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Annexin levels in GCF determine the imbalance of periodontal inflammatory regulation

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Objective: Annexin-1 (ANXA1), a member of the annexin family, plays a role in the resolution of inflammation and the regulation of anti-inflammatory responses, while annexin-2 (ANXA2) is involved in the initiation of the inflammatory responses. The aim of this study was to determine the effects of annexin family (ANXA1 and ANXA2) in periodontal disease. Methods: Healthy participants (n:25) and stage III, grade B periodontitis (n:25) patients enrolled for this study. Clinical periodontal parameters and the periodontal inflamed surface area (PISA) levels were noted. Serum, saliva, and gingival crevicular fluid (GCF) samples were collected to measure the ANXA1, ANXA2 and IL-1β levels. Results: Salivary and serum concentrations of ANXA1 was significantly lower in the periodontitis group than in the control group (respectively, $p = 0.0177$ and $p = < 0.0001$). Periodontitis patients demonstrated higher serum ANXA2 and IL-1β concentrations compared to controls (respectively, $p = 0.0002$ and $p = 0.0017$). As an inflammatory index; saliva, serum and GCF ANXA1/ANXA2 ratio were significantly lower in the periodontitis group compared to healthy controls. Conclusions: The data suggest that periodontitis is associated with a disruption of the balance between pro-inflammatory mechanisms (ANXA2 and IL-1beta) and inflammation resolution (ANXA1), in parallel with PISA levels. Clinical Trial Registration: ClinicalTrials.gov NCT06554756 (15/08/2024).

Keywords Periodontitis, Annexin, Cytokine

Annexins are proteins that respond to calcium ions, binding to phospholipids in cell membranes to connect calcium signaling with membrane functions, thereby creating networks on the surface of the membrane. Annexin-1 (ANXA1) is a 37-k Da protein, belongs to the annexin superfamily[1]. It plays various roles in biological processes such as regulating vesicle trafficking, and cellular structure[2]. Activation of ANXA1 leads to inhibition of neutrophil and monocyte adhesion to the endothelium, among other anti-inflammatory effects[2]. For a while, ANXA1 has been proposed as an intrinsic inhibitor of pro-inflammatory agents, such as IL-1β, a cytokine capable of direct cellular action or triggering the secretion of other pro-inflammatory cytokines[3]. Another study showed that ANXA1 plays a crucial role in suppressing the expression of cytokines in macrophages, such as IL-6 and TNF, and its anti-inflammatory effects are achieved via GILZ (Glucocorticoid-Induced Leucine Zipper)[4]. Similar to its counterparts in the annexin family, annexin A2 (ANXA2) is a versatile protein involved in a range of cellular processes, including cell motility, endocytosis, fibrinolysis, ion channel formation, and interactions with the cell matrix[5,6]. In the presence of inflammation, ANXA2 accelerates the migration of monocytes and macrophages to the site of inflammation and induces the chemotaxis of neutrophils[7,8]. Furthermore, a different study showed that ANXA2 tetramer stimulates the release of many chemokines, including TNF-alpha, IL-1 β, and IL-6, which can activate different kinds of white blood cells[9]. It has been shown that the decrease in ANXA1 production during inflammatory bone diseases can promote the progression of inflammation[10]. A study conducted on patients with rheumatoid arthritis demonstrated that the levels of ANXA2 in the serum, synovial fluid, and tissue samples of these patients were higher compared to healthy individuals[11].

Periodontal disease is the progressive degeneration of the periodontal complex's soft and hard tissues, which is caused by dysbiotic microbial populations and aberrant immune responses[12]. For instance, osteoclastic resorption in periodontitis has been linked to proinflammatory cytokines such as TNF-alpha, IL-1 family, IL-6, and IL-17[13]. While these cytokines and various mediators increase local inflammation and cause periodontal tissue destruction, they can also pass into the systemic circulation and trigger other inflammatory processes in the body. Reports show that periodontal disease can be linked to many serious health problems, such as

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cardiovascular disease, diabetes, and even some types of cancer[14]. It has been reported that periodontal diseases are more severe in diabetic patients, and periodontal infections can make blood glucose control difficult[14]. The procedures performed as part of periodontal therapy help reduce the pathogenic microbial load, thereby facilitating the resolution of the inflammatory process. Following periodontal therapy, Rabelo et al. have reported that, the production of anti-inflammatory cytokines[15]. These factors promote tissue healing and facilitate the resolution of inflammation[16]. Additionally, several studies have reported that pro-inflammatory mediators (e.g., TNF-α, IL-8, IL-6) in body fluids (serum and GCF) significantly decrease in periodontitis patients after periodontal treatment[17].

