Journal of Inflammation Research

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Association Between Salivary Cytokines, Chemokines and Growth Factors and Salivary Gland Function in Children with Chronic Kidney Disease

Julita Szulimowska[1], Anna Zalewska[1], Katarzyna Taranta-Janusz[2], Grażyna Marczuk-Kolada[3], Małgorzata Żendzian-Piotrowska[4], Mateusz Maciejczyk[4]

[1]Department of Restorative Dentistry, Medical University of Bialystok, Bialystok, Poland; [2]Department of Pediatrics and Nephrology, Medical University of Bialystok, Bialystok, Poland; [3]Department of Pedodontics, Medical University of Bialystok, Bialystok, Poland; [4]Department of Hygiene, Epidemiology and Ergonomics, Medical University of Bialystok, Bialystok, Poland

Correspondence: Mateusz Maciejczyk, Department of Hygiene, Epidemiology and Ergonomics, Medical University of Bialystok, Bialystok, 2c Mickiewicza Street, Bialystok, 15-233, Poland, Email mat.maciejczyk@gmail.com

Introduction: Chronic kidney disease (CKD) is a systemic inflammatory disease that leads to multiple organ complications not only in the kidneys and the cardiovascular system, but also in the oral cavity. CKD children experience reduced saliva secretion (hyposalivation), which leads to increased incidence of dental caries and significant impairment of patients' quality of life. However, the causes of salivary gland dysfunction in children with CKD are unknown. The present study is the first to evaluate the inflammatory and anti-inflammatory profile in the saliva of children with CKD at different stages of renal failure with normal and reduced salivary gland function.

Methods: Thirty children with CKD (age 9–16) and thirty age- and gender-matched healthy children were classified for the study. Salivary inflammatory and anti-inflammatory profile were assayed using the multiplex ELISA assay.

Results: We demonstrated statistically significant changes in salivary pro-inflammatory (↑TNF-α, ↓IL-7), anti-inflammatory (↑IL-10), Th1 (↑INF-γ, ↑IL-15), Th2 (↑IL-4, ↑IL-5, ↑IL-6, ↑IL-9) and Th17 (IL-17) cytokines as well as chemokines (↑MCP-1/CCL-2, ↑MIP-1α/CCL3, ↓MIP-1β/CCL4, ↓EOTAXIN/CCL11) and growth factors (↑G-CSF, ↑FGF) in unstimulated saliva of children with CKD compared to the controls. Although the evaluation of the salivary inflammatory profile does not indicate a particular dominance of any of the branches of the immune system, we observed a statistically significant increase in the concentration of all Th2 cytokines assayed. The multivariate regression analysis showed that the content of salivary cytokines, chemokines and growth factors depends on the secretory function of the salivary glands, ie, salivary flow, total protein concentration and amylase activity in the saliva. Salivary MIP-1α/CCL3 was the most effective to differentiate children with CKD and hyposalivation from patients with normal saliva secretion.

Discussion: Inflammation is involved in salivary gland dysfunction in children with CKD, although further studies on in vitro and in vivo models are necessary to confirm this hypothesis.

Keywords: chronic kidney disease, saliva, inflammation, salivary gland dysfunction, hyposalivation

Introduction

Chronic kidney disease (CKD) is a multisymptomatic syndrome that results from permanent damage or a reduction in the number of active nephrons, causing a progressive decrease in glomerular filtration rate (GFR).[1,2] According to epidemiological data, the number of children with CKD is constantly increasing, as is the number of patients who require dialysis.[3,4] Unfortunately, the cost of renal replacement therapy is very high, and the progression of the disease also results in numerous systemic complications, such as cardiovascular diseases, disorders of calcium and phosphate

Received: 6 December 2022
Accepted: 25 February 2023
Published: 14 March 2023

metabolism and diseases within the oral cavity.[5,6] Oral pathologies involve both hard tissues (teeth and jawbones) and the oral mucosa. However, children with CKD mainly suffer from reduced saliva secretion (hyposalivation), which is responsible for increased incidence of dental caries, periodontal disease or oral fungal infections.[7] Importantly, periodontitis and its mediators affect the course of the underlying disease, thus making CKD more severe.[8] Yet, hyposalivation is often overlooked in routine nephrology practice, particularly when facing other health needs of a patient.[9] It should not be forgotten that saliva is the natural environment of the oral cavity, serving not only a lubricating and protective, but also antimicrobial and remineralizing function. It participates in taste recognition, water regulation and plaque formation.[10,11] Given the role of saliva in maintaining oral health, elucidating the causes of hyposalivation in children with CKD may be of great importance to researchers and clinicians.

