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Evaluation of annexin A1, carbonic anhydrase 1, and elongation factor 1-gamma levels in periodontal diseases

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Abstract

Background Periodontitis arises from dysbiotic subgingival microbiota and an unresolved inflammatory response. Annexin A1 (ANXA1), Carbonic Anhydrase I (CA1), and Elongation Factor 1-gamma (EF1-ɣ) may play a role in periodontal inflammation and disease pathogenesis. This study aimed to investigate the levels of these molecules in gingival crevicular fluid (GCF) of individuals with different periodontal conditions.

Methods GCF samples were collected from 20 patients with Stage III Grade B periodontitis, 20 with Stage III Grade C periodontitis, 19 gingivitis patients, and 21 periodontally healthy individuals. ANXA1, CA1, and EF1-ɣ levels were measured using ELISA.

Results Clinical parameters were significantly higher in periodontitis groups compared to gingivitis and healthy groups ($p < 0.001$). GCF EF1-ɣ total amount differed among groups, with higher levels in gingivitis compared to periodontitis and healthy control groups ($p < 0.001$). Elevated levels of EF1-ɣ were found in gingivitis compared to Stage III/B and Stage III/C periodontitis ($p < 0.001$). GCF ANXA1 and CA1 levels were similar across study groups ($p > 0.05$).

Conclusion Within the limitations of this study, it might be suggested that the decreased levels of EF1-ɣ in diseased sites of periodontitis and its elevated levels in gingivitis are associated with the pathogenesis of periodontal disease.

Keywords Periodontitis, Gingivitis, Gingival crevicular fluid, Annexin-a1, Carbonic anhydrase-1, Elongation factor-1 gamma, Pathogenesis

Introduction

The current understanding in relation to initiation and progression of periodontitis involves a sophisticated interaction between dysbiotic microbiota and dysregulated immune-inflammatory in a predisposed host that trigger periodontal attachment loss and marginal alveolar bone destruction that can end up, in severe cases, in tooth mobility and final tooth loss, as well as higher risk of non-communicable diseases [1]. Multiple interactions between host cells and resident oral microbiome regulate the protective functions that lead to inflammation in the oral cavity [2]. Failure of appropriate regulation of inflammation can lead to chronic inflammatory diseases

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that result from an imbalance between pro-inflammatory and pro-resolution mediators [3].

Annexin A1 (ANXA1), a prominent member of the annexin superfamily, was initially identified in leukocytes as a glucocorticoid-inducible protein, formerly known as lipocortin [4]. Structurally, annexins are calcium-dependent, phospholipid-binding proteins [5]. ANXA1 is known for its ability to modulate various aspects of innate and adaptive immunity [4]. As a pleiotropic molecule, it influences inflammatory processes by interacting with multiple signaling pathways within the cytoplasm. This interaction can either promote proinflammatory responses through the release of arachidonic acid or inhibit inflammatory processes during the resolution phase [6]. ANXA1, a potent endogenous anti-inflammatory agent, is closely associated with periodontal inflammation. Bostanci et al. (2010), were the first to detect ANXA1 in gingival tissue exudates from healthy individuals using proteomic techniques, observing its frequent presence in health but its absence in severe periodontitis [7]. This biomarker has shown promise in various oral biofluids and gingival tissue samples analyzed through proteomics [8, 9].

Carbonic anhydrases (CAs), a superfamily of zinc metalloenzymes, are crucial for maintaining acid–base homeostasis in both healthy and pathological conditions [10, 11]. They regulate pH by catalyzing the reversible conversion of carbon dioxide and water to bicarbonate and protons, a fundamental physiological reaction. CA1, a cytosolic isoenzyme of the α-CA family, is extensively studied due to its role in diverse physiological and pathological processes [12]. Dysregulation of CA1 can disrupt pH homeostasis in the local environment, potentially impacting the growth and virulence of periodontal pathogens [10]. Although CA1 has been identified in gingival crevicular fluid and tissue samples from both periodontitis patients and healthy controls, its role in oral conditions remains underexplored [8, 9].

