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Salivary Protease Activity in Children with Cystic Fibrosis

Kistik Fibrozisli Çocuklarda Tükürük Proteaz Aktivitesi

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Abstract

Objective: Patients with cystic fibrosis (CF) present with impaired protease-antiprotease balance in their lungs. However, salivary protease equilibrium in children with CF with poor oral health has not been reported. The current study investigated salivary matrix metalloproteinase-8 (MMP-8), tissue inhibitor of matrix metalloproteinases-1 (TIMP-1), neutrophil elastase (NE), and myeloperoxidase (MPO) levels in children with CF with or without gingivitis.

Materials and Methods: Eleven CF and 11 systemically healthy children aged 3-12 years were evaluated. Clinical periodontal examinations including probing pocket depth (PPD), gingival index (GI), plaque index (PI), and bleeding on probing (BOP) were recorded and saliva samples were obtained. Salivary MMP-8, TIMP-1, NE, and MPO levels were analyzed by immunofluorometric assay and enzyme-linked immunosorbent assay.

Results: Salivary levels of MMP-8, TIMP-1, NE, MPO, and MMP-8/TIMP-1 molar ratios were similar in CF and systemically healthy children ($p>0.05$). Levels of MMP-8, NE, and MPO were significantly higher in the saliva of children with gingivitis compared to periodontally healthy children in both CF and systemically healthy groups ($p<0.05$). MMP-8 and MMP-8/TIMP-1 levels were positively correlated with GI, PI, and BOP ($p<0.05$), while NE and MPO levels were related to all periodontal parameters ($p<0.01$).

Conclusion: CF may not alter the activity of MMP-8, NE, and MPO. On the other hand, gingival inflammation had a pronounced effect on salivary levels of these enzymes and the MMP-8/TIMP-1 molar ratio in children, irrespective of CF. Further investigations in larger cohorts are needed to better clarify this issue.

Keywords: Gingivitis, matrix metalloproteinases, leukocyte elastase, cystic fibrosis, biomarkers, saliva

Öz

Amaç: Kistik fibrozisli (KF) hastalar, akciğerlerde bozulmuş proteaz-antiproteaz dengesi ile kendini göstermektedir. Bununla birlikte, ağız sağlığı kötü olan KF'li çocuklarda tükürük proteaz dengesini gösteren bir çalışma henüz bulunmamaktadır. Bu çalışmanın amacı, gingivitise sahip olan veya olmayan KF'li çocuklarda tükürük matriks metalloproteinaz-8 (MMP-8), matriks metalloproteinaz-1 doku inhibitörü (TIMP-1), nötrofil elastaz (NE) ve miyeloperoksidaz (MPO) seviyelerinin araştırılmasıdır.

Gereç ve Yöntemler: Üç - on iki yaşları arasında 11 KF ve 11 sistemik sağlıklı çocuk değerlendirildi. Klinik periodontal muayenede, sondalanabilir cep derinliği, gingival indeks (Gİ), plak indeksi (Pİ) ve sondalamada kanama (SK) değerleri kaydedildi; ve tükürük örnekleri alındı. Tükürük MMP-8, TIMP-1, NE ve MPO seviyeleri, immünoflorometrik analiz ve enzime bağlı immünosorbent analiz metodlarıyla değerlendirildi.

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Bulgular: KF ve sistemik sağlıklı çocuklarda tükürük MMP-8, TIMP-1, NE, MPO ve MMP-8/TIMP-1 molar oranı düzeyleri benzerdi ($p>0,05$). Gingivitise sahip olan çocuklarda tükürük MMP-8, NE ve MPO seviyeleri periodontal olarak sağlıklı çocuklara göre hem KF hem de sistemik olarak sağlıklı gruplarda anlamlı olarak daha yüksekti ($p<0,05$). MMP-8 ve MMP-8/TIMP-1 seviyeleri Gİ, Pİ ve SK ile pozitif korelasyon gösterirken ($p<0,05$), NE ve MPO seviyeleri tüm klinik periodontal parametrelerle ilişkili bulundu ($p<0,01$).

Sonuç: KF, MMP-8, NE ve MPO'nun aktivitesini değiştirmeyebilir. Ancak çocuklarda gingival enflamasyon, KF'den bağımsız olarak bu enzimlerin tükürük seviyeleri ve MMP-8/TIMP-1 molar oranı üzerinde belirgin bir etkiye sahipti. Bu konuyu açıklığa kavuşturmak için daha büyük kohort çalışmalarına ve ileri araştırmalara ihtiyaç vardır.