Although several molecules, agents or bacteria related to the pathogenesis of periodontal disease and its possible systemic effects have being investigated and tried to be reported as a responsible, there have been a very limited number of studies conducted on ANXA1 and ANXA2, which are members of the annexin family. According to our knowledge, ANXA1 has been only examined in pregnant patients with gingivitis in periodontology field on annexins[18], and a very recent study reported that before any clinical signs of periodontal loss, grandchildren of individuals with periodontal disease showed lower salivary ANXA1 levels compared to healthy individuals[19].

Due to the limited data on this subject, we aimed to assess ANXA1, ANXA2 and IL-1 β in human body fluid samples (serum, gingival crevicular fluid (GCF) and saliva) from individuals with periodontal disease and healthy controls for early diagnosis of periodontal disease and additionally; determine whether there is a relationship between the periodontal inflamed surface area (PISA) and annexin levels. The hypothesis of our study; pro-inflammatory mediators ANXA2 and IL-1β will be found at higher levels in periodontitis patients than in healthy individuals, and ANXA1 will have an opposite effect to these molecules.

Materials and methods

Subjects and diagnosis

The present study was approved by the human subject ethics board of Istanbul Medipol University (date: 01.08.2023; Number: 635) for the use and access of human subjects in research and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013. Voluntary participants were included in the study. The informed consent form intended for this study was signed by each participant.

Sample size analysis was performed a priori, using specific software (3.1.9.2 G*Power; https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/ gpower.html). 2 separate papers were tried to be selected for references for power analysis in our study (before), it was observed that these studies were not appropriate because the reasons (1) there was no mean ± SD for concentrations[19] and (2) protein modulation was added to the analysis[18]. Total of 48 subjects are necessary to provide a statistical power (1–β) of 0.9 and a type I error rate of 0.05 according to the clinical differences[20]. Therefore, the study was designed to include 50 patients. Group 1 was the control group with a healthy periodontium($n=25$) and Group 2 was the Stage III Grade B generalized periodontitis($n=25$). Every individual involved in this research was systemically healthy. The exclusion criteria were as follows: the use of antibiotics and/or anti-inflammatory nonsteroidal anti-inflammatory drugs, steroids, immunosuppressants, beta-blockers, calcium channel blockers, anticoagulants, and hormonal contraceptives within 3 months preceding the study; having nonsurgical periodontal treatment (previous 6 mo); having surgical periodontal treatment (previous 12 mo); having less than 20 natural teeth excluding the third molars; and having a diabetes diagnosis, rheumatoid arthritis diagnosis, or pregnancy, lactation, or systemic conditions, smokers. All measurements were performed using a periodontal probe with William's markings by two calibrated periodontists (MY, NB). Clinical and radiographic criteria were used to make the clinical diagnosis of periodontitis and a healthy periodontium, as previously mentioned[21]. A healthy periodontium was characterized as having a pocket probing depth (PD) of ≤3 mm and <10% bleeding sites[22]. The clinical diagnostic of patients' periodontal state was categorized in accordance with the 2018 World Workshop, as previously mentioned[21]. Individuals with a evident interdental clinical attachment level (CAL) at least two non-adjacent teeth were classified as having periodontitis. Interdental CAL at the side of maximum loss was measured for each tooth, and CAL ≥ 5 mm was classified as stage III periodontitis. Patients were graded based on the bone loss/age index as Grade B (index score between 0.25 and1.00). The clinical periodontal parameters of the plaque index (PI), PD, CAL, and bleeding on probing (BOP) were documented. PISA scores, which are presented in millimeter-squared, served as a summary of the periodontal exam results. An EXEL sheet from Nesse's publication is used to calculate PISA based on PD and BOP[23].

Saliva, serum and GCF sampling

After an overnight fast, unstimulated saliva samples were taken from each patient between 9:00 and 11:00 in the morning in order to assess the levels of the chosen markers in the saliva. Saliva and blood samples were collected as applied in our previous study[24]. GCF samples were taken from the vestibular, mesial, and distal surfaces of a single-rooted tooth with standard paper strips (Periopaper, Oraflow Inc., Plainview, NY, USA). Periodontal parameters were noted prior to GCF sample, and a sterile curette was used to remove the supragingival plaque. Paper strips were placed into the crevice and allowed to remain there for 30 s after the cotton roll had isolated the teeth that were to be sampled. Samples contaminated with blood and saliva were eliminated. GFC volumes were determined using a calibrated tool (Periotron 8000, Oraflow Inc., Plainview, NY, USA). Next, each sample was put into an eppendorf tube.(*20*) All samples were then stored at − 80 °C until analysis.

Laboratory determinations

ANXA-1, ANXA2, IL-1 β in collected samples were determined by ELISA kits (SUNRED, cat 201-12-3158, 201-12-1089, 201-12-0144; respectively) and analyzed according to manufacturers' instructions, with colorimetric

assessment performed using a microplate reader at 450 nm. The respective assay standard curve was used to calculate concentrations. Every sample was examined twice, and the average was used in subsequent calculations.