Elongation factor-1-gamma (EF1-ɣ), a subunit of the EF1 complex, is integral to the first step of protein elongation in eukaryotic cells [13]. EF1-ɣ is involved in various cellular functions, including growth, motility, protein turnover, signal transduction, DNA replication/repair, and apoptosis [14, 15]. Given its pivotal role in protein synthesis, EF1-ɣ may also influence the production of cytokines, antibodies, and other immune-related proteins crucial for responding to periodontal pathogens. While EF1-ɣ has been implicated in several diseases such as tumors, neurodegenerative, and cardiovascular diseases [13, 16, 17] its specific role in periodontal disease remains unexplored. Although studies using proteomic methodologies have demonstrated significant differences in the levels of ANXA1, CA1, and EF1-ɣ in gingival crevicular fluid (GCF) and tissue biopsy samples between periodontal health and disease [7–9, 18, 19], there is limited data on the validation of these molecules in GCF using immunoassay methods.

In the present study, molecules involved in different aspects such as antioxidant activity, stress response, immune response, and apoptosis, and which might be potential biomarkers for early-stage periodontal disease based on current data, were investigated. Few studies have investigated the roles of ANXA1, CA1, and EF1- ɣ [9, 20, 21] in periodontal diseases; however, no research has simultaneously assessed the levels of ANXA1, CA1, and EF1-ɣ in GCF from patients with periodontitis, gingivitis, and healthy periodontium within a single cohort. The aim of our study, to investigate the levels of ANXA1, CA1, and EF1-ɣ in GCF in different periodontal diseases. Our hypothesis is that the molecules ANXA1, CA1, and EF1-ɣ are associated with periodontal disease pathogenesis, based on their potential involvement in inflammatory processes as indicated by the literature.

Methods

Study population

The diagnosis of periodontal diseases and conditions was made based on the radiographic and clinical diagnostic criteria proposed by the 2017 World Workshop on the Classification of Periodontal and Peri-implant Diseases and Conditions. This cross-sectional study included 20 patients with Stage III Grade C generalized periodontitis (P-Stage III/C), 20 patients with Stage III Grade B generalized periodontitis (P-Stage III/B), 19 patients with gingivitis, and 21 periodontally healthy volunteers. The controls/Healthy group were the individuals with well-maintained oral hygiene without no periodontal disease history or symptoms with a probing pocket depth (PPD) of $\leq$3 mm and bleeding on probing (BOP) of (+) $\leq$10. Only generalized gingivitis patients were included in this study. Individuals presenting with a BOP $\geq$30% and PPD $\leq$3 mm without radiographic bone loss and attachment loss were considered to have gingivitis. Stage III periodontitis patients had at least 4 interdental sites clinical attachment level (CAL) >5 mm due to periodontitis, radiographic bone loss reaching to the mid-third of the root or beyond, teeth loss less than 4 teeth due to periodontitis. These patients were graded according to the bone loss (%)/age index (Grade B, 0.25–1.00). Since the bone loss (%)/age values were >1.0, all periodontitis patients were considered grade C [22].

The study protocol was approved by the Ethics Committee of the Ege University School of Medicine, Izmir (22-9 T/25). Patients were selected from Ege University, Faculty of Dentistry, Periodontology Clinic, Izmir, Turkey between September 2022 and December 2022. All

patients were informed about the study, and a written informed consent form in compliance with the 1964 Helsinki Declaration and its later amendments was obtained. This study has been registered at ClinicalTrials.gov with the identifier NCT05680441.

Clinical examination, inclusion and exclusion criteria The clinical periodontal examination of the healthy and periodontitis subjects consisted of plaque index (PI) [23], probing pocket depth (PPD), clinical attachment level (CAL), and bleeding on probing (BOP) recorded. Averages for full mouth PPD, CAL, and the percentage of sites with BOP were calculated for each subject. A single calibrated examiner (VÖÖ) conducted a full mouth periodontal examination of all participants. The measurements were performed using a Williams periodontal probe (Hu-Friedy, Chicago, IL, USA). All measurements were performed full mouth and at six sites for each tooth. Before clinical measurements, intra-examiner calibration was performed by measuring PPD and CAL values twice on five patients with one day interval resulting in intraclass correlation coefficients were 0.92 for PPD and 0.90 for CAL.