Anahtar Kelimeler: Gingivitis, matriks metalloproteinazlar, lökosit elastaz, kistik fibrozis, biyomarkerlar, tükürük

Introduction

Cystic fibrosis (CF) is an autosomal recessive inflammatory disease associated with the cystic fibrosis transmembrane conductance regulatory (CFTR) gene dysfunction affecting many organs, especially lungs, causing chronic destructive pulmonary infections as the main reason for early death (1). The CFTR dysfunction causes impaired ion channel function and thus leads to the hyper-viscous secretions from exocrine glands, including salivary glands, resulting in changed salivary properties and profile (2,3). Mucus hyper-viscosity furthermore facilitates increased colonization in the airways by Gram-negative bacteria like *Pseudomonas aeruginosa* leading to non-resolving neutrophilic inflammation (4). In the pathogenesis of CF, proteolytic enzymes released by neutrophils are believed to have a central role in processes causing tissue damage and abnormal tissue remodeling (5,6). In this context, matrix metalloproteases (MMPs), which exert numerous biological functions including both physiological remodeling of tissues and pathological tissue destruction in chronic inflammatory conditions including periodontal disease, seem to be of specific importance (7,8). MMP secretion and activity are strictly regulated by specific tissue inhibitors of metalloproteinases (TIMPs) and a dysfunction of TIMPs and/or a MMP-TIMP disbalance promotes the pathological destruction of gingival tissue (8).

Neutrophil elastase (NE) is well-known as one of the major destructive proteases degrading extracellular matrix (ECM) components, and is believed to have effects on increased MMP activity and degradation of TIMPs. NE has also been shown to be a biomarker of CF (7). In addition to NE, myeloperoxidase (MPO) is the other important destructive enzyme released by degranulation of neutrophils. MPO has a pivotal role in oxidative stress mechanisms and it can activate both latent proMMP-8 and proMMP-9 through this way as well as oxidatively inactivate TIMPs (8). Therefore, NE and MPO have the ability to modify inflammatory reactions by promoting MMPs.

Studies have shown that CF patients have increased MMP-9 and lower TIMP-1 concentrations due to the NE action in their sputum or bronchoalveolar lavage fluid (BAL) (7,9). On the other hand, clinical research clearly indicated altered salivary flow rate and its components during the course of CF (10). Saliva is an important fluid reflecting changes in the oral cavity and thus periodontal inflammation (11). It was hypothesized that changes in the salivary content and increased levels of MMPs in CF might affect the periodontal health. Therefore, this study aimed to investigate salivary MMP-8, TIMP-1, MMP-8/TIMP-1 molar ratio, NE and MPO levels in CF and systemically healthy children, in the presence or absence of gingival inflammation.

Materials and Methods

Study population

Eleven children with CF (aged 3 to 12 years) monitored by the Aydın Adnan Menderes University Faculty of Medicine, Department of Pediatric Gastroenterology, Aydın, Turkey between May 2016 and December 2018 were enrolled for the present study. All children with CF had a verified diagnosis by abnormal sweat test in combination with the presence of characteristic clinical properties compatible with the CF phenotype and/or a genotype with mutations. CF patients who were clinically and symptomatically stable with no acute respiratory infections for at least 4 weeks and with no other systemic disease were included. Patients were excluded if they used probiotics or any anti-inflammatory drugs, need for a lung transplantation, had pulmonary exacerbation and have taken oral/intravenous antibiotics or any other medications that may affect the periodontium and study findings in the previous four months (12). For the control group (C), eleven age and gender matched systemically healthy children applied for dental checkups to the Aydın Adnan Menderes University Faculty of Dentistry, Aydın, Turkey were included. In both groups, the exclusion criteria also included the received periodontal treatment, use of medications (antibiotic or other anti-inflammatory drugs) in the last four months, presence of caries and having less than 10 fully-erupted teeth. The ethical principles stated in the World Medical Association's Declaration of Helsinki were followed; and this study protocol was approved by the Ethics Committee on Clinical Researches of Ege University (decision number: 17-5/9, date: 11.09.2017). Following the aim and the procedures of this study were explained, informed consent was provided from all participants before the enrollment [parents (in writing) and children (orally)].

