SALIVARY BIOMARKERS IN THE CONTEXT OF GINGIVAL INFLAMMATION IN CHILDREN WITH CYSTIC FIBROSIS

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One-sentence summary: Children with cystic fibrosis had poorer gingival health as reflected by higher gingival bleeding scores and an altered salivary biomarker profile, especially in calprotectin levels, that correlated with systemic inflammatory markers.

Authors contribution

Zeynep Pinar Keles Yucel and Angelika Silbereisen contributed to data acquisition, interpretation, drafted and critically revised the manuscript. Gulnur Emingil contributed to design, data interpretation, and critically revised the manuscript. Yavuz Tokgoz contributed to data acquisition, interpretation and critically revised the manuscript. Timur Kose contributed to data analysis, interpretation and critically revised the manuscript. Timo Sorsa and Georgios Tsilingaridis contributed to data interpretation and critically revised the manuscript. Nagihan Bostanci contributed to conception and design, data analysis and interpretation and drafted and critically revised the manuscript. All authors gave final approval and agreed to be accountable for all aspects of the work.

Footnotes

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Abbreviations: CF, Cystic fibrosis; TREM-1, Triggering receptor expressed on myeloid cells 1; PGLYRP1, peptidoglycan recognition protein 1; GI, gingival index; PI, plaque index; BOP, bleeding on probing; CFTR, cystic fibrosis transmembrane conductance regulator; TNF, tumor necrosis factor; IL, interleukin; CRP, C-reactive protein; MCV, mean cell volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; RDW, red cell distribution width; PCT, procalcitonin; MPV, mean platelet volume; C, control group; PPD, probing pocket depth; H, periodontally healthy; G, gingivitis; SNs, supernatants; ELISA, enzyme-linked immunosorbent assay; RT, room temperature; TMB, tetramethylbenzidine; GCF, gingival crevicular fluid

ABSTRACT

Background: Cystic fibrosis (CF) is a life-threatening chronic inflammatory disease in children due to respiratory complications. Saliva could serve as reservoir of bacterial colonization and potentially reflect systemic inflammation. This study investigated whether salivary triggering receptor expressed on myeloid cells 1 (TREM-1), peptidoglycan recognition protein 1 (PGLYRP1), interleukin (IL)-1β and calprotectin are associated with CF or reflect concomitant gingival inflammation.

Methods: Ten CF (age:3-12yrs) and ten systemically healthy age-and-gender-matched children (C) were enrolled in the study. Individuals with CF underwent routine laboratory determinations. Probing pocket depth (PPD), gingival index (GI), plaque index (PI) and bleeding on probing (BOP) were recorded on fully erupted teeth and saliva samples collected. Salivary TREM-1, PGLYRP1, IL-1β and calprotectin were analysed by ELISA.

Results: Children with CF had significantly higher BOP scores (P=0.001) and calprotectin levels (P=0.017) compared to the C group. TREM-1, PGLYRP1 and IL-1β could not distinguish between CF and SH but showed positive correlation with GI, PI and BOP in both groups. Calprotectin levels positively correlated with procalcitonin (P=0.014), thrombocyte counts (P=0.001), mean platelet volume (P=0.030) and with PGLYRP1 (P=0.019) and IL-1β (P=0.013) in CF children. Receiver operating characteristic curve analysis for calprotectin (CFvsC) showed an area under the curve of 0.79 (95% CI 0.58-0.99, P=0.034).

Conclusions: CF children presented with higher gingival inflammation scores and salivary calprotectin levels, that correlated with systemic inflammatory markers. Salivary calprotectin levels were not associated with periodontal parameters. Hence, preliminary data demonstrate that salivary calprotectin might have a chairside diagnostic potential for CF in children.