Impaired renal function as a persistent low-grade inflammation leads to accumulation of uremic toxins in the body, increased oxidative stress or overproduction of pro-inflammatory cytokines.[12,13] However, complement components: monocyte chemoattractant protein-1 (MPC-1) and interleukins (ILs) are particularly important in CKD progression. They enhance the activity of adhesion molecules in endothelial cells of renal capillaries.[14,15] Vascular cell adhesion protein 1 (VCAM) and intercellular adhesion molecule 1 (ICAM) have been shown to bind to the receptors of activated T lymphocytes, which stimulates fibroblast activity, thus leading to renal tissue fibrosis and progression of CKD.[14–16] However, little is known about the causes of salivary gland hypofunction in the course of CKD, particularly in the population of children and adolescents. Although previous studies have confirmed the involvement of oxidative stress in the pathogenesis of salivary gland dysfunction in children with CKD,[9,17] the exact cause of these disorders is unknown. Despite the undoubted role of inflammation in the pathogenesis of CKD,[14,18] the literature to date lacks studies assessing the role of salivary cytokines, chemokines and growth factors in hyposalivation in children with CKD. The presented study is the first to evaluate the salivary inflammatory and anti-inflammatory profile in unstimulated saliva of CKD children at different stages of renal failure compared to healthy children. Our study may contribute to understanding the causes of salivary gland hypofunction that considerably impairs the quality of life of children with CKD. Moreover, given the increased interest in saliva in the field of laboratory medicine,[19,20] an additional goal of this study was to evaluate the diagnostic utility of salivary cytokines, chemokines and growth factors in young patients with CKD. Saliva is an excellent diagnostic material because it is non-infectious, readily available and collected in a non-invasive manner, which is particularly important in pediatrics.[21,22]

Materials and Methods

Patients

The study was conducted in accordance with the Declaration of Helsinki. The researchers obtained approval from the Local Bioethics Committee in Bialystok (permission number R-I-002/43/2018), and all participants and/or their legal guardians gave their written consent to take part in the experiment.

Only children with normal weight (body mass index (BMI) adequate for age and height) were included in the study. The exclusion criteria in both groups were: the presence of chronic autoimmune diseases (diabetes mellitus, Sjogren's syndrome, systemic sclerosis, rheumatoid arthritis, lupus, psoriasis), as well as infectious (viral and bacterial), lung, thyroid, liver, gastrointestinal tract, oral cavity and periodontium (including active caries) diseases, and taking such medications as corticosteroids, non-steroidal anti-inflammatory drugs (NSAIDs), hormones and antibiotics, as well as vitamins and dietary supplements for a period of 3 months before the test.

Children with CKD (n = 30) treated at the Department of Pediatrics and Nephrology of the Children's Hospital of the Medical University of Bialystok were qualified for the study. CKD was defined according to the Kidney Disease Improving Global Outcomes (KDIGO) criteria based on different Eger distribution.[23] The estimated glomerular filtration rate (eGFR) was calculated pursuant to the updated Schwartz formula: eGFR (mL/min/1.73 m^2) = 0.413 x [height in cm/sCr].[24] Renal function was also assessed by serum creatinine (sCr) and urea levels, as well as urine protein levels (albuminuria, proteinuria). Upon being diagnosed with CKD, all patients were on a renal diet that was low in sodium, phosphorous and/or protein depending on patients' condition and CKD stage.[25]

The control group consisted of generally healthy children (n = 30) coming for follow-up visits to the Specialized Dental Clinic of the Medical University of Bialystok. The controls were matched by age and gender to the study group.