Clinical Periodontal Assessment

Clinical periodontal examination was performed for each participant in CF and C groups to identify their periodontal status. For the clinical periodontal examination, probing

pocket depth (PPD), gingival index (GI) (13), plaque index (PI) (14) and bleeding on probing (BOP) (15) parameters were measured and recorded at four sites (mesial, distal, buccal, lingual/palatinal) on each fully-erupted tooth present by a single calibrated periodontist (ZPKY) using a Williams periodontal probe (Hu-Friedy, Chicago, IL). Clinical attachment level was also evaluated, however data was not shown since none of the children had any attachment loss. Additionally, the determination of calculus formation was performed by a visual assessment on the surface of each tooth and noted as present or absent (16). This data was not presented either since no calculus was observed on any tooth surface in both CF and systemically healthy children. The children were defined as periodontally healthy (H) if they had clinically healthy gingiva (GI=0), good oral hygiene, and PPD ≤3 mm with no clinical attachment loss and radiographic bone loss. Children showing GI ≥1 and PPD ≤3 mm were diagnosed with gingivitis (G). After the completion of the periodontal examination, children were further divided into subgroups based on their periodontal conditions: CF and periodontally healthy (CF-H, n=6); CF and gingivitis (CF-G, n=5); systemically healthy and periodontally healthy (C-H, n=6); systemically healthy and gingivitis (C-G, n=5).

Saliva Sampling

Saliva samples (n=22) were obtained by asking the patients to expectorate into sterile 50 mL polypropylene tubes for five minutes in the morning hours (8.00-10.00 am) following an overnight fast one day after the clinical periodontal measurements (17). All children were requested to avoid eating, drinking and any oral hygiene procedures two hours before the sampling under supervision of their parents. Obtained saliva was centrifuged at 10,000 x g for 15 minutes at 4 °C and the supernatants were snap frozen (-20 °C) and stored at -80 °C.

MMP-8 Analysis by Immunofluorometric Assay

Salivary MMP-8 levels were measured by a time-resolved immunofluorometric assay (Medix Biochemica, Espoo, Finland), as previously described (18). The detection limit for MMP-8 was 0.08 ng/mL.

TIMP-1, NE and MPO Analyses by Enzyme-linked Immunosorbent Assay

Salivary TIMP-1, NE and MPO levels were determined by commercial enzyme-linked immunosorbent assays (ELISAs) (Human TIMP-1, Human Biotrak ELISA Systems, GE Healthcare, Buckinghamshire, UK; Human NE, Platinum ELISA, Bender MedSystemss GmbH, Vienna, Austria; Human MPO, Immunodiagnostic AG, Bensheim, Germany) according to the manufacturer's instructions, as previously described (19). The detection limit for TIMP-1 was 1.25 ng/mL, for NE 1.98 pg/mL and for MPO 1.6 ng/mL.

The calculation of MMP-8/TIMP-1 molar ratio was performed to convert ng/mL levels to mol/L (18).

Statistical Analysis

The distribution of all numerical variables was tested by Shapiro-Wilk normality test. For data of BOP and NE variables that were not normally distributed, normality was achieved by logarithmic transformation. Then, 2x2 factorial ANOVA was carried out for intergroup comparisons of all descriptive variables including the effect of periodontal status, systemic condition and interactions between CF and the periodontal status. Sex ratio was assessed using chi-square test. The possible correlations of biochemical variables with clinical periodontal parameters were analyzed using Pearson correlation test. All data analyses were performed using the statistical software program (SPSS, v22.0, IBM, Chicago, IL) at α=0.05 significance level.

Results

Demographic Characteristics and Clinical Findings

Demographics of the children with CF and systemically healthy and their related subgroups are shown in Table 1. Age and sex distribution were not significantly different among the study groups (CF and C) as the groups were matched ($p>0.05$). For subgroups, mean age (years) of CF-G, C-G, CF-H and C-H was 8.20±2.59, 8.60±2.51, 6.00±3.16 and 6.00±1.67, respectively. The mean age of CF-H and C-H was significantly lower than the CF-G and C-G ones ($p<0.05$). Male-to-female ratio was similar among the subgroups ($p>0.05$).