Keywords: Calprotectin, TREM-1 protein, biomarkers, cystic fibrosis, gingivitis, saliva

1. Introduction

Cystic fibrosis (CF), a life-threatening chronic inflammatory disease, is linked to a mutation in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein[1]. In CF children, the most significant reason of morbidity or mortality is due to persistent bacterial colonization of the lung and nasal mucosa[2]. However, the mechanisms that allow persistent colonization of pathogens are not well understood. The oral cavity harbours more than 700 species and has therefore been suggested as one of the potential reservoirs[1]. The association between CF and caries risk has been established in young children, but relatively less information exists on its association with periodontal inflammation[3-6]. Although children with CF are reported to have a higher prevalence of calculus due to deregulated calcium and phosphate levels in their saliva[3], it is not conclusive if other salivary factors are associated with and/or reflect CF. There is an increasing evidence that expression of triggering receptor expressed on myeloid cells 1 (TREM-1), along with its putative ligand peptidoglycan recognition protein 1 (PGLYRP1), is significantly increased in saliva in the presence of chronic inflammatory diseases including periodontal disease[7,8]. Activation of TREM-1 triggers amplification of proinflammatory cytokine production such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β[7], which in return can help to eliminate pathogens. Interestingly, TREM-1 has been shown to be down-regulated in monocytic cells from patients with CF or was not detectable at all in their serum[9]. However, a small pilot study from 2017[10] showed significantly elevated TREM-1 levels in plasma of CF patients. PGLYRP1, recently identified as a ligand for TREM-1, is an antimicrobial peptide expressed in neutrophils[11]. Earlier work indicates that PGLYRP1 is involved in exacerbation of airway inflammation and the development of allergic asthma[12], yet its association with CF is unclear. Another characteristic of patients with CF is increased levels

of CF antigen or namely calprotectin in their plasma, sputum and, to a lesser extent in their saliva[13,14]. Nonetheless, the regulation of TREM-1, PGYLRP1 and calprotectin in saliva of CF patients in the presence or absence of gingival inflammation has not been studied so far. Therefore, in the present study we investigated whether salivary TREM-1, PGYLRP1 and calprotectin are associated with CF or are rather a result of or reflect concomitant gingival inflammation.

2. Materials and Methods

2.1. Study Population

Ten children with CF aged 3 to 12 years old followed by the Department of Pediatric Gastroenterology, Faculty of Medicine, Adnan Menderes University, Aydın, Turkey from May 2016 until April 2018 were recruited for this study. CF patients having no other systemic disease, who did not need a lung transplantation and were clinically and symptomatically stable, free of acute respiratory infections for at least 4 weeks were included. Their routine clinical tests included the following measurements: C-reactive protein (CRP), haemoglobin, haematocrit, mean cell volume (MCV), erythrocyte, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), leukocyte, neutrophil, lymphocyte, monocyte, eosinophil, basophil, thrombocyte, procalcitonin (PCT), mean platelet volume (MPV). Patients were excluded who had been administered probiotics or anti-inflammatory drugs or were on the waiting list for lung transplantation or had pulmonary exacerbation and used oral or intravenous antibiotics within the past four months[15]. Ten systemically healthy age and sex matched children who visited the Faculty of Dentistry, Adnan Menderes University, Aydın, Turkey for dental check-ups were enrolled as a control group (C). A history of periodontal treatment, use of antibiotic or other anti-

inflammatory drugs within the past four months, having caries or less than ten fully-erupted teeth were criteria for exclusion. The Ethics Committee of Ege University approved the study protocols following the ethical principles stated in the World Medical Association Declaration of Helsinki (Protocol Number: 18-9.1/30). The design and the aim of this study were explained in detail prior to the clinical periodontal evaluation and informed consent was obtained from all participants (parents (in writing) and children (orally)).

2.2. Clinical Assessment

Full-mouth periodontal evaluation and radiographic examination were performed for all children (CF and control (C) group) to define their periodontal status. Clinical periodontal parameters included probing pocket depth (PPD), gingival index (GI)[16], plaque index (PI)[17] and bleeding on probing (BOP)[17]. All measurements were recorded at four sites (mesial, distal, buccal, lingual/palatinal) on each fully erupted tooth[19,20] present by a single calibrated examiner (ZPKY) using a Williams periodontal probe.* Although the clinical attachment level was assessed, data was not presented due to absence of any attachment loss. Calculus formation was also determined by visually assessing the surface of each tooth and noted as present or absent[21]. Children in the periodontally healthy (H) group showed clinically healthy gingiva (GI=0), good oral hygiene, and PPD ≤3 mm with no clinical attachment and radiographic bone loss. Children with GI scores ≥1 and PPD ≤3 mm were defined as gingivitis (G). After completion of the periodontal examination, children were further classified into subgroups: CF and periodontally healthy (CF-H, N=6); CF with gingivitis (CF-G, N=4); systemically healthy and periodontally healthy (C-H, N=5); systemically healthy with gingivitis (C-G, N=5).