Statistical analysis

Statistical calculations were performed using the GraphPad Prism version 10 statistical software packages. Shapiro-Wilk test was applied to determine data normality. When intergroup comparisons, student's t test was used to analyze normally distributed data while Mann Whitney U was used to analyze non-normally distributed data. Spearman correlation test was used to evaluate the correlation between all markers, clinical periodontal parameters, and PISA levels. To confirm sample size analysis mentioned at material and method section in detail, post hoc power analysis was performed according to ANXA levels after our study, the power calculation was found to be 93%. It was observed that there was no problem for sample size in our study. The ROC curve was employed to distinguish between periodontitis and healthy subjects, and evaluation of the area under the curve (AUC) for selected biomarkers was also conducted. All tests were performed at a significance level of $\alpha = 0.05$.

Results

The study population's demographic and clinical periodontal parameters are reported in Table 1. Briefly, clinical periodontal parameters (PD, BOP, CAL, PI) and PISA levels were significantly higher in the periodontitis group (P) than in the control (C) ($p < 0.001$). Periodontal pockets were measured from a total of 2802 sites in 25 periodontitis patients participating in the study. 497 of the 2802 sites had a pocket depth of more than 4 mm (17.73%). In addition, the mean of PD measured more than 4 mm was 5.07 ± 0.42. Age or sex differences were not statistically significant among the groups.

Salivary and serum concentrations of ANXA1 was lower in the periodontitis group than in the control group (respectively, $p = 0.0177$ and $p = < 0.0001$), and this difference was statistically significant (Fig. 1). Periodontitis patients demonstrated higher serum ANXA2 and IL-1β concentrations compared to controls (respectively, $p = 0.0002$ and $p = 0.0017$). In the P group, GCF ANXA2 and IL-1β levels were significantly higher than those in the C group (respectively, $p = 0.0127$, $p = 0.0005$) (Fig. 1). As an inflammatory index; saliva, serum and GCF ANXA1/ANXA2 ratio were significantly lower in the periodontitis group compared to healthy controls. (Fig. 2)

	C n=25	P n=25	*p*
Age (year)	37.2 ± 7.23	42.3 ± 6.1	0,1075
Gender (F/M)	13/12	11/14	0,777
PD (mm)	1.36 (IQR 1.21 – 1.68)	3.15 (IQR 2.91 – 3.55)	**<0,0001**
BOP (%)	4.7 ± 2.09	58.19 ± 16.06	**<0,0001**
CAL (mm)	1.36 (IQR 1.21 – 1.68)	3.51 (IQR 3.32 – 4.20)	**<0,0001**
PI	0.86 (IQR 0.82 - 0.00)	2.36 (IQR 2.28 – 2.50)	**<0,0001**
GCF (µL)	21 ± 19.8	97 ± 34	**0,0001**
PISA (mm^2)	51.67 ± 7.09	449.13 ± 62.41	**<0,0001**
PD> 4 mm in periodontitis (mm)	-	5.07 ± 0.42	-
Sites> 4 mm in periodontitis (%)	-	17.73	-

Table 1. Demographic and clinical results of periodontitis and control groups. C, group of periodontally and systemically healthy volunteers; P, group of systemically healthy patients with stage III Grade B generalized periodontitis; PI, plaque index; PD, probing depth; BOP, bleeding on probing; CAL, clinical attachment level. Normally distributed data are expressed as mean ± SD. Non-normally distributed data are expressed as interquartile range (IQR). Statistically significant difference with control group are indicated in bold ($p < 0.05$).