Material from the study and control group was collected in parallel from January 2018 to January 2022. After collecting material from children with CKD, appropriate control samples were selected.

Sialochemistry

The study material was whole unstimulated saliva collected from children via the spitting method.[21,26] Saliva was obtained between 7 and 9 a.m., one day after admission to the Department of Pediatrics and Nephrology at the Children's Hospital of the Medical University of Bialystok, or during a routine dental visit. Samples were taken in a separate, child-friendly room to ensure maximum patient comfort. After rinsing the mouth with deionized water at room temperature and taking a 5-minute break, the children spat out saliva accumulated at the bottom of the mouth into a Falcon tube placed in an ice container. The collection time for saliva samples was 10 min. The time that had passed since their last meal, tooth brushing and medication intake was at least 8 h.[21,26] Immediately after sample collection, the volume of saliva was measured using a calibrated pipette with the accuracy of 100 µL. Next, salivary flow rate (SFR) was calculated by dividing the volume of the sample by the time necessary to collect it (mL/min).

The saliva samples were then centrifuged (MPW 351, MPW Med. Instruments, Warszawa, Poland; 5000 x g, 20 min, 4°C). To prevent sample oxidation, 0.5 M butylated hydroxytoluene (10 µL/mL) was added to the saliva.[21,26] The obtained supernatants were frozen (−80°C) and preserved for further determinations.

Hyposalivation (reduced saliva secretion) was defined as SFR below 0.2 mL/min.[9,27] In all control children, SFR was above 0.2 mL/min. To evaluate the salivary gland function, total protein (TP) content and salivary amylase activity were also determined in saliva samples. TP content was assayed via the bicinchoninic (BCA) method using a commercial Thermo Scientific PIERCE BCA Protein Assay kit (Rockford, IL, USA).[28] Salivary amylase activity (SA, EC 3.2.1.1) was assayed colorimetrically using 3',5'-dinitrosalicylic acid (DNS).[29] The determinations were performed in duplicate samples. Transferrin concentration was also measured in saliva samples to identify those containing blood.[30] However, transferrin was not detected in any of the samples.

After saliva collection, dental examinations were performed. According to the criteria of the World Health Organization,[31] they were conducted in artificial lighting by means of a mirror, an explorer and a periodontal probe. Every examination was conducted by the same pediatric dental specialist (J. S.) and included the determination of DMFT (decayed, missing, filled teeth) and SBI (Sulcus Bleeding Index) indices according to Muhlemann and Son,[32] GI (Gingival Index) according to Löe and Silness[33] as well as API (Approximal Plaque Index) according to Lange.[34] The DMFT index is the sum of teeth with caries (D), teeth extracted due to caries (M), and teeth filled because of caries (F). DMFT was also calculated for deciduous teeth (dmft). The PBI shows the intensity of bleeding from the gingival sulcus after probing. GI criteria include qualitative changes in the gingiva, and API determines the percentage of tooth surface with plaque. API was assessed at teeth 11, 22, 16, 26, 36, 41, 32, 36 and 46. Periodontal indicators were examined at the anterior tangent, posterior tangent, buccal side and lingual side. All teeth were not assessed due to the discomfort caused to the child and the fact that not all permanent teeth are present in children. In 15 patients, the inter-rater agreements between the examiner (J. S.) and another experienced pedodontist (A. Z.) were assessed. The reliability for DMFT was r = 0.96; for SBI: r = 0.95; for GI: r = 0.93 and for API: r = 0.98.

Salivary Inflammatory and Anti-Inflammatory Profile

Salivary inflammatory and anti-inflammatory profile were assayed using the Bio-Plex Pro Human Cytokine 27-plex Assay commercial diagnostic kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA) (Figure 1). Bio-Plex assay is a multiplex ELISA test in which captured antibodies directed against a specific biomarker are covalently bound to magnetic beads. The coupled beads then react with a sample containing the selected biomarker. A series of rinses is performed to remove the unbound protein. Next, the biotinylated detection antibody is added to form a sandwich complex. The final complex is formed by adding a streptavidin-phycoerythrin conjugate, and the result is read using a specialized plate reader (Bio-Plex 200). The effectiveness of the method can be compared to a typical ELISA.[35]