Clinical periodontal findings of CF and systemically healthy children and their related subgroups are outlined in Table 2. Clinical periodontal measurements including PPD, GI and PI values showed no significant difference between CF and C groups ($p>0.05$), whereas BOP scores of the CF children were significantly higher compared to the C children ($p<0.05$). In the subgroups, CF-G and C-G had higher scores of PPD, GI, BOP and PI compared to CF-H and C-H children ($p=0.003$ for PPD and $p<0.0001$ for GI, BOP, PI).

Biochemical Findings

The biochemical findings of the CF and systemically healthy children and their related subgroups are outlined in Table 3. No significant differences were found between the CF and C groups regarding salivary levels of MMP-8, TIMP-1, MMP-8/TIMP-1 molar ratio, NE and MPO ($p>0.05$). The CF-G and C-G subgroups exhibited elevated MMP-8, MMP-8/TIMP-1, NE and MPO in saliva when compared to the periodontally healthy subgroups (CF-H and C-H) ($p<0.05$); while no statistically significant difference was observed in salivary concentrations of TIMP-1 among all four subgroups ($p>0.05$).

Correlations Between Clinical Parameters and Biochemical Data

Correlations of age and clinical parameters with biochemical data are presented in Table 4. Salivary NE and MPO levels had a strong positive relationship with

all clinical periodontal parameters (PPD, GI, BOP and PI) ($p<0.01$). MMP-8 levels and MMP-8/TIMP-1 molar ratio were also positively associated with GI, BOP and PI ($p<0.05$). No correlation was detected between age and the biochemical parameters (MMP-8, TIMP-1, MMP-8/TIMP-1 molar ratio, NE and MPO) ($p>0.05$).

Discussion

This age matched case-control study evaluating the possible association between CF and periodontal health showed that salivary MMP-8, MMP-8/TIMP-1, NE and MPO levels were elevated in the presence of gingival inflammation in both CF and systemically healthy children. Moreover, salivary MMP-8, MMP-8/TIMP-1, NE and MPO levels were positively correlated with GI, BOP and PI and also NE and MPO levels in saliva were positively associated with PPD.

Earlier studies examining clinical periodontal status in CF children reportedly stated no significant differences in plaque accumulation (20,21), gingival bleeding (21-24) or calculus occurrence (22,23) between the patients of CF and systemically healthy controls. In contrast to the earlier reports, BOP scores were significantly increased in children with CF compared to systemically healthy ones. However, other periodontal parameters such as PPD and PI were not significantly different between CF children and systemically healthy controls. These results may be due to the differences in age ranges or clinical indexes evaluated in previous studies (20-24). On the other hand, the current findings of high BOP scores in CF patients suggested that the systemic inflammation of CF could exacerbate gingival inflammation and lead to the manifestation or worsening of the clinical signs of periodontal disease. This is supported by a recently published study that demonstrates a higher BOP as well as an increased pro-inflammatory host response in CF patients with gingivitis than those of non-CF (25).

There is a considerable evidence that function of salivary glands are affected in patients with CF due to the defective

Table 1. Demographic data of groups and related subgroups

	Cystic fibrosis (CF) (n=11)		Systemically healthy (Control) (C) (n=11)	
Age (years)	7.00±3.00		7.18±2.40	
Sex (F/M)	6/5		5/6	
	CF-H (n=6)	CF-G (n=5)	C-H (n=6)	C-G (n=5)
Age (years)	6.00±3.16	**8.20±2.59**	6.00±1.67	**8.60±2.51**
Sex (F/M)	3/3	3/2	3/3	2/3

CF-H: Cystic fibrosis-periodontally healthy, CF-G: Cystic fibrosis-gingivitis, C-H: Control-periodontally healthy, C-G: Control-gingivitis. Values in bold: Significant differences ($p<0.05$) compared to CF-H and C-H

Table 2. Clinical periodontal evaluation of groups and related subgroups

	Cystic fibrosis (CF) (n=11)		Systematically healthy (Control) (C) (n=11)		Cystic fibrosis effect p-value	
GI	1.13±0.67		0.83±0.68		0.070	
PI	0.97±0.45		0.81±0.44		0.140	
BOP (%)	1.33±0.39		1.10±0.43		**0.001**	
PPD (mm)	2.07±0.37		2.03±0.41		0.695	
	CF-H (n=6)	CF-G (n=5)	C-H (n=6)	C-G (n=5)	Periodontal status effect p-value	Cystic fibrosis-periodontal status interaction p-value
GI	0.61±0.21	1.74±0.45	0.32±0.15	1.44±0.53	**<0.001**	0.984
PI	0.63±0.20	1.37±0.32	0.46±0.15	1.24±0.22	**<0.001**	0.833
BOP (%)	1.01±0.13	1.72±0.10	0.75±0.17	1.52±0.16	**<0.001**	0.607
PPD (mm)	1.81±0.24	2.38±0.22	1.86±0.42	2.22±0.33	**0.003**	0.446