2.3. Saliva Sampling

All saliva samples (n=20) were collected in the morning hours (8.00-10.00 am) following overnight fasting one day after the clinical periodontal recordings. Children and their parents were requested not to drink or eat and to omit any oral hygiene procedures including brushing, flossing or mouth rinsing up to two hours prior to sampling. To obtain saliva samples, each participant expectorated for five minutes into sterile 50 mL polypropylene tubes after rinsing their mouth with tap water. Then, samples were centrifuged at 10,000 x g for 15 minutes at 4 °C. The supernatants (SNs) were immediately frozen and stored at -80 °C until further analysis.

2.4. Analysis of TREM-1, PGLYRP1, Calprotectin, IL-1β and Total Protein Levels in Saliva

Salivary TREM-1, PGLYRP1, Calprotectin and IL-1β levels were analysed by commercial enzyme-linked immunosorbent assay (ELISA) kits[†,‡,§,ǁ] according to the manufacturer's instructions and as described before[22]. The TREM-1 ELISA kit is detecting both soluble and membrane-bound TREM-1. Briefly, standards and diluted saliva SNs (TREM-1 (1:3), PGLYRP1 (1:60), Calprotectin (1:2000) and IL-1β (1:20)) were applied to pre-coated (capturing antibodies) 96 well plates (Nunc-Immuno™ MicroWell™ 96 well solid plates, Sigma) and incubated for two hours at room temperature (RT) after washing and blocking the plates. Biotin-conjugated detection antibodies were applied (1h at RT) followed by a 20 minutes incubation with horseradish peroxidase (HRP)-conjugated streptavidin at RT. Tetramethylbenzidine (TMB)-substrate solution was used to develop the assay and the reaction was stopped by applying 2N H_2SO_4. Absorbance was measured at 450 nm (wavelength correction = 540 nm). Finally, to determine the concentrations of TREM-1, PGLYRP1, Calprotectin and IL-1β, a four-parametric logistic standard curve was applied. Total protein levels were measured using the Pierce™ BCA Protein Assay Kit.[¶]

2.5. Statistical Analysis

The distribution of all variables were examined by Shapiro-Wilk normality test. Normality was provided by logarithmic transformation for data that was not normally distributed. Then, 2x2 factorial ANOVA was performed for comparisons of all descriptive variables between groups including the effect of systemic condition and periodontal status and interactions between CF and the periodontal status. As a categorical parameter, sex ratio was evaluated with chi-square test. Correlations between biochemical parameters and clinical periodontal parameters were determined by Pearson and Spearman's rank correlation tests. All analyses were carried out using a statistical software program[#] at $\alpha = 0.05$ significance level. Receiver operating characteristic curves (ROC) were constructed to assess the ability of calprotectin as diagnostic aid. Statistical power calculations were performed for TREM-1 using one-way ANOVA revealing a minimum sample size of 3 for each group to detect differences among the four groups at 3.5 f-type effect size level, with a power of 99% at 0.01 Type 1 error level for TREM-1 (pg/mL)[8].

3. Results

3.1. Demographics and Clinical Findings

Demographic characteristics of CF and systemically healthy (C) children and related subgroups are presented in Table 1. Age and sex showed no differences between CF and C groups as the groups were matched ($P>0.05$). In the subgroups, CF-G and C-G had a higher average age than CF-H and C-H children ($P=0.033$). RDW, neutrophil, lymphocyte and thrombocyte levels were slightly above the normal (reference) range but are considered

normal (non pathological) for CF children, potentially influenced by common components of the disease, such as anemia, chronic infections and malabsorption.

Table 2 shows the clinical periodontal findings of the CF and C groups. PPD, GI and PI were similar in CF and C children (P>0.05) while BOP scores were higher in the CF compared to the C group (P=0.001). Clinical findings for the subgroups are also presented in Table 2. All clinical periodontal parameters were higher in the gingivitis subgroups (CF-G and C-G) compared to the periodontally healthy subgroups (CF-H and C-H) (GI, PI, BOP: P<0.001; PPD: P=0.005). No calculus was detected in both groups.