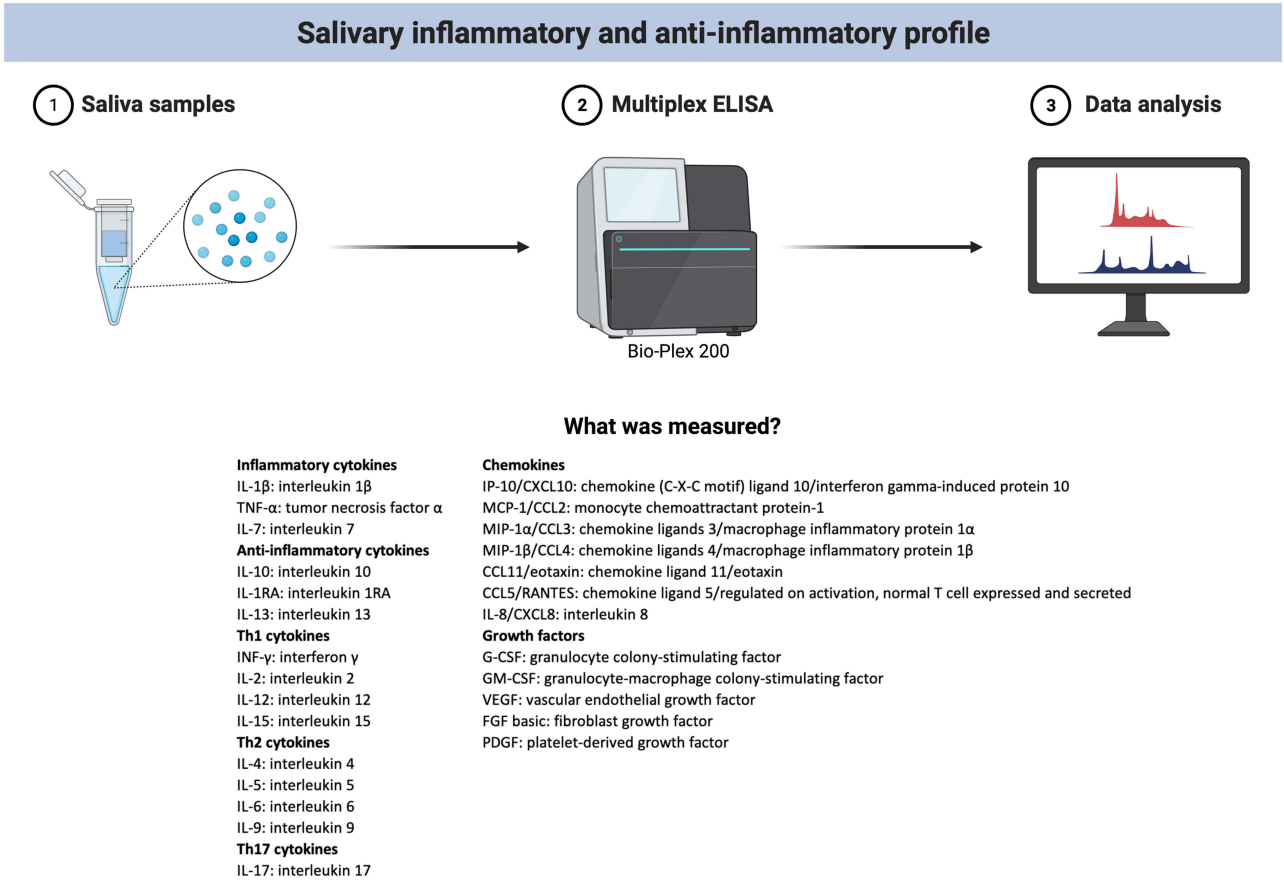

Figure 1 Measurement of salivary inflammatory and anti-inflammatory profile by multiplex ELISA. Created with BioRender.com.