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

CFTR gene (26). Such genetic alteration can affect the composition, properties and flow of saliva in patients with CF (10,27-29). In addition, researchers have confirmed altered salivary protein profiles in patients suffering from CF (10,29). However, the literature dealing with periodontal status in CF is based solely on clinical assessments and specific proteins were not studied in saliva. In our previous report, we evaluated whether salivary triggering receptor expressed on myeloid cells-1, its putative ligand peptidoglycan recognition protein-1 and also calprotectin

Table 3. Biochemical findings of groups and related subgroups

	Cystic fibrosis (CF) (N=11)		Systematically healthy (Control) (C) (N=11)		Cystic fibrosis effect p-value	
MMP-8 (ng/mL)	671.49±208.91		499.35±327.88		0.101	
TIMP-1 (ng/mL)	358.41±213.02		277.50±145.84		0.316	
MMP-8/TIMP-1	1.11±0.72		0.92±0.77		0.534	
NE (ng/mL)	2.53±0.56		2.23±0.56		0.193	
MPO (ng/mL)	3623.65±1318.07		2817.82±1358.66		0.096	
	CF-H (n=6)	CF-G (n=5)	C-H (n=6)	C-G (n=5)	Periodontal status effect p-value	Cystic fibrosis-periodontal status interaction p-value
MMP-8 (ng/mL)	527.48 169.84±	844.31±67.90	359.83±382.69	666.77±146.46	**0.006**	0.961
TIMP-1 (ng/mL)	391.12±268.64	319.16±140.38	323.89±171.85	221.83±95.69	0.289	0.852
MMP-8/TIMP-1	0.87±0.67	1.40±0.74	0.41±0.38	1.52±0.67	**0.007**	0.284
NE (ng/mL)	2.34±0.65	2.76±0.36	1.93±0.50	2.59±0.41	**0.023**	0.588
MPO (ng/mL)	3021.02±1231.48	4346.81±1117.64	1856.39±777.91	3971.54±898.97	**0.001**	0.379

MMP-8: Matrix metalloproteinase-8, TIMP-1: Tissue inhibitor of matrix metalloproteinases-1, NE: Neutrophil elastase, MPO: Myeloperoxidase, CF-H: Cystic fibrosis-periodontally healthy, CF-G: Cystic fibrosis-gingivitis, C-H: Control-periodontally healthy, C-G: Control-gingivitis. For two main groups: No 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 and biochemical parameters

	Age (years)	PPD (mm)	GI	PI	BOP (%)	MMP-8 (ng/mL)	TIMP-1 (ng/mL)	MMP-8/ TIMP-1	NE (ng/mL)	MPO (ng/mL)
Age (years)	1	0.310	0.412	**0.600†**	**0.472***	0.310	0.133	0.320	0.352	0.203
PPD (mm)	0.310	1	**0.582†**	**0.591†**	**0.565†**	0.357	-0.253	0.313	**0.593†**	**0.485†**
GI	0.412	**0.582†**	1	**0.833†**	**0.930†**	**0.502***	-0.100	**0.475***	**0.554†**	**0.758†**
PI	**0.600†**	**0.591†**	**0.833†**	1	**0.876†**	**0.662†**	-0.163	**0.656†**	**0.541†**	**0.724†**
BOP (%)	**0.472***	**0.565†**	**0.930†**	**0.876†**	1	0.618†	-0.071	**0.549†**	**0.606†**	**0.704†**
MMP-8 (ng/mL)	0.310	0.357	**0.502***	**0.662†**	**0.618†**	1	0.089	**0.647†**	**0.687†**	**0.684†**
TIMP-1 (ng/mL)	0.133	-0.253	-0.100	-0.163	-0.071	0.089	1	**-0.547†**	0.074	0.001
MMP-8/TIMP-1	0.32	0.313	**0.475***	**0.656†**	**0.549†**	**0.647†**	**-0.547†**	1	0.357	**0.445***
NE (ng/mL)	0.352	**0.593†**	**0.554†**	**0.541†**	**0.606†**	**0.687†**	0.074	0.357	1	**0.748†**
MPO (ng/mL)	0.203	**0.485***	**0.758†**	**0.724†**	**0.704†**	**0.684†**	0.001	**0.445***	**0.748†**	1

