correlation results.

A key objective of assessing the factors that influence the host response to bacterial plaque, which leads to periodontal disease, is to achieve periodontal health. Psychological stress is one of these influencing factors that can affect individual oral care behaviors [9, 34]. Our research findings indicated that the frequency of self-reported toothbrushing cessation due to stress was significantly higher in the Stage III periodontitis group; however, this behavioral indicator of stress was not accompanied by a corresponding increase in CgA at the individual level. This further supports the notion that CgA may not capture stress-driven behavioral changes that indirectly impact oral health, such as reduced oral hygiene.

Smoking is a well-established confounder in periodontal disease and has been shown to modulate salivary biomarker levels. In our study, the proportion of smokers was significantly higher in the Stage III periodontitis group, which may have contributed to the observed elevation in salivary CgA levels. Smoking can influence both systemic stress physiology and local inflammatory processes, complicating the interpretation of salivary biomarker concentrations. Recent findings further support this notion: a study demonstrated that smokers with periodontitis had higher salivary cortisol levels than serum cortisol, along with elevated salivary IL-1β compared to non-smokers with periodontitis. Moreover, stress levels were higher in smokers and positively correlated with salivary cortisol in both smoking and non-smoking individuals [35]. These findings reinforce the possibility that smoking amplifies psychoneuroimmunological responses in periodontitis, which may also extend to CgA dynamics. This issue requires further research in larger or stratified populations to better understand the role of smoking as a potential confounder.

In contrast to the absence of significant associations between CgA and clinical or psychological measures, strong positive correlations were observed among periodontal clinical indices. GI demonstrated robust correlations with PD, BOP, and CAL, indicating high internal consistency among these indicators of periodontal disease severity. These findings align with the established understanding of periodontal pathophysiology, wherein

gingival inflammation often accompanies and precedes deeper tissue destruction, as reflected in increased probing depth and attachment loss [18].

Saliva has emerged as a valuable medium in psychoneuroimmunological research due to its noninvasive collection and rich measurable biomarkers profile [36]. Numerous stress-related molecules, including cortisol, alpha-amylase, β-endorphin, immunoglobulin A, and CgA have been identified in saliva [4, 32, 37]. While these biomarkers can be detected in various biological fluids such as blood, serum, and urine, saliva provides a simple, stress-free sampling method that does not require invasive procedures. This is especially important when assessing psychological variables [15, 38].

Among the markers related to psychological stress, cortisol is widely recognized for its role as a biomarker of hypothalamic-pituitary-adrenal (HPA) axis activation and has been extensively used in studies evaluating psychological and physiological stress [32, 39]. However, the SAM system, which responds more rapidly to stressors, triggers the release of catecholamines that are difficult to detect due to their short half-life and low concentration in saliva. In contrast, salivary CgA, which is co-released with catecholamines, offers a promising alternative as it can more reliably reflect SAM activation. The studies have suggested that CgA may be a more sensitive and temporally accurate biomarker of acute psychological stress than cortisol [40, 41].

One significant challenge in salivary biomarker research is how circadian rhythms affect analyte concentrations [23, 42]. To address this, the current study employed a standardized sampling protocol, ensuring that all samples were collected at the same time of day under uniform conditions. Importantly, research has shown that CgA is less sensitive to diurnal variations than cortisol and other markers, which enhances its reliability in cross-sectional studies [43].

This study offers valuable insights into the link between psychological stress and salivary CgA levels, but its cross-sectional design limits the ability to determine causality. We found that salivary CgA levels were significantly higher in individuals with advanced periodontal disease. Nevertheless, despite differences between groups, there were no significant correlations between individual CgA levels and specific clinical measures or psychological stress scores. This inconsistency could stem from high individual variability in stress responses, immune functions, and salivary secretion, which can mask linear relationships. Additionally, the study's cross-sectional nature, which assesses only a single time point, might overlook fluctuations over time in stress biomarkers or disease progression. Although a positive correlation was observed between the BDI score and periodontal parameters such as PI, GI, and PD, these relationships did not reach statistical significance. This may be due to the limited sample size. Future studies with larger cohorts may help clarify whether this association reaches significance and supports the potential role of CgA as a biomarker for periodontal disease severity.

Conclusion

This study demonstrated that salivary CgA levels were significantly elevated in individuals with advanced periodontal disease, particularly in Stage III periodontitis. Although no correlations were found between CgA levels and individual periodontal or psychological parameters, the observed group-level differences suggest that CgA may reflect systemic stress responses associated with disease severity. Psychological stress, as measured by self-reported behaviors, also appeared to influence oral hygiene habits. These findings underscore the potential role of stress as a contributing factor in periodontal progression and highlight the relevance of psychoneuroimmunological markers in periodontal research.

Abbreviations

CgA	Chromogranin A
SAM	Sympathetic-Adrenomedullary
PMNs	Polymorphonuclear Leukocytes
HPA	Hypothalamic-Pituitary-Adrenal
BDI	Beck Depression Inventory
PI	Plaque Index
GI	Gingival Index
BOP	Bleeding On Probing
PD	Pocket Depth
CAL	Clinical Attachment Level
DEPC	Diethylpyrocarbonate
PCR	Polymerase Chain Reaction
PSS	Perceived Stress Scale
STAI	State-Trait Anxiety Inventory
IL-1β	Interleukin 1-Beta

Acknowledgements

Not applicable.

Authors' contributions

F. Soysal contributed to the conceptualization, methodology, investigation, and writing of the original draft.B. Unsal contributed to supervision and writing – review, and editing.S.C. Isler and G. Akca contributed to the investigation, writing – review and editing, and resource acquisition.Z. Guney was responsible for figure preparation, formal analysis, and resource provision. All authors have read and approved the final version of the manuscript.

Funding

Not applicable.

Data availability

The datasets generated and/or analyzed during the current study are available in the Zenodo repository at https://doi.org/10.5281/zenodo.15303029.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Ankara Medipol University (Approval No: E-85859696-604.01.01-2756). All procedures were conducted in accordance with the principles of the Declaration of Helsinki.

Prior to inclusion in the study, written informed consent was obtained from all participants and/or their legal guardians.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 22 April 2025 / Accepted: 3 September 2025
Published online: 06 October 2025

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