3.2. Biochemical Findings

Salivary levels of TREM-1, PGLYRP1, IL-1β, calprotectin and total protein were analysed (Table 3). Of the samples, 75% could detect TREM-1, 100% PGLYRP1, 90% IL-1β, 95% calprotectin and 100% total protein with the rest showing values below the detection limit (23.1 pg/mL, 12.5 pg/ mL, 1.8 pg/mL, 36.1 pg/mL and 6.5 μg/mL, respectively). Mean concentrations (± SD, μg/mL) of calprotectin were 15.66±15.30 in CF children and 3.99±4.58 in C children. Higher (by 3.9-fold) calprotectin levels were observed in the CF compared to the C group (P=0.017). No significant differences were observed in salivary levels of TREM-1, PGLYRP1, IL-1β and total protein between the CF and C group (all P>0.05).

The findings of the subgroups are demonstrated in Table 3. Mean salivary concentrations (± SD, pg/mL) of TREM-1 were 49.10±47.64 in the CF-H group, 169.44±123.26 in the CF-G group, 38.04±42.13 in the C-H group and 242.17±141.21 in the C-G group (3.4-fold higher in CF-G compared to CF-H and 6.3-fold higher in C-G compared to C-H). Mean IL-1β concentrations (± SD, pg/mL) in the CF-H, CF-G, C-H and C-G groups were 58.46±55.22, 345.28±269.81, 45.90±38.71 and 279.76±222.75, respectively (5.9-fold

higher in CF-G compared to CF-H and 6.1-fold higher in C-G compared to C-H). Salivary concentrations of TREM-1 and IL-1β were elevated in the CF-G and C-G subgroups compared to the periodontally healthy (CF-H and C-H) subgroups (P=0.003). No significant difference was found in salivary PGLYRP1 and total protein levels among any of the subgroups (P>0.05). CF groups had higher salivary calprotectin levels compared to C groups (P=0.017). However no significant difference in calprotectin levels was observed between the gingivitis subgroups (CF-G and C-G) and the periodontally healthy (CF-H and C-H) subgroups (P>0.05).

The ROC analysis (continuous analysis without threshold) for calprotectin showed an area under the curve (AUC) of 0.79 (95% CI 0.58-0.99, P=0.034) for the CF group compared to the C group.

3.3. Correlation Analysis

Table 4 presents the correlations of clinical periodontal parameters with biochemical findings in CF and C children. In the CF group, concentrations of salivary TREM-1 positively correlated with GI (P=0.009) and BOP (P=0.009), PGLYRP1 positively correlated with GI (P=0.038), BOP (P=0.038), IL-1β (P=0.011) and calprotectin (P=0.019), and IL-1β positively correlated with PI (P=0.022), BOP (P=0.048), PGLYRP1 (P=0.011) and calprotectin (P=0.013). Calprotectin positively correlated with PGLYRP1 (P=0.019) and IL-1β (P=0.013), but did not show an association with any of the clinical periodontal parameters (P>0.05). In the C group, levels of TREM-1 positively correlated with GI (P=0.003), PI (P=0.001), BOP (P=0.001), PGLYRP1 (P=0.043) and IL-1β (P=0.003), PGLYRP1 positively correlated with GI (P=0.048), PI (P=0.029), TREM-1 (P=0.043), IL-1β (P=0.004) and age (P=0.020), and IL-1β positively correlated with GI (P=0.008), PI (P=0.014), BOP (P=0.001), TREM-1 (P=0.003), PGLYRP1 (P=0.004) and age (P=0.001). Calprotectin positively correlated with

total protein levels (P=0.036) but, did not correlate with any clinical periodontal or biochemical parameter (P>0.05).

Correlations of medical, biochemical and clinical periodontal parameters of CF patients are presented in the supplement (see Supplementary Table 1 in online Journal of Periodontology). The primary interest was to investigate whether any of these medical or clinical periodontal parameters correlate with any of the four tested molecules (TREM-1, PGLYRP1, IL-1β, calprotectin). And indeed, salivary calprotectin levels were positively correlated with PCT (P=0.014), MPV (P=0.030) and thrombocyte levels (P=0.001). Salivary PGLYRP1 levels were positively correlated with thrombocyte levels (P=0.033). Furthermore, basophil levels positively correlated with GI (P=0.027) and BOP (P=0.036). All other correlations did not reach to significance (P>0.05).