GI: Gingival index, PI: Plaque index, BOP: Bleeding on probing, PPD: Probing pocket depth, MMP-8: Matrix metalloproteinase-8, TIMP-1: Tissue inhibitor of matrix metalloproteinases-1, NE: Neutrophil elastase, MPO: Myeloperoxidase. *Correlation (in bold) is significant at the 0.05 level (2-tailed); †Correlation (in bold) is significant at the 0.01 level (2-tailed)

were associated with CF; and observed that CF children showed a varying salivary biomarker profile, particularly regarding the levels of calprotectin, in addition to elevated gingival inflammation scores (30). We therefore investigated the salivary analysis of proteolytic mediators with clinical periodontal measurements to assess, for the first time, the relationship of the periodontal status with CF.

Proteolytic enzymes released from neutrophils are taken part in the pathophysiology of CF and the balance of MMPs with their inhibitors or the activators is believed to reflect the proteolytic processes of the disease (5). Increased MMP-9 levels and MMP-9/TIMP-1 molar ratios have been shown to correlate with NE activation in sputum (7) and BAL (9) samples of CF children. This is corroborated by elevated serum concentrations of MMP-8 and MMP-9 in cases with CF lung disease (1). Contrary to sputum, BAL or serum fluids, lower salivary MMP-9 concentrations have been shown in CF patients than controls (26). The current findings showed that CF does not appear to influence the salivary levels of proteinases significantly, although these enzymes tend to rise in CF. This observation may point out the distinctiveness of the oral cavity from the lower airway and blood or might be linked to the local responses of CF rather than the systemic inflammatory response. Yet, salivary MMP-8 and MMP-8/TIMP-1 molar ratio were significantly increased in the gingivitis subgroup of CF in comparison to the periodontally healthy CF children. Additionally, in the systemically healthy group, children with gingival inflammation had also higher levels of these molecules compared to periodontally healthy children. These findings are in accordance with the literature that reveals the involvement of MMP-8 with gingival inflammation (18,31-36).

It is also important to note that NE and MPO concentrations in saliva were significantly elevated in addition to MMP-8 in gingivitis subgroups in the present study. Evidence shows that neutrophils release NE and MPO which in turn activate MMP-8 and -9, respectively, in response to the accumulation of plaque (8). Therefore, the increased MMP-8 and MMP-8/TIMP-1 molar ratio might be a consequence of higher levels of NE and MPO activity in children with gingivitis. Our data also presented a significant correlation of MMP-8 levels and MMP-8/TIMP-1 molar ratio with GI, BOP and PI. Besides, the levels of NE and MPO also had a positive relationship with all clinical periodontal parameters. If all these findings of the study are taken into consideration, it is likely that the increased levels of these enzymes in saliva of children seems to be arised from the microbiological condition regardless of the systemic condition. An activated systemic inflammatory response by CF disease could have a tendency to influence the gingival health, however in the present study we evaluated these molecules solely in saliva and also with a relatively small sample size. The results of current study need to be verified with larger size of CF patients.

Conclusion

Within the limitations of this study, the current findings suggest that salivary MMP-8, TIMP-1, NE, MPO are similar between children with CF or without CF. Importantly, children with CF had elevated bleeding scores, and gingival inflammation had a pronounced effect on salivary proteolytic activity. Larger scale studies analysing neutrophilic enzymes in biofluids are needed to determine the validity of these results and to clarify the association between CF and gingival inflammation.

Ethics

Ethics Committee Approval: The ethical principles stated in the World Medical Association's Declaration of Helsinki were followed; and this study protocol was approved by the Ethics Committee on Clinical Researches of Ege University (decision number: 17-5/9, date: 11.09.2017).

Informed Consent: Following the aim and the procedures of this study were explained, informed consent was provided from all participants before the enrollment [parents (in writing) and children (orally)].