Exclusion criteria included the following: having any diagnosed medical disorders such as cardiovascular diseases, diabetes mellitus, rheumatoid arthritis, mucocutaneous diseases, immunological diseases, hepatitis, and human immunodeficiency virus infection, taken antibiotics, nonsteroidal antiinflammatory drugs, steroids, immunosuppressants, beta-blockers, calcium channel blockers, anticoagulants, and hormonal contraceptives within the past 6 months, having any destructive periodontal disease, nonsurgical or surgical periodontal treatment received in the past year, having a restorative and endodontic therapy requirement, having a removable partial denture and orthodontic appliances, current pregnancy or lactation and smoking.

Collection of GCF samples GCF samples were taken from the buccal aspects of two nonadjacent interproximal sites in single-rooted teeth. In periodontitis groups, GCF was sampled from two deepest pockets of single-rooted teeth. Samples were collected from the sites with visible signs of inflammation in gingivitis group and without BOP in the healthy group. The selected areas were carefully cleared of supragingival plaque using sterile curettes, isolated with cotton rolls, and slightly air-dried to avoid contamination.

Standardized filter paper strips (PerioPaper, Proflow, Amityville, NY) were used for GCF sampling. Sterile paper strips were gently inserted into the gingival sulcus or pocket until mild resistance was felt and left there for 30 s. Mechanical irritation was avoided and strips visually contaminated with blood were discarded. A precalibrated electronic device measured the absorbed fluid volume (Periotron 8010, Oraflow, Amityville, NY). The readings were converted to an actual volume (microliter, µL) by reference to the standard curve. The paper strips were individually placed into a sterile polypropylene tube and stored at −80∘C for further analysis. The clinical data collection and sample collection phases were completed with the same cohort of 80 participants without any dropouts.

Measurement of ANXA1, CA1, and EF1- ɣ levels in GCF samples ANXA1 levels were studied with commercially available kits using the ELISA method (Bioscience SRB, catalog no: 201–12–3158). The optical density was measured spectrophotometrically at a wavelength of 450 nm (Tecan). The assay ranges for the Annexin-A1 kit were 0.20–20 ng/mL, sensitivity 0.2 ng/mL, and the intra- and interassay coefficients of variance (CV%) were < 10%. The results were presented as ng. CA1 levels were studied with commercially available kits using the ELISA method (Bioscience SRB, catalog no: SRB-T-88927). The optical density was measured spectrophotometrically at a wavelength of 450 nm (Tecan). The assay ranges for the CA1 kit were 3.12–200 ng/mL, sensitivity < 1.875 ng/mL, and the intra- and interassay coefficients of variance (CV%) were < 10%. The results were presented as ng. EF1- ɣ levels were studied with commercially available kits using the ELISA method (Bioscience SRB, catalog no: 201–12–3732). The optical density was measured spectrophotometrically at a wavelength of 450 nm (Tecan). The assay ranges for the EF1- ɣ kit were 31.25–2000 pg/mL, sensitivity < 12.4 pg/mL, and the intra- and interassay coefficients of variance (CV%) were < 10%. The results were presented as pg.

Statistical analysis

The minimum sample size was calculated based on a 50% difference in the mean GCF levels of biochemical markers between the healthy and diseased groups, with standard deviations assumed to be up to 80% of the mean values. Power analysis was performed using specialized software (G*Power version 3.1.9), assuming a significance level of $p = 0.05$ and with a power of 80%. To detect a difference of at least 0.4 effect size (Ellis, 2010) using the one-way ANOVA method, the calculation determined that a minimum of 19 patients per group was required.

In the data analysis, SPSS 26.0 statistical package program was utilized. Descriptive statistics for the evaluation results were presented as mean and standard deviation. The Kolmogorov–Smirnov test was applied to assess the normality of the data distribution. For comparisons of numerical variables across three or more independent groups, the Kruskal–Wallis test was used due to the violation of normal distribution assumptions. In cases where the Kruskal–Wallis test indicated significant differences,

post hoc comparisons were conducted using the Bonferroni correction. The chi-square test was employed to analyze independent categorical variables. The Spearman test was applied for correlation analysis. A statistical alpha significance level of $p < 0.05$ was considered for all tests. *P* values < 0.05 were considered statistically significant, and 95% confidence intervals (CIs) were calculated.

Results

The P-Stage III/C group had the lowest mean age compared to the P-Stage III/B, gingivitis, and healthy groups in Table 1 ($p < 0.001$).