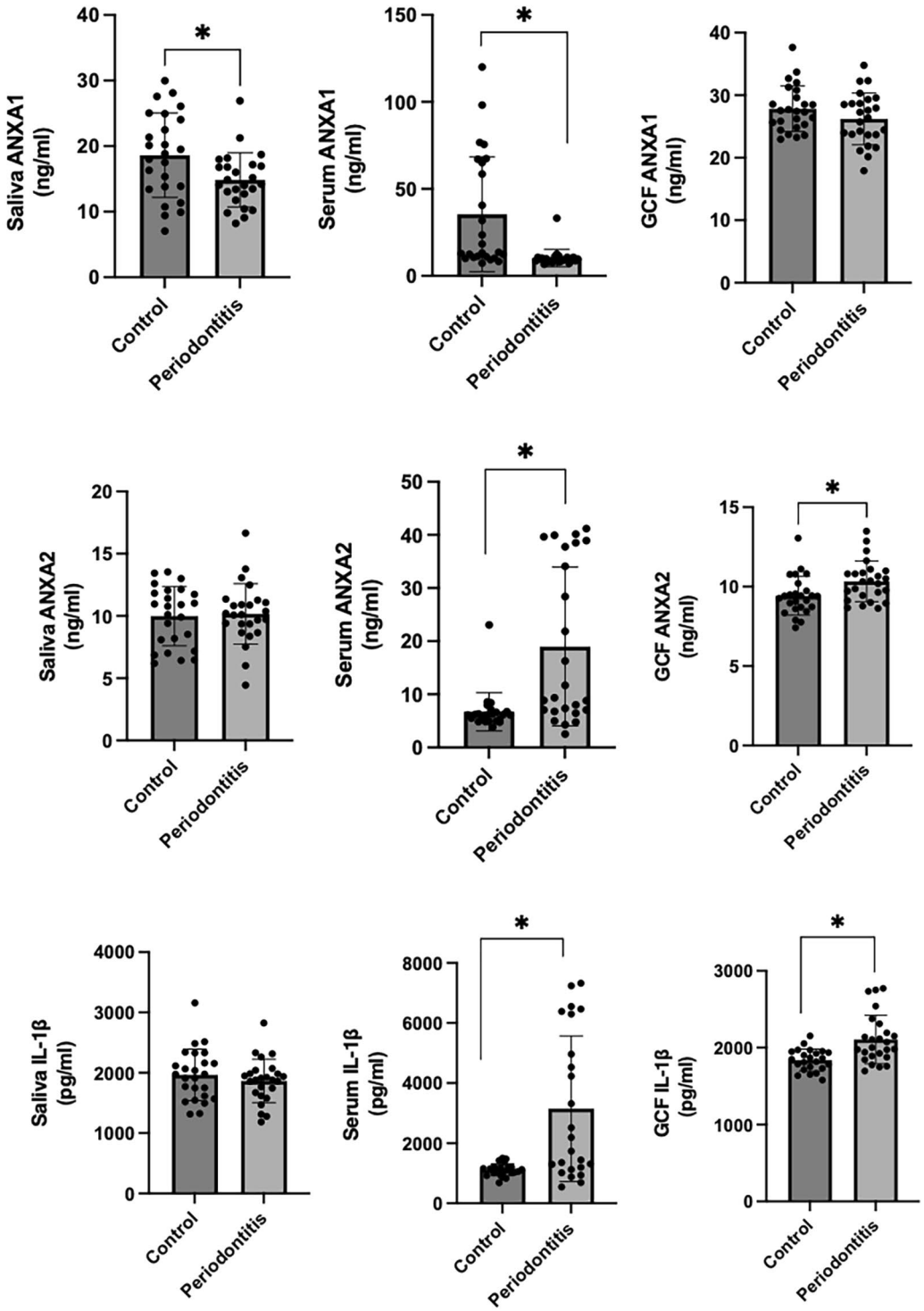

Fig. 1. ANXA1, ANXA2, and IL-1β levels in saliva, serum, and GCF samples from periodontitis and healthy participants.

Table 2 shows the correlation between annexin levels and clinical periodontal parameters for all patients. Briefly, serum ANXA1 was negatively and ANXA2 positively correlated with all clinical parameters except the bleeding on probing. There was a negative low degree of correlation between serum ANXA1 and ANXA2 (r= -0.282, $p<0.05$). GCF ANXA2 was positively correlated with PI and BOP (respectively, $r=0.155$, $r=0.033$, $p<0.05$). PISA was significantly correlated with PI ($r=0.523$, $p<0.05$), BOP ($r=0.098$, $p<0.05$), serum ANXA2 ($r=0.439$, $p<0.05$), GCF ANXA2 ($r=0.366$, $p<0.05$), serum IL-1β ($r=0.441$, $p<0.05$) while negatively

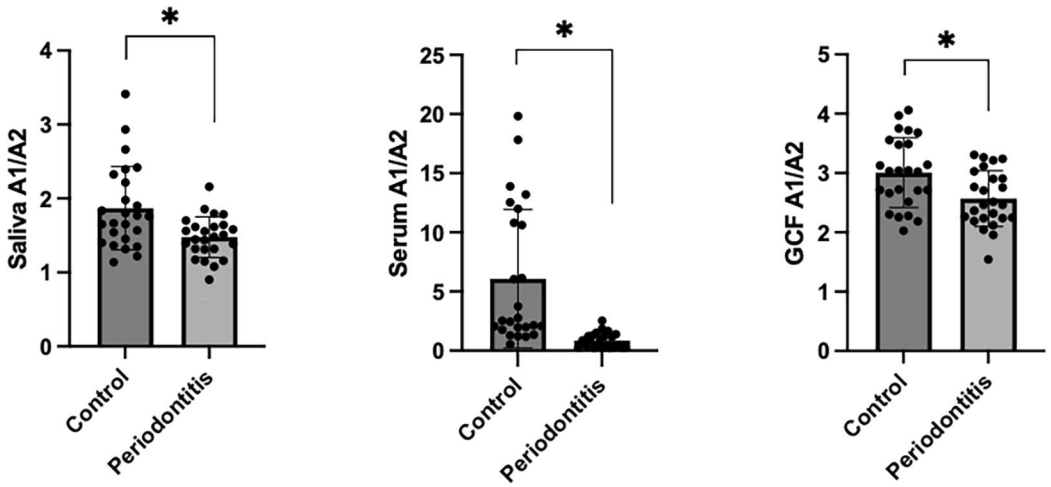

Fig. 2. Saliva, serum and GCF ANXA1/ANXA2 ratio of periodontitis and healthy participants.