4. Discussion

To the best of our knowledge the present study investigated, for the first time, the possible link between CF and periodontal disease based on periodontal parameters and biomarkers in saliva to unravel potential inflammatory response patterns. Interestingly, BOP scores were significantly elevated in CF patients compared to systemically healthy controls. This indicates that the systemic inflammation in CF patients can aggravate gingival inflammation and worsen the oral manifestation of periodontal disease. However, other periodontal parameters (PI and PPD) measured in this study did not show significant differences between CF patients and systemically healthy controls. It is known that in CF a mutation in the gene encoding the CFTR protein can negatively affect the salivary glands and the protective role of saliva that thus may result in a hyperinflammatory response with reduced host defence[5,23]. Earlier literature investigating the periodontal conditions in CF

children and adolescents revealed no significant differences in plaque levels[4,5,23] and BOP[4] between children with CF and systemically healthy controls.

We further investigated whether biochemical changes in saliva could support a possible link between CF and gingival inflammation in periodontal disease. Previous studies reported that salivary protein profiles differed between CF patients and healthy individuals[24,25]. Since salivary biomarkers are known to be important for diagnosis and prognosis of various autoimmune and inflammatory disorders including periodontal disease[24], the study of such salivary markers might highlight a possible association between CF and gingival inflammation. In line with earlier reports, the present findings also showed that calprotectin as a marker for CF and present in sputum or serum of CF patients was significantly elevated in CF children compared to systemically healthy controls[13,14]. More interestingly, salivary calprotectin levels were positively correlated with procalcitonin levels, thrombocyte counts and mean platelet volume in CF patients. There is evidence that CF patients have elevated circulating platelet levels and platelet activation[26], which is suggested to be an effect of the CFTR gene mutation. In CF patients, procalcitonin has also been evaluated as a marker of inflammation[27], therefore the observed correlation between calprotectin and procalcitonin in the present study could reflect the ongoing systemic inflammatory responses in CF children. Furthermore, the ROC analysis identified a high discriminating capacity of calprotectin to differentiate between CF and systemically healthy children, supporting salivary calprotectin as a potential marker for the diagnosis of CF in children. Age dependency has been described for faecal calprotectin levels in CF and systemically healthy children below the age of four years[28]. However, since only one child within our study group was below the age of four (CF-H, 3 years of age) and the children in the C group were age and gender matched to the CF children, the age-dependent calprotectin effect in this study should have been controlled by this.

When subdividing the groups based on their periodontal status, calprotectin levels remained higher in CF patients compared to systemically healthy controls (with or without gingivitis) but the differences were no longer significant. A reason for this might be the rather small sample size after subdivision into the four subgroups (4-6 individuals). Different studies have already investigated calprotectin in periodontal disease and identified higher salivary calprotectin levels in experimental gingivitis[29] and higher calprotectin levels in gingival crevicular fluid (GCF) of both periodontitis and gingivitis patients compared to healthy controls[30]. However, a recent study showed no significant differences in calprotectin levels in neither serum nor saliva in gingivitis patients compared to healthy controls, even though higher serum calprotectin levels were observed in aggressive periodontitis[31]. In this study, neither in CF children nor in the healthy controls, calprotectin was associated with any of the clinical periodontal parameters (PPD, PI, GI, BOP) indicating that salivary calprotectin may not be influenced by the periodontal status in these children. However, even though this study indicated for the first time that salivary calprotectin could be a potential marker to distinguish CF children with or without gingivitis from systemically healthy children, further studies are required to investigate the role of calprotectin in gingivitis or periodontal disease in general.