Clinical findings

The whole mouth clinical parameters of the 80 participants are presented in Table 2. P-Stage III/C and P-Stage III/B groups had significantly higher mean PPD and CAL scores compared to the gingivitis and healthy groups ($p < 0.001$). All patient groups had significantly higher BOP and PI scores than the healthy group ($p < 0.001$). Periodontitis and gingivitis groups had similar BOP scores ($p > 0.05$).

The mean clinical data for the sampling areas are shown in Table 3. As expected, the mean PPD scores of sampling sites in Periodontitis groups were significantly higher than the healthy and gingivitis group ($p < 0.001$). Stage III/C and P-Stage III/B groups had similar PPD scores, and higher scores than the gingivitis group ($p < 0.001$). The mean CAL of sampling sites in Periodontitis groups was significantly higher than the gingivitis and healthy group ($p < 0.001$). P-Stage III/B and P-Stage III/C groups had similar CAL scores ($p > 0.05$). All patient groups had significantly higher BOP and PI scores than the healthy group ($p < 0.001$). Periodontitis and gingivitis groups had similar BOP and PI scores ($p > 0.05$). Periodontitis and gingivitis groups had significantly higher mean GCF volume than the healthy group ($p < 0.001$). Gingivitis group had also significantly higher mean GCF volume compared to P-Stage III/B ($p < 0.008$). P-Stage III/B and P-Stage III/C groups had similar mean GCF volumes ($p > 0.05$).

Table 1 Demographics of participants

Patient characteristic	P-Stage III/C	P-StageIII/B	Gingivitis	Healthy	*p* value
Age	30.00 ± 5.01[a]	44.10 ± 7.85	38.11 ± 11.37	40.95 ± 6.17	< 0.001
Sex (males/females)	9/11	9/11	9/10	11/10	0.972

[a] Shows difference from P-Stage III/C group

Table 2 Whole mouth clinical parameters of the study groups

	P-Stage III/C	P-Stage III/B	Gingivitis	Healthy	*p* value
PPD (mm)	4.61 ± 0.83[a]	4.67 ± 0.83[ac]	2.72 ± 0.09[a]	1.90 ± 0.17	< 0.001
CAL (mm)	5.10 ± 0.97[b]	5.38 ± 0.79[b]	2.72 ± 0.09[a]	1.90 ± 0.17	< 0.001
BOP (%)	71.21 ± 19.01[a]	79.38 ± 17.20[a]	71.47 ± 13.46[a]	8.59 ± 1.44	< 0.001
PI	2.39 ± 0.97[a]	2.60 ± 0.34[ac]	2.07 ± 0.33[a]	0.44 ± 0.22	< 0.001

Abbreviations: *PPD* Probing pocket depth, *CAL* Clinical attachment level, *BOP* Bleeding on probing, *PI* Plaque index

[a] Significant difference from healthy group

[b] Significant difference from gingivitis and healthy groups

[c] Significant difference between P-Stage III/B and gingivitis groups

Table 3 Clinical parameters of the sampling areas in study groups

	P-Stage III/C	P-Stage III/B	Gingivitis	Healthy	*p* value	*p* value
PPD (mm)	7.32 ± 1.33[b]	6.19 ± 0.40[b]	2.68 ± 0.47	2.14 ± 0.72	< 0.001	< 0.001
CAL (mm)	7.95 ± 1.68[b]	6.71 ± 0.95[b]	2.68 ± 0.47	2.14 ± 0.72	< 0.001	< 0.001
BOP (%)	2.11 ± 0.73[a]	2.48 ± 0.60[a]	2.63 ± 0.49[a]	_	< 0.001	< 0.001
PI	2.63 ± 0.49[a]	2.67 ± 0.48[a]	2.74 ± 0.45[a]	_	< 0.001	< 0.001
GCF (µl)	0.29 ± 0.12[a]	0.32 ± 0.24[ac]	0.59 ± 0.21[a]	0.10 ± 0.04	< 0.001	< 0.001

Abbreviations: *PPD*, Probing pocket depth, *CAL* Clinical attachment level, *BOP* Bleeding on probing, *PI* Plaque index, *GCF* Gingival crevicular fluid