Variables	PI.ORT	BOP	PD	CAL	Saliva ANXA1	Serum ANXA1	GCF ANXA1	Serum ANXA2	Saliva ANXA2	GCF ANXA2	Serum IL-1β	Saliva IL-1β	GCF IL-1β
Saliva ANXA1	-0,105	0,239	-0,032	-0,058									
Serum ANXA1	**-0,425**	0,202	**-0,354**	**-0,352**	0,111								
GCF ANXA1	-0,197	0,075	**-0,305**	**-0,314**	-0,032	0,245							
Serum ANXA2	**0,419**	-0,060	**0,425**	**0,395**	-0,108	**-0,282**	-0,105						
Saliva ANXA2	-0,152	0,042	0,014	-0,017	0,598	0,0480	-0,155	0,015					
GCF ANXA2	**0,155**	**0,033**	0,235	0,254	-0,110	-0,020	-0,126	0,089	0,037				
Ser IL-1β	**0,392**	-0,052	**0,491**	**0,464**	-0,115	-0,277	-0,116	0,988	0,026	0,028			
Saliva IL-1β	-0,116	0,155	0,051	0,011	0,756	0,110	-0,107	0,059	0,699	-0,052	0,060		
GCF IL-1β	0,106	-0,150	0,260	0,224	-0,147	-0,209	-0,013	0,155	0,099	0,109	0,147	0,037	
PISA	**0,523**	**0,098**	0,662	0,648	-0,312	**-0,474**	-0,213	**0,439**	0,071	**0,366**	**0,441**	-0,112	0,503

Table 2. Correlations between biomarkers and periodontal clinical parameters (Spearman correlation coefficients, r). PI, plaque index; PD, probing depth; BOP, bleeding on probing; CAL, clinical attachment level. Spearman correlation test. Statistically significant difference with control group are indicated in bold ($p < 0.05$).

correlated with serum ANXA1 (r = -0.474, $p < 0.05$). PISA shows moderate correlation with other variables except GCF ANXA2.

In order to determine which of the three biochemicals is a better predictor of the course of the disease and whether these biochemicals may be used as biomarkers for periodontitis, receiver operating characteristic (ROC) analyses were performed. Figure 3; Table 3 illustrate the ROC analysis results. None of the aforementioned biomarkers in saliva showed statistically significant diagnostic accuracy in differentiating between periodontitis patients and controls except for ANXA1 (sensitivity = 0.92, specificity = 0.52, cutoff value ≤ 18.7, AUC = 0.682). ANXA1, ANXA2, and IL-1β in serum showed statistically significant diagnostic accuracy in differentiation between periodontitis and controls. (Respectively; sensitivity = 0.84, specificity = 0.81, cutoff value ≤ 10.7, AUC = 0.851; sensitivity = 0.80, specificity = 0.80, cutoff value > 6.7, AUC = 0.808; sensitivity = 0.70, specificity = 0.83, cutoff value > 1212.08, AUC = 0.759). In GCF, ANXA2 was found the most accurate biomarker in differentiating between periodontitis patients and controls (sensitivity = 0.72, specificity = 0.72, cutoff value > 9.55, AUC = 0.714).