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Authorship Contributions

Surgical and Medical Practices: Z.P.K.Y., T.T., A.S., Y.T., Concept: N.B., T.S., G.E., Design: N.B., T.S., G.E., Data Collection or Processing: Z.P.K.Y., T.T., A.S., Y.T., Analysis or Interpretation: Z.P.K.Y., T.T., A.S., T.K., G.T., Literature Search: Z.P.K.Y., Y.T., T.K., G.T., Writing: Z.P.K.Y.

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References

1. Rath T, Zwaschka L, Hage L, Kügler M, Menendez K, Naehrlich L, et al. Identification of neutrophil activation markers as novel surrogate markers of CF lung disease. PLoS One 2014; 9: e115847.
2. Aps JK, Delanghe J, Martens LC. Salivary electrolyte concentrations are associated with cystic fibrosis transmembrane regulator genotypes. Clin Chem Lab Med 2002; 40: 345-50.
3. Alkhateeb AA, Mancl LA, Presland RB, Rothen ML, Chi DL. Unstimulated Saliva-Related Caries Risk Factors in Individuals with Cystic Fibrosis: A Cross-Sectional Analysis of Unstimulated Salivary Flow, pH, and Buffering Capacity. Caries Res 2017; 51: 1-6.
4. Fischer N, Hentschel J, Markert UR, Keller PM, Pletz MW, Mainz JG. Non-invasive assessment of upper and lower airway infection and inflammation in CF patients. Pediatr Pulmonol 2014; 49: 1065-75.
5. Devereux G, Steele S, Jagelman T, Fielding S, Muirhead R, Brady J, et al. An observational study of matrix metalloproteinase (MMP)-9 in cystic fibrosis. J Cyst Fibros 2014; 13: 557-63.
6. Voynow JA, Fischer BM, Zheng S. Proteases and cystic fibrosis. Int J Biochem Cell Biol 2008; 40: 1238-45.
7. Jackson PL, Xu X, Wilson L, Weathington NM, Clancy JP, Blalock JE, et al. Human neutrophil elastase-mediated cleavage sites of MMP-9

and TIMP-1: implications to cystic fibrosis proteolytic dysfunction. Mol Med 2010; 16: 159-66.