[a] Significant difference from healthy group

[b] Significant difference from gingivitis and healthy groups

[c] Significant difference between P-Stage III/B and gingivitis groups

Biochemical findings ANXA1, CA1 and EF1-ɣ were detected in all GCF samples analyzed (80 out of 80 GCF samples). The medians, interquartile ranges, means and standard deviations of GCF ANXA1, CA1 and EF1-ɣ are presented in Table 4. GCF ANXA1 and CA1 total amount of the study groups were similar ($p > 0.05$). GCF EF1-ɣ total amount was different among the study groups. The total amount of GCF EF1-ɣ was higher in the gingivitis group than in the P-Stage III/C, P-Stage III/B and healthy groups. Periodontitis and healthy groups had similar GCF EF1-ɣ total amount ($p > 0.05$). Elevated levels of GCF EF1-ɣ were found in gingivitis group compared to periodontitis group ($p < 0.001$) (Fig. 1).

The correlation between GCF ANXA1 and CA1 total amount and clinical parameters is presented in Table 5. There was no correlation between GCF EF1-ɣ, CA1, and clinical parameters ($p > 0.05$), but ANXA1 negatively correlated with the PPD and CAL ($p < 0.05$) although no correlation was present between ANXA1, BOP, and PI ($p > 0.05$).

Discussion

The present study demonstrated the presence of ANXA1, CA1, and EF1-ɣ in GCF in individuals with different periodontal diseases. Increased GCF EF1-ɣ levels were found in individuals with gingivitis, while GCF ANXA1, CA1 levels were similar among the groups. There were no differences between Grade B and Grade C periodontitis. The current study provided to the best of our knowledge the first evidence about the presence of CA1 and EF1-ɣ in the GCF of individuals with in periodontal disease stage III periodontitis, gingivitis, and periodontal health. Elevated GCF EF1-ɣ levels in gingivitis group compared to the healthy group might suggest a potential role of EF1-ɣ as a biochemical marker that could offer insights into the progression from gingivitis to periodontitis.

The presence of ANXA1 in biofluids and gingival tissue in health and disease has been previously demonstrated using proteomic analysis [7–9, 24, 25]. Nevertheless, previous studies indicated that ANXA1 appears to be more associated with a healthy status, since it was more frequently detected in health compared to disease [7, 8, 19]. Moreover, ANXA1 has been suggested as a potential biomarker for distinguishing between periodontal disease and health status in previous studies [19, 24, 26]. It appears to have an important role in inflammation, particularly in regulating the balance between pro- and anti-inflammatory responses [27, 28]. Chronic inflammation, such as that seen in periodontitis, promotes tissue destruction when unresolved, and ANXA1 mitigates this by inhibiting leukocyte extravasation and regulating cytokine synthesis [29]. Furthermore, ANXA1 reduces osteoclast differentiation through the inhibition of NF-κB and activation of PPAR-γ pathways, potentially limiting bone loss [30].

The current results demonstrated no marked differences in the GCF ANXA1 levels according to disease profile. Although statistically not significant, there is a trend toward increased amounts of ANXA1 in gingivitis and decreased amounts in periodontitis compared to the healthy group. It is noteworthy that proteolytic degradation of ANXA1 has been observed in inflammatory conditions [31] which may contribute to lower levels in highly inflamed sites. This observation aligns with previous studies indicating ANXA1's protective role during inflammation, as suggested by Aboodi et al. (2016), who found increased salivary levels of ANXA1 in experimental gingivitis model, highlighting its protective role [26]. However, da Silva et al. (2023) reported decreased salivary levels [25] and Grant et al. (2010) found stable GCF ANXA1 levels during 21-day experimental gingivitis [32]. Furthermore, ANXA1 levels were found to be significantly lower in individuals with periodontitis and their children, indicating its potential as a biomarker for early disease detection [19]. Conversely, pregnant women with gingivitis exhibited elevated salivary ANXA1 levels compared to healthy controls and non-pregnant women with gingivitis [27]. This elevation may reflect ANXA1's protective role in managing inflammation, as it is known to have anti-inflammatory properties [33]. The presence of ANXA1 in gingivitis likely represents a mechanism aimed at controlling infection and promoting tissue repair, aligning with the emerging paradigm that emphasizes the role of inflammation in periodontal disease pathogenesis [34]. These findings support ANXA1's potential as both a biomarker and therapeutic target in early periodontal disease.