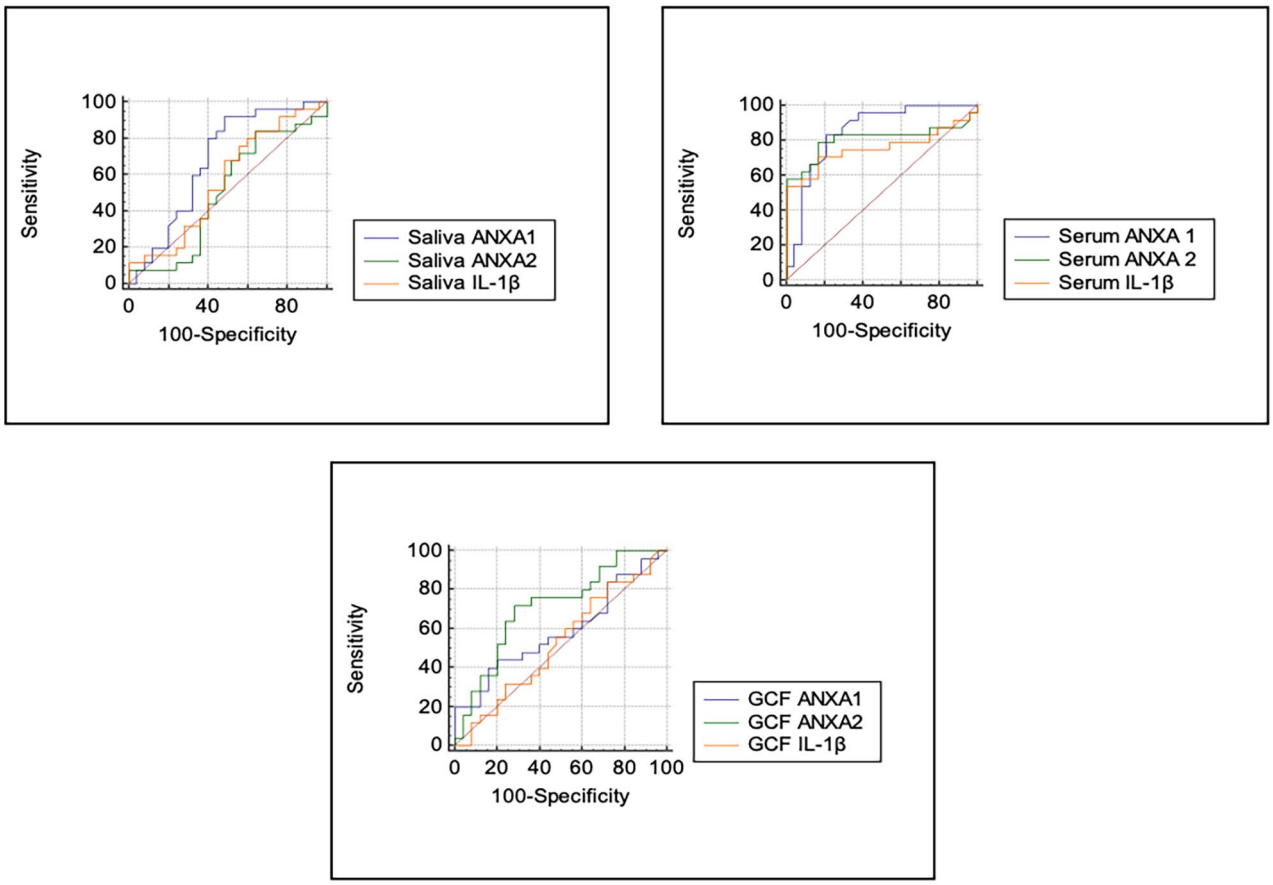

Fig. 3. Receiver operating characteristic (ROC) analysis of serum, saliva and GCF ANXA1, ANXA2 and IL-1β in healthy controls and periodontitis patients.

Discussion

A complicated immune-inflammatory disease known as periodontitis is characterized by alveolar bone loss, which frequently leads to tooth loss, and disruption of the periodontal ligament, that creates periodontal pockets[12]. In this process, increased neutrophil infiltration and macrophage migration are prominently observed at the cellular level, and the activation of these cells plays a critical role in the pathogenesis of the disease[12]. It has been shown that ANXA1 has an anti-inflammatory effect and prevents neutrophil and monocyte adhesion during inflammation, conversely ANXA2 induces the migration of macrophages and neutrophils to the diseased area[2,7]. To best our knowledge, there is no study in the literature that evaluates the role of ANXA1 and ANXA2 together in periodontal disease. For this purpose, this study investigated the levels of ANXA1, ANXA2 and IL-1β in GCF, serum, and saliva during periodontal disease and their relationship with PISA. For the periodontitis group, participants with stage 3 periodontitis, representing an advanced stage of the disease but offering a more balanced environment to assess the systemic effects of periodontal disease and understand the damage to local tissues, were targeted. Additionally, participants with grade B periodontitis were selected to avoid deviations in actual results due to modifying factors (such as smoking or diabetes), ensuring the disease remains active but less influenced by these factors.

Inflammation of the periodontal tissues is thought to be a risk factor for systemic inflammation and disease at distant organs. For this reason, in 2008, a new classification was developed, systemic inflammatory burden is determined by the quantity of inflamed periodontal tissue[23]. This classification is based on a symptom of periodontal disease activity such as bleeding on probing, in addition to linear metrics like clinical attachment loss (CAL) and gingival recession (GR). Therefore, PISA represents the bleeding pocket epithelium's surface area in square millimeters (mm^2)[23]. Early diagnosis and prevention of periodontal disease are important due to the burden it places on the healthcare system and its impact on individuals' systemic health. Therefore, evaluating potential biomarkers from easily obtainable body fluids in the clinic before destruction occurs in oral tissues will enable early diagnosis and treatment.