8. Sorsa T, Tjäderhane L, Konttinen YT, Lauhio A, Salo T, Lee HM, et al. Matrix metalloproteinases: contribution to pathogenesis, diagnosis and treatment of periodontal inflammation. Ann Med 2006;38:306-21.
9. Garratt LW, Sutanto EN, Ling KM, Looi K, Iosifidis T, Martinovich KM, et al. Matrix metalloproteinase activation by free neutrophil elastase contributes to bronchiectasis progression in early cystic fibrosis. Eur Respir J 2015; 46: 384-94.
10. da Silva Modesto KB, de Godói Simões JB, de Souza AF, Damaceno N, Duarte DA, Leite MF, et al. Salivary flow rate and biochemical composition analysis in stimulated whole saliva of children with cystic fibrosis. Arch Oral Biol 2015; 60: 1650-4.
11. Javaid MA, Ahmed AS, Durand R, Tran SD. Saliva as a diagnostic tool for oral and systemic diseases. J Oral Biol Craniofac Res 2016; 6: 66-75.
12. Garg M, Leach ST, Coffey MJ, Katz T, Strachan R, Pang T, et al. Age-dependent variation of fecal calprotectin in cystic fibrosis and healthy children. J Cyst Fibros 2017; 16: 631-6.
13. Silness J, Löe H. Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 1964; 22: 121-35.
14. Löe H, Silness J. Periodontal disease in pregnancy I. Prevalence and severity. Acta Odontol Scand 1963; 21: 533-51.
15. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. Int Dent J 1975; 25: 229-35.
16. Aps JK, Van Maele GO, Martens LC. Caries experience and oral cleanliness in cystic fibrosis homozygotes and heterozygotes. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002; 93: 560-3.
17. Navazesh M. Methods for collecting saliva. Ann N Y Acad Sci 1993; 694: 72-7.
18. Gursoy UK, Könönen E, Pradhan-Palikhe P, Tervahartiala T, Pussinen PJ, Suominen-Taipale L, et al. Salivary MMP-8, TIMP-1, and ICTP as markers of advanced periodontitis. J Clin Periodontol 2010; 37: 487-93.
19. Akcalı A, Bostanci N, Özçaka Ö, Gümüş P, Öztürk-Ceyhan B, Tervahartiala T, et al. Gingival Inflammation and Salivary or Serum Granulocyte-Secreted Enzymes in Patients With Polycystic Ovary Syndrome. J Periodontol 2017; 88: 1145-52.
20. Dabrowska E, Błahuszewska K, Minarowska A, Kaczmarski M, Niedźwiecka-Andrzejewicz I, Stokowska W. Assessment of dental status and oral hygiene in the study population of cystic fibrosis patients in the Podlasie province. Adv Med Sci 2006; 51 Suppl 1: 100-3.
21. Martens LJ, Aps JKM, Van Maele GOG. Is oral health at risk in people with cystic fibrosis. Eur J Paediatr Dent 2001; 4: 21-7.
22. Ferrazzano GF, Orlando S, Sangianantoni G, Cantile T, Ingenito A. Dental and periodontal health status in children affected by cystic fibrosis in a southern Italian region. Eur J Paediatr Dent 2009; 10: 65-8.
23. Narang A, Maguire A, Nunn JH, Bush A. Oral health and related factors in cystic fibrosis and other chronic respiratory disorders. Arch Dis Child 2003; 88: 702-7.
24. Pawlaczyk-Kamieńska T, Borysewicz-Lewicka M, Śniatała R, Batura-Gabryel H, Cofta S. Dental and periodontal manifestations in patients with cystic fibrosis - A systematic review. J Cyst Fibros 2019; 18: 762-71.
25. Duruel O, Berker E, Özşin-Özler C, Gharibzadeh-Hızal M, Gürpınar Ö, Eryılmaz-Polat S, et al. Levels of pro- and anti-inflammatory cytokines in cystic fibrosis patients with or without gingivitis. Cytokine 2020; 127: 154987.
26. Nie S, Zhang H, Mayer KM, Oppenheim FG, Little FF, Greenberg J, et al. Correlations of salivary biomarkers with clinical assessments in patients with cystic fibrosis. PLoS One 2015; 10: e0135237.
27. Herman K, Kowalczyk-Zając M, Pytrus T. Oral cavity health among cystic fibrosis patients: Literature overview. Adv Clin Exp Med 2017; 26: 1147-53.
28. Gonçalves AC, Marson FA, Mendonça RM, Ribeiro JD, Ribeiro AF, Paschoal IA, Levy CE. Saliva as a potential tool for cystic fibrosis diagnosis. Diagn Pathol 2013; 8: 46.
29. Livnat G, Bentur L, Kuzmisnsky E, Nagler RM. Salivary profile and oxidative stress in children and adolescents with cystic fibrosis. J Oral Pathol Med 2010; 39: 16-21.
30. Yucel ZPK, Silbereisen A, Emingil G, Tokgoz Y, Kose T, Sorsa T, et al. Salivary biomarkers in the context of gingival inflammation in children with cystic fibrosis. J Periodontol 2020; 91: 1339-47.
31. Rai B, Kharb S, Jain R, Anand SC. Biomarkers of periodontitis in oral fluids. J Oral Sci 2008; 50: 53-6.
32. Gursoy UK, Könönen E, Huumonen S, Tervahartiala T, Pussinen PJ, Suominen AL, et al. Salivary type I collagen degradation end-products and related matrix metalloproteinases in periodontitis. J Clin Periodontol 2013; 40: 18-25.
33. Gupta N, Gupta ND, Gupta A, Khan S, Bansal N. Role of salivary matrix metalloproteinase-8 (MMP-8) in chronic periodontitis diagnosis. Front Med 2015; 9: 72-6.
34. Johnson N, Ebersole JL, Kryscio RJ, Danaher RJ, Dawson D 3rd, Al-Sabbagh M, et al. Rapid assessment of salivary MMP-8 and periodontal disease using lateral flow immunoassay. Oral Dis 2016; 22: 681-7.
35. de Morais EF, Pinheiro JC, Leite RB, Santos PPA, Barboza CAG, Freitas RA. Matrix metalloproteinase-8 levels in periodontal disease patients: A systematic review. J Periodontal Res 2018; 53: 156-63.
36. Nascimento GG, Baelum V, Sorsa T, Tervahartiala T, Skottrup PD, López R. Salivary levels of MPO, MMP-8 and TIMP-1 are associated with gingival inflammation response patterns during experimental gingivitis. Cytokine 2019; 115: 135-41.