In the present study GCF CA1 total amount showed similar profile to ANXA1 in study groups. All groups had similar CA1 total amount although in gingivitis those levels tended to be higher. CA1, an enzyme far less investigated in oral conditions. Limited number of proteomic studies found increased CA1 levels in GCF and in gingival tissue of subjects affected by periodontal diseases [8, 9, 18, 21, 35]. In contrast to our findings, Baliban et al. (2013) found GCF CA1 to be one of the proteins for differentiating periodontitis from periodontal health. Gingival tissue levels of CA1 was also found to be upregulated in periodontitis compared to healthy ones [9, 18]. In a very recent study by Blanco-Pintos et al. (2024), CA1 levels were reported to show variations among untreated Stages III–IV and Grades B–C generalized periodontitis, treated periodontitis, and periodontal health, reflecting differences in protein expression across these conditions [21].

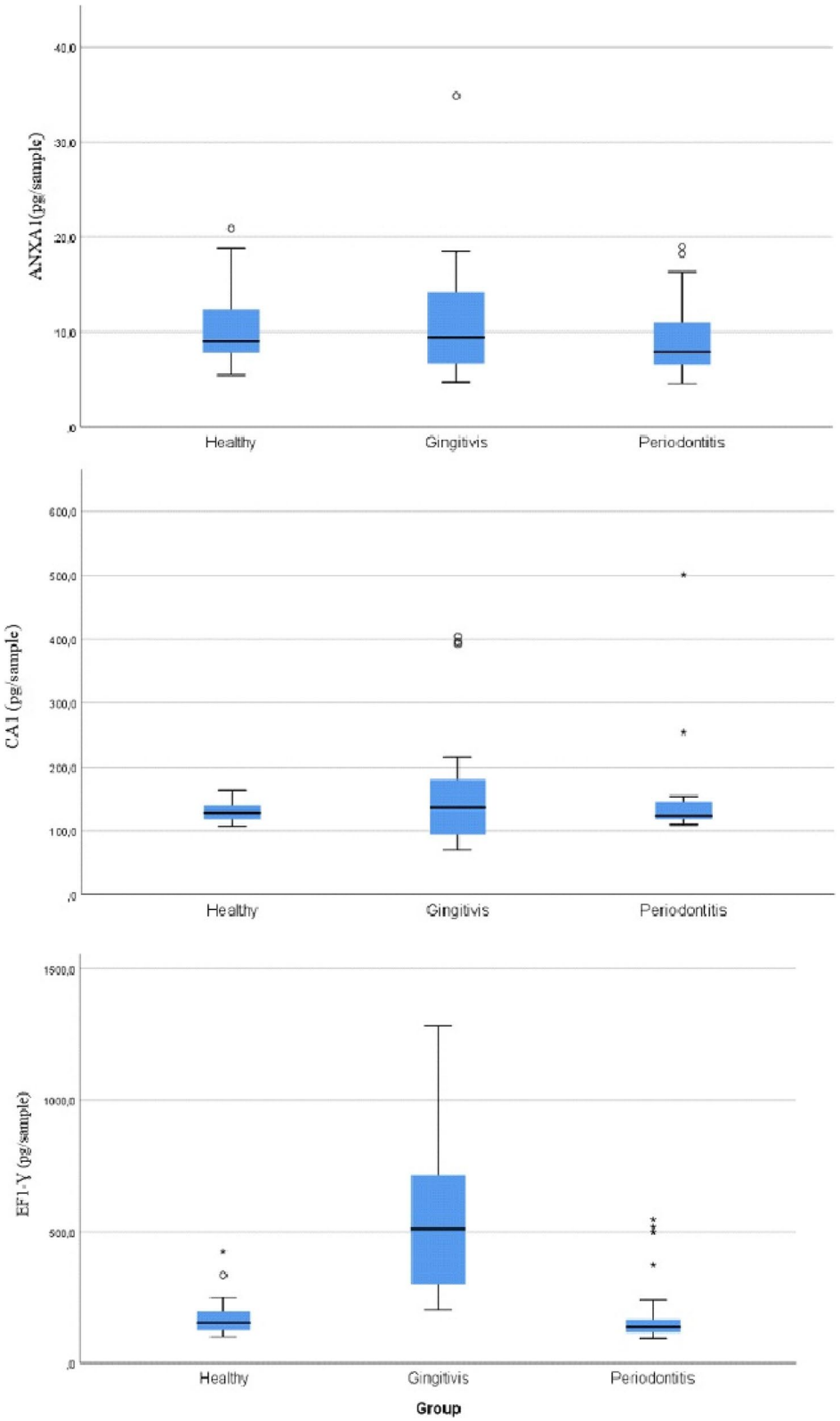

Fig. 1 GCF ANXA1 and CA1 total amount of the study groups were similar ($p>0.05$). Elevated levels of GCF EF1-ɣ Were found in gingivitis group compared to periodontitis and healthy groups ($p<0.001$)