According to our results, individuals affected by periodontal disease showed lower levels of ANXA1 in both saliva and serum compared to healthy participants. Conversely, ANXA2 levels were found to be significantly elevated in the serum and GCF in the periodontitis group. ANXA1 contributes to the reduction of inflammation by suppressing inflammatory responses, while ANXA2 generally stimulates pro-inflammatory signaling. The results of our study demonstrate contrasting interactions between these two proteins in a manner consistent

Saliva	AUC	Std. Error	p	95% Confidence Interval	
				Lower Bound	Upper Bound
ANXA1	0.682	0.08	0.027*	0.536	0.807
ANXA2	0.508	0.09	0.923	0.363	0.652
IL-1β	0.571	0.08	0.388	0.423	0.710
ANXA1/ANXA2 p	0.012*				
ANXA1/ IL-1 β p	0.074				
ANXA2/ IL-1 β p	0.380				
Serum					
ANXA1	0.851	0.06	0.001*	0.718	0.937
ANXA2	0.808	0.07	0.001*	0.669	0.907
IL-1β	0.759	0.08	0.002*	0.614	0.870
ANXA1/ANXA2 p	0.647				
ANXA1/ IL-1 β p	0.348				
ANXA2/ IL-1 β p	0.163				
GCF					
ANXA1	0.590	0.08	0.277	0.442	0.727
ANXA2	0.714	0.07	0.010*	0.568	0.833
IL-1β	0.526	0.08	0.749	0.380	0.669
ANXA1/ANXA2 p	0.248				
ANXA1/ IL-1 β p	0.488				
ANXA2/ IL-1 β p	0.073				

Table 3. Receiver operating characteristic (ROC) analysis showing serum, saliva and GCF ANXA1, ANXA2 and IL-1β in healthy controls and periodontitis patients. Significantly different values are marked as * ($P < 0.05$).

with existing literature, thereby supporting their roles. In addition, considering that ANXA1 and ANXA2 are predominantly serum derived[2,6], the changing levels of these molecules in serum in periodontitis patients indicate the systemic effect of periodontal disease. (Fig. 1)

The main strength of the present study is the simultaneous determination of serum, saliva, and GCF ANXA1, ANXA2, IL-1β levels. This approach provided an opportunity to observe annexin family members roles on periodontitis progress in a systemic and local manners from serum to GCF. Examining the levels of annexins, which plays roles in cellular processes, monocyte migration, inflammatory response allowed us to illuminate the missing parts of the mechanisms by which periodontal disease development. In addition, measuring the ANXA1/ANXA2 ratio as an inflammatory index has become an important indicator in explaining which mechanism (inhibition of neutrophil and monocyte adhesion/macrophage and monocyte migration) is more dominant in the periodontal disease process. Although there was no significant difference between the groups in sociodemographic data in our study, the fact that the effect of cofounding factors such as age and sex on the relationship between periodontal disease and annexin levels may cause bias in this study design can be considered as a limitation of the study. Several studies showed that dysregulation in human ANXA1 levels is linked to atherosclerosis, rheumatoid arthritis and inflammatory bowel disease. Additionally, it has been reported that ANXA1 levels in gingival crevicular fluid are five fold higher in healthy individuals compared to those with

periodontal disease[25]. Casarin et al. indicated that descendants of periodontitis-affected patients showed a lower abundance of saliva ANXA1 than periodontally healthy individuals, before any clinical evidence of periodontal loss[19]. In our study results, serum and saliva levels of ANXA1 were significantly lower in periodontitis group compared to health individuals. Studies emphasize that ANXA1 has anti-inflammatory effects and is associated with health, and our results also parallel with the literature. Moreover, the negative correlation of serum and GCF ANXA1 levels with clinical periodontal parameters supports the association of this molecule with health. It has been shown that ANXA1 plays a role in the resolution of inflammation and anti-inflammatory effects by activating pathways such as Fpr2/ALX signaling axis and p38/MAPK/IL-10[26,27]. Casarin et al. reported that they identified the p38/MAPK pathways in saliva samples of periodontal healthy individuals[19]. When evaluating the importance of resolving inflammation in the pathogenesis of periodontal disease, it is recognized that a deficiency in these pathways can predispose individuals to periodontal disease. The lower levels of salivary ANXA1, particularly indicative of local inflammatory response, in individuals with periodontal disease compared to healthy individuals, may be interpreted as a deficiency in resolving inflammation, contributing to the development of periodontal disease.