Apart from calprotectin, TREM-1, its ligand PGLYRP1 and IL-1β, a downstream molecule of the TREM-1 signalling pathway, were investigated in saliva. We observed a positive correlation among TREM-1, PGLYRP1 and IL-1β in healthy control children but none of these molecules associated with calprotectin. The former association is supported by previous findings[7,8,22] and could be attributed to the involvement of all three molecules in the TREM-1 signalling pathway. TREM-1, PGLYRP1 and IL-1β are also known to be regulated during gingival inflammation[22]. This fact together with our findings that the periodontal status seemingly does not effect salivary calprotectin levels, might explain why calprotectin

did not correlate with TREM-1, PGLYRP1 and IL-1β in systemic health. In CF children however, PGLYRP1, IL-1β and calprotectin positively correlated with each other. An earlier study in patients with CF also proofed a positive association between TREM-1 and PGLYRP1 in plasma[10]. So it is not too surprising that calprotectin and PGLYRP1, an antimicrobial molecule also expressed by neutrophils and secreted upon neutrophil degranulation[11], and IL-1β, a proinflammatory cytokine, positively correlate with each other during inflammation and/or infection. The fact, that TREM-1 monocyte-expression might be down-regulated in CF could explain why calprotectin and TREM-1 did not correlate.

Although, salivary TREM-1, PGLYRP1 and L-1β levels were not able to distinguish between CF patients and systemically healthy controls, TREM-1 and IL-1β levels were significantly higher in the gingivitis subgroups compared to individuals with healthy periodontium, independently of CF. Therefore, salivary TREM-1 levels might closely reflect the presence of gingival inflammation in children. Up till now there is no study analysing TREM-1 levels in children with gingivitis or CF with periodontal health to compare our findings to. However, previous studies in adults or elderly are conform with our findings. Elevated TREM-1 levels in GCF in gingivitis[32] and in saliva, GCF and serum in periodontitis[8,33] have been reported compared to periodontal health in systemically healthy individuals. Recently, it also has been demonstrated in a human model of induced gingival inflammation that TREM-1 and PGLYRP1 in saliva are positively regulated in response to biofilm accumulation and removal[22]. So, it is also not surprising that TREM-1, PGLYRP1 and IL-1β positively correlated with periodontal parameters, both in CF and healthy control children. Hence, it was highlighted for the first time that these molecules may have a crucial role in the early stage of periodontal disease and salivary TREM-1 may be substantial to reflect gingival inflammation in children. According to our findings, salivary TREM-1,

PGLYRP1 and IL-1β might directly reflect gingival inflammation while their features to reflect systemic inflammation in CF in children might rather be indirectly. The results of the current study need to be verified in larger studies of CF patients, however, it should be considered that CF is an autosomal recessive disease which is very rare[34].

5. Conclusion

Children with CF had higher gingival inflammation scores and an altered salivary biomarker profile, especially in calprotectin levels, that is correlated with systemic inflammatory markers. In children with CF, pediatricians could check for signs of gingivitis by a simple oral examination and, if gingival inflammation is present, refer them to a dentist for dental care consultation. While these preliminary findings demonstrate that salivary calprotectin might have a chairside diagnostic potential for CF in children, further investigations in larger cohorts would be needed to determine the validity of these results.

Footnotes

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[†] Human TREM-1 DuoSet ELISA, R&D systems (DY1278B)

[‡] Human PGLYRP1/PGRP-S DuoSet ELISA, R&D systems (DY2590)

[§] Human IL-1 beta/IL-1F2 DuoSet ELISA, R&D systems (DY201)

[‖] Human S100A8/S100A9 Heterodimer DuoSet ELISA, R&D systems (DY8226)

[¶] Thermo Fisher Scientific, Rockford, USA

[#] SPSS, version 20, IBM Corporation, Armonk, NY, USA

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Conflict of interest statement

The authors report no conflicts of interest related to this study.

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Table 1. The Study Population Characteristics

	Cystic Fibrosis (CF) (N=10)	Systemically Healthy (Control) (C) (N=10)	Reference range
Age (yr.)	7.00±3.16	7.10±2.51	NA
Sex (F/M)	6/4	5/5	NA
CRP (mg/L)	2.86±2.11	ND	(0-6)
Haemoglobin (gr/dL)	12.59±0.83	ND	(13.7-17.5)
Haematocrit (%)	39.83±3.51	ND	(40.0-53.0)
MCV(fL)	79.06±8.14	ND	(80.4-95.9)
Erythrocyte (10^6/mkrL)	5.08±0.61	ND	(4.38-5.77)
MCH (pg)	25.00±2.42	ND	(27.2-33.5)
MCHC (g/dL)	31.63±1.30	ND	(32.7-35.6)
RDW (%)	14.70±1.87*	ND	(11.8-14.3)
Leukocyte (10^3/mkrL)	12.11±2.57	ND	(6.0-13.5)