Table 4 GCF EF1- ɣ, CA1 and ANXA1 total amounts in study groups

	EF1-ɣ (pg/sample)		CA1 (pg/sample)		ANXA1 (pg/sample)	
	Median (IQR)	**Mean (SD)**	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**	**Mean (SD)**
P-Stage III/C	125.08(30.32)[a]	181.41 ± 147.39	120.53(16.72)	142.19 ± 85.33	8.08 (2.80)	9.19 ± 3.68
P-Stage III/B	150.98(74.54)[a]	181.06 ± 97.14	131.49(27.39)	139.80 ± 30.48	7.74 (6.87)	9.66 ± 4.33
Gingivitis	510.45(444.75)	532.63 ± 294.87	136.10(95.16)	169.19 ± 109.07	9.42 (7.94)	11.57 ± 7.04
Healthy	154.14 (76.75)[a]	175.51 ± 80.79	128.61(12.68)	130.91 ± 14.91	9.00 (5.60)	10.76 ± 4.34
p value	< 0.001		0.610		0.49	

Abbreviations: *GCF* Gingival crevicular fluid, *EF1-ɣ* Elongation Factor-1 Gamma, *CA1* Carbonic anhydrase-1, *ANXA1* Annexin A1

[a] Significant difference from gingivitis group

Table 5 Correlations between EF1- ɣ, CA1 and ANXA1 total amount and clinical parameters

Clinical Parameters	EF1- ɣ	CA1	ANXA1
PPD	−0.204	−0.011	[a] −0.235
CAL	−0.19	−0.032	[a] −0.262
BOP%	0.029	−0.02	−0.118
PI	−0.056	0.068	−0.024
GCF (µl)	[‡] 0.428	−0.003	−0.028

[‡] $p < 0.05$

Abbreviations: *PPD* Probing pocket depth, *CAL* Clinical attachment level, *BOP* Bleeding on probing, *PI* Plaque index, *GCF* Gingival crevicular fluid, *EF1-ɣ* Elongation Factor-1 Gamma, *CA1* Carbonic anhydrase-1, *ANXA1* Annexin A1

[a] ANXA1 negatively correlated with the PPD and CAL, EF1- ɣ positively correlated with GCF

Abnormal CA1 activity can lead to disrupted pH homeostasis, of the local environment, potentially affecting bacterial growth and the inflammatory milieu [10]. CA1 can influence the inflammatory response by modulating the pH of the local environment, which in turn affects the activity of immune cells and the production of pro-inflammatory cytokines [36]. Alterations in local pH mediated by CA1 can create an environment more favorable for the growth and virulence of periodontal pathogens, thereby promoting the development of periodontal disease [10, 37, 38]. Therefore, CA1 may contribute to the development and progression of periodontal diseases. In the present study, a trend of elevated CA1 levels, similar to ANXA1, was observed in gingivitis, although not statistically significant. This finding could suggest a potential protective role for CA1 as an early-stage biomarker for periodontal diseases.

In the present study, we demonstrate for the first time the presence of GCF EF1-ɣ in different periodontal diseases. GCF EF1-ɣ total amount was significantly about threefold increased in gingivitis compared to others. On the other hand, periodontitis groups had decreased levels compared to gingivitis and similar levels compared to health ones. In contrast to our findings, a proteomic study examining GCF samples in periodontitis and periodontal health groups by Baliban et al. (2013) has shown that GCF EF1-ɣ levels are associated with periodontitis [8]. This discrepancy with Baliban et al. [8], who identified EF1-ɣ as a periodontitis-associated protein, may be attributed to differences in study design, sample collection methods, or disease classification criteria. While proteomic methodologies provide a broad screening of protein expression, targeted immunoassays such as ELISA allow for a more quantitative assessment, potentially revealing disease-stage-specific variations in biomarker levels. We might suggest that the EF1-ɣ enzyme plays a potential role in the pathogenesis of periodontal disease, particularly in the transition from gingivitis to periodontitis, though this connection remains to be further explored [3]. It might be also speculated that EF1-ɣ activity of gingiva does not completely flow to GCF from gingivitis to periodontitis.