The expression of ANXA2 is highly variable among many tissues and cells, with monocytes and macrophages exhibiting the highest levels of expression[5,6]. Studies indicates that extracellular ANXA2 binds to TLR4 receptors on macrophage surface, stimulating the production of TNF-α, IL-1β, and IL-6[9,28]. Pan et al. reported that ANXA2 triggers the inflammatory response by inducing STAT3 phosphorylation[29]. When the levels of ANXA1, ANXA2, IL-1β, IL6, and TNF-α in the serum samples of individuals with Covid-19 disease were evaluated, it was reported that ANXA2 and other pro-inflammatory mediators showed a positive correlation with the severity of the disease, while ANXA1 showed an opposite effect[30]. Lin et al. reported increased levels of ANXA2 in serum samples of people with coronary heart disease[31]. In our study, serum and GCF ANXA2 levels in the periodontitis group, which is a chronic inflammatory disease, were significantly higher than in healthy individuals. Additionally; IL-1β, which has been showed to have pro-inflammatory effects in periodontal disease, was also found to be elevated in the serum and GCF samples of the periodontitis group compared to healthy individuals, similar to ANXA2. These results indicate that ANXA2 is involved in the induction of inflammation in periodontal disease. Given that STAT3 activation increases bone resorption in periodontal disease, the induction of STAT3 by ANXA2 suggests that ANXA2 has a destructive effect in the pathogenesis of periodontal disease[32]. ANXA2 also activates the NF-κB signaling pathway, leading to the release of pro-inflammatory mediators such as IL-1β and TNF-α[33]. This signaling pathway, which can also be activated in periodontal disease, may be induced by the effect of ANXA2, potentially leading to an exacerbation of the periodontal disease[34]. Gingival crevicular fluid (GCF), being in close proximity to the tissues where periodontal disease initiates, holds great promise as a valuable source of information for diagnosing periodontal diseases. Additionally, its ease of collection and non-invasive nature make it particularly attractive as a diagnostic method. Therefore, the high level of GCF ANXA2 in the periodontitis group indicates the local effect of ANXA2 and suggests that it can be used as a diagnostic marker of periodontal disease. Additionally, the demonstration that GCF ANXA2 can be used to distinguish periodontitis and healthy individuals according to ROC analysis supports this interpretation. (Table 3)

In our study, we also calculated the ratio between ANXA1 and ANXA2 as an inflammatory index. The ANXA1/ANXA2 ratio of serum, saliva and GCF samples was significantly lower in the periodontitis group than in the control group. Considering ANXA1's anti-inflammatory nature and its association with health, and ANXA2's inflammation-triggering mechanism, these results are not surprising. Similarly, in line with the aforementioned COVID-19 study, our study also found that serum ANXA1 levels were higher in healthy participants, whereas ANXA2 levels were higher in the periodontitis group[30]. Additionally, the significant negative correlation between serum ANXA1 and ANXA2 further supports the opposing effects of these two molecules.

According to our results, the periodontitis group exhibits significantly higher PISA levels than the control group. This result is not surprising since PISA is a value that shows the activity of inflammation. In a study conducted by Leira et al. in 2017, it was reported that individuals with a PISA value ≥ 130.33 mm^2 could be classified as having periodontitis[35]. The fact that the PISA average of our periodontitis group was 449.13 can be interpreted as the general group having moderate inflammation. Among our findings that support each other is that the inflammation in periodontitis patients is moderate and that inflammatory mediators are also more reasonable in parallel. In addition to the positive correlation between PISA and serum ANXA2 and IL-1β, the negative correlation with ANXA1 further supports the suggestion that PISA can be used as an indicator of systemic inflammation. Furthermore, the positive correlation between PISA levels and clinical periodontal parameters (BOP and PI) and GCF ANXA2 also demonstrates the impact of local inflammation caused by periodontal disease. Our study's data shed light on the imbalance between pro-inflammatory mechanisms (ANXA2 and IL-1β) and the resolution of inflammation (ANXA1) associated with periodontal disease.

Conclusion

In the current study, the findings have underlined the importance of proinflammatory (ANXA2 and IL-1β) and anti-inflammatory mechanisms (ANXA1) in periodontitis and their correlation with and PISA. Additionally, together with these results, lower levels of the ANXA1/ANXA2 ratio in periodontitis group compared to healthy controls may suggest that the effects of inflammatory cell migration are higher in the periodontitis. Detection and monitoring of GCF ANXA2 and additionally saliva, serum, GCF ANXA1/ANXA2 levels might be potential indicators of predict the susceptibility for periodontitis in the future with more precisely defined testing method and large-cohort studies with a longitudinal design.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Author contributions

MY contributed to the design of the study, recorded the clinical periodontal parameters, performed biochemical analysis and wrote the manuscript with input from other authors. IB and SH collected the blood, GCF and

saliva samples. NB contributed to the design of the study, wrote the manuscript with input from other authors, performed biochemical analysis and recorded the clinical periodontal parameters. ET contributed to the design of the study, performed statistical analysis, helped interpret the results. HT contributed to the design of the study and helped interpret the results. All authors reviewed and approved the submitted manuscript.

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Declarations

Ethics approval and consent to participate
All subjects were signed the informed consent from designed for this study. The present study was approved by the human subject ethics board of Istanbul Medipol University (date: 11.08.2023; Number: 635) for the use and access of human subjects in research and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013.

Competing interests
The authors declare no competing interests.

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