Neutrophil (10^3/mkrL)	7.09±3.45*		ND		(2.1-6.1)
Lymphocyte (10^3/mkrL)	3.84±2.03*		ND		(1.2-3.6)
Monocyte (10^3/mkrL)	0.94±0.24		ND		(0.3-0.9)
Eosinophil (10^3/mkrL)	0.15±0.11		ND		(0.0-0.5)
Basophil (10^3/mkrL)	0.04±0.02		ND		(0.0-0.2)
Thrombocyte (10^3/mkrL)	381.70±106.29*		ND		(150.0-350.0)
PCT (%)	0.36±0.08		ND		(0.0-100.0)
MPV (fL)	9.69±1.15		ND		(6.8-10.8)
	CF-H (N=6)	CF- G (N=4)	C-H (N=5)	C-G (N=5)	
Age (yr.)	6.00±3.16	**8.50±2.88**	5.60±1.51	**8.60±2.51**	
Sex (F/M)	3/3	3/1	3/2	2/3	

CRP: C-reactive protein; MCV: mean cell volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; RDW: red cell distribution width leukocyte; PCT: procalcitonin; MPV: mean platelet volume; CF-H: Cystic Fibrosis- Periodontally Healthy; CF-G: Cystic Fibrosis- Gingivitis; C-H: Control- Periodontally Healthy; C-G: Control- Gingivitis; Reference range: normal range of the blood

parameters in children. *Values outside the reference range but not considered as a pathological finding; Values in bold: Significant differences (P<0.05) compared to CF-H and C-H; ND: not determined; NA: not applicable.

Table 2. Clinical Periodontal Evaluation of Groups and Related Subgroups

	Cystic Fibrosis (CF) (N=10)		Systemically Healthy (Control) (C) (N=10)		Cystic fibrosis effect P value	
GI	1.04±0.64		0.85±0.71		0.093	
PI	0.94±0.47		0.84±0.45		0.137	
BOP (%)	28.98±25.06		20.32±18.81		**0.001**	
PPD (mm)	2.02±0.33		2.13±0.25		0.573	
	CF-H (N=6)	CF-G (N=4)	C-H (N=5)	C-G (N=5)	Periodontal status effect P value	Cystic fibrosis-periodontal status interaction P value
GI	0.61±0.21	1.68±0.50	0.27±0.08	1.44±0.53	**<0.001**	0.763
PI	0.63±0.20	1.40±0.37	0.45±0.16	1.24±0.22	**<0.001**	0.918

BOP (%)	10.63±2.83	56.50±13.67	5.28±1.88	35.36±15.06	**<0.001**	0.485
PPD (mm)	1.81±0.23	2.32±0.19	2.03±0.09	2.22±0.33	**0.005**	0.152

GI: gingival index; PI: plaque index; BOP: bleeding on probing; PPD: probing pocket depth; CF-H: Cystic Fibrosis- Periodontally Healthy; CF-G: Cystic Fibrosis- Gingivitis; C-H: Control- Periodontally Healthy; C-G: Control- Gingivitis. Values in bold for two main groups: Significant difference (P<0.05) from C group. Values in bold for subgroups: Significant differences (P<0.05) compared to CF-H and C-H.

Table 3. Biochemical Findings of Groups and Related Subgroups

	Cystic Fibrosis (CF) (N=10)	Systemically Healthy (Control) (C) (N=10)	Cystic fibrosis effect P value	
TREM-1 (pg/mL)	97.24±100.92	140.11±145.69	0.827	
PGLYRP1 (ng/mL)	29.20±31.79	19.04±16.36	0.291	
IL-1β (pg/mL)	173.18±218.85	162.83±194.71	0.609	
Calprotectin (μg/mL)	15.66±15.30	3.99±4.58	**0.017**	