Recent studies highlight the role of translation elongation factors in immune cell function and inflammation [39]. Efficient protein synthesis is critical for the rapid production of these molecules during an inflammatory response and the resolution of inflammation, which plays a key role in pathogenesis of periodontal diseases [40, 41]. Any dysregulation in EF1-ɣ function could potentially affect the ability of immune cells to produce necessary proteins, thereby influencing the inflammatory process. It is reported that NF-κB transcription factor inhibits EF2 kinase, an essential elongation factor, to enhance translation elongation and protein synthesis, linking elongation factors directly to inflammation [42]. Similarly, the negative elongation factor (NELF) complex is identified as a regulator of anti-inflammatory gene expression in macrophages, underscoring elongation factors'role in inflammation modulation [43]. Moreover, the significance of elongation in the NF-κB activation pathway highlights its complex involvement in the inflammatory response [44]. The transition from gingivitis to periodontitis has been reported to involve

molecular pathways regulating inflammation that determine disease outcome and healing potential [3]. Proposing a role for EF1-ɣ dysregulation in this transition is plausible, considering the threefold increase in GCF levels in gingivitis compared to periodontitis and the potential role of EF1-ɣ in inflammation. Alternatively, the observed decrease in EF1-ɣ levels in the periodontitis group could also be interpreted as indicative of compensatory mechanisms in inflammation.

Within the limitations of the present study, these findings provide valuable insights into the presence and measurement of these three molecules in GCF, contributing to our understanding of both periodontal disease pathogenesis. One limitation is the cross-sectional nature of the study, which precludes knowledge of the effect of periodontal treatment on GCF ANXA1, CA1, and EF1-ɣ levels. These analyses of specific proteins with GCF do not identify a protein as a 'potential' biomarker, as certain diagnostic accuracy parameters are mandatory [45]. Therefore, although the current findings are not sufficient to establish a causal relationship between EF1-ɣ and the pathogenesis of periodontal disease, they are noteworthy. Another limitation is the small sample size. However, according to power calculation it was large enough to detect a statistically significant difference between groups. Furthermore, the episodic nature of periodontal disease, characterized by periods of remission and aggravation, poses a challenge in understanding its progression and underlying molecular dynamics [46]. Finally, the statistically significant age difference among the groups should be recognized as a limitation, as age may serve as a confounding factor influencing the observed outcomes [47].

Conclusion

Despite the limitations of the study, EF1-ɣ levels increased in gingivitis and decreased to levels similar to the healthy condition in stage III periodontitis, drawing attention to the possible protective role of EF1-ɣ in periodontal diseases. Further studies are needed to elucidate the role of these molecules in the pathogenesis of periodontal diseases.

Abbreviations

ANXA1	Annexin A1
CA1	Carbonic Anhydrase I
EF1-ɣ	Elongation Factor 1-gamma
GCF	Gingival crevicular fluid
CAL	Clinical attachment level
BOP	Bleeding on probing
PI	Plaque index
PPD	Probing pocket depth
P-Stage III/C	Stage III Grade C generalized periodontitis
P-Stage III/B	Stage III Grade B generalized periodontitis
NF-κB	Nuclear factor kappa B
NELF	Negative elongation factor

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Authors' contributions

BCUS: Writing – review and editing; data curation; writing – original draft. BY: Writing – review and editing. GE: Methodology; writing – original draft; writing–review and editing;project administration; supervision; data curation; conceptualization, funding acquisition. VÖÖ: Conceptualization; resources; investigation. HA: Validation; formal analysis.

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Data availability

Data is provided within the manuscript (tables and figures).

Declarations

Ethics approval and consent to participate

All patients were informed about the study, and a written informed consent form in compliance with the 1964 Helsinki Declaration and its later amendments was obtained. The study protocol was approved by the Ethics Committee of the Ege University School of Medicine, Izmir (22-9 T/25).

Consent for publication

Not Applicable.

Competing interests

The authors declare no competing interests.

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