Total Protein (µg/mL)	1383.24±562.23		918.84±483.59		0.097	
	CF-H (N=6)	CF-G (N=4)	C-H (N=5)	C-G (N=5)	Periodontal status effect P value	Cystic fibrosis-periodontal status interaction P value
TREM-1 (pg/mL)	49.10 ±47.64	169.44±123.26	38.04±42.13	242.17±141.21	**0.003**	0.368
PGLYRP1 (ng/mL)	15.25±3.14	50.06±45.26	18.47±24.04	19.61±4.84	0.106	0.900
IL-1β (pg/mL)	58.46±55.22	345.28±269.81	45.90±38.71	279.76±222.75	**0.003**	0.728
Calprotectin (µg/mL)	9.76±6.78	24.50±21.27	3.05±4.57	4.75±4.97	0.262	0.834
Total Protein (µg/mL)	1525.48±447.29	1169.87±716.73	947.51±503.69	890.17±520.04	0.409	0.548

CF-H: Cystic Fibrosis- Periodontally Healthy; CF-G: Cystic Fibrosis- Gingivitis; C-H: Control- Periodontally Healthy; C-G: Control- Gingivitis. Values in bold for two main groups: Significant difference ($P<0.05$) from C group. Values in bold for subgroups: Significant differences ($P<0.05$) compared to CF-H and C-H.

Table 4. Correlations Between Clinical Periodontal and Biochemical Parameters in CF patients and Controls

		Age (yr.)	PPD (mm)	GI	PI	BOP (%)	TREM-1 (pg/mL)	PGLYRP1 (ng/mL)	IL-1β (pg/mL)	Calprotectin (µg/mL)	Total protein (µg/mL)
Cystic Fibrosis (CF)											
TREM-1 (pg/mL)	*r*	-0.104	0.474	**0.770**†	0.515	**0.770**†		0.515	0.624	0.297	-0.152
	p	0.775	0.166	0.009	0.128	0.009		0.128	0.054	0.405	0.676
PGLYRP1 (ng/mL)	*r*	-0.122	0.292	**0.661***	0.309	**0.061***	0.515		**0.758***	**0.721***	0.345
	p	0.736	0.413	0.038	0.385	0.038	0.128		0.011	0.019	0.328
IL-1β **(pg/mL)**	*r*	0.165	0.590	0.612	**0.709***	**0.636***	0.624	**0.758***		**0.745***	0.164
	p	0.648	0.073	0.060	0.022	0.048	0.054	0.011		0.013	0.651
Calprotectin (µg/mL)	*r*	-0.080	0.225	0.455	0.304	0.455	0.297	**0.721***	**0.745***		0.600
	p	0.827	0.532	0.187	0.260	0.187	0.405	0.019	0.013		0.067
Total protein (µg/mL)	*r*	-0.416	-0.517	-0.127	-0.333	-0.200	-0.152	0.345	0.164	0.600	
	p	0.232	0.126	0.726	0.347	0.580	0.676	0.328	0.651	0.06	

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Systemically Healthy (Control) (C)											
TREM-1 (pg/mL)	*r*	0.611	0.333	**0.830**†	**0.891**†	**0.863**†		**0.648***	**0.833**†	0.317	0.164
	p	0.060	0.347	0.003	0.001	0.001		0.043	0.003	0.406	0.651
PGLYRP1 (ng/mL)	*r*	**0.716***	0.079	**0.636***	**0.685***	0.620	**0.648***		**0.815**†	0.467	0.515
	p	0.020	0.829	0.048	0.029	0.056	0.043		0.004	0.205	0.128
IL-1**β (pg/mL)**	*r*	**0.870**†	0.304	**0.778**†	**0.742***	**0.860**†	**0.833**†	**0.815**†		0.510	0.468
	p	0.001	0.393	0.008	0.014	0.001	0.003	0.004		0.160	0.172
Calprotectin (µg/mL)	*r*	0.306	0.417	-0.033	0.317	0.176	0.317	0.467	0.510		**0.700***
	p	0.423	0.265	0.932	0.406	0.651	0.406	0.205	0.160		0.036
Total protein (µg/mL)	*r*	0.309	0.588	0.018	-0.067	0.061	0.164	0.515	0.468	**0.700***	
	p	0.385	0.074	0.960	0.855	0.868	0.651	0.128	0.172	0.036	

GI: gingival index; PI: plaque index; BOP: bleeding on probing; PPD: probing pocket depth; *Correlation (in bold) is significant at the 0.05 level (2-tailed); †Correlation (in bold) is significant at the 0.01 level (2-tailed).