Statistics

The analysis of the obtained data was performed by means of the GraphPad Prism 8.4.3. statistical software for MacOS (GraphPad Software, La Jolla, USA) using the Shapiro–Wilk test to assess normality of distribution, and the Levene's test to evaluate homogeneity of variance. Mann–Whitney *U*-test was used to compare the groups; its results are presented in box plots as the median (minimum – maximum). Concentrations of evaluated cytokines, chemokines and growth factors below detection levels were not included in the analysis. Correlations between the biomarkers and secretory function of the salivary glands (SFR, TP, SA) were assessed using the Spearman correlation coefficient. The multivariate analysis of simultaneous impact of many independent variables on one quantitative dependent variable was carried out by means of linear regression, and 95% confidence intervals were reported along with regression parameters. The analysis of the diagnostic utility of salivary cytokines, chemokines and growth factors was assessed by examining the receiver operating characteristic (ROC). The value of $p < 0.05$ was considered statistically significant in all tests.

The number of patients in the groups (test and control) was determined based on previously conducted our pilot study (n = 15). Variables used for sample size calculation were salivary TNF-α, IL-5, and IL-10. The *ClinCalc* online calculator was used, and the value of 0.8 (α = 0.05) was determined as the power of the test. The minimum number of patients per group was 25; as a result, 30 patients were qualified for the study.

Results

Clinical Data

The patients' clinical data are presented in Table 1. SFR, TP and SA activity were significantly decreased in children with CKD compared to the controls. Hyposalivation was found in 46.67% of children with CKD. However, children with CKD and healthy children did not differ in terms of oral hygiene and periodontal status. Low values of periodontal and

Table 1 Clinical Data of Children with Chronic Kidney Disease (CKD) Compared to Healthy Children (C)

	C (n = 30)	CKD (n = 30)	*P*-value
Sex (male/female)	16/14	16/14	ND
Age	13 (9–16)	13 (9–16)	>0.9999
Reasons of CKD			
Glomerulopathies n (%)	0 (0)	10 (33.3)	ND
Urologic defects n (%)	0 (0)	9 (30)	ND
Nephropathies n (%)	0 (0)	5 (16.7)	ND
Renal dysplasia n (%)	0 (0)	5 (16.7)	ND
Undetermined etiology n (%)	0 (0)	1 (3.3)	ND
Kidney function			
Serum Cr (mg/dL)	0.397 (0.3595–0.4642)	1.66 (0.9575–3.753)	**<0.0001**
Serum Urea (mg/dL)	17.38 (15.77–19.96)	58 (29.75–113)	**<0.0001**
eGFR (mL/min/1.73 m^2)	136.1 (131.2–141.5)	38.74 (16.2–89.6)	**<0.0001**
Albuminuria (mg/24 h)	ND	121.2 (10.38–335.5)	ND
Proteinuria (mg/24 h)	ND	150 (95.25–1029)	ND
Biochemical tests			
Hemoglobin (g/dL)	ND	11.7 (10.6–14.1)	ND
Hematocrit (%)	ND	36.35 (31.38–39.33)	ND
Iron (μg/dL)	ND	82 (55.5–90)	ND
Drugs			
ACEI n (%)	0 (0)	20 (66.7)	ND
β-blockers n (%)	0 (0)	7 (23.3)	ND
CCB n (%)	0 (0)	8 (26.7)	ND
Loop diuretics n (%)	0 (0)	21 (70)	ND
Iron n (%)	0 (0)	18 (60)	ND
Sialochemistry			
SFR (mL/min)	0.475 (0.4–0.6175)	0.225 (0.1475–0.35)	**<0.0001**
TP (μg/mL)	1814 (1550–2054)	1280 (1041–1590)	**<0.0001**
SA (μg/mg protein)	0.225 (0.1775–0.3)	0.09 (0.07–0.1125)	**<0.0001**
Hyposalivation n (%)	0 (0)	14 (46.67)	ND
Dental examination			
DMFT	10 (2–12)	10 (1–12)	>0.9999
dmft	3 (0–4)	3 (0–4)	>0.9999
PBI	0.2 (0–0.4)	0.2 (0–0.3)	>0.9999

(*Continued*)

Table 1 (Continued).

	C (n = 30)	CKD (n = 30)	*P*-value
GI	0.1 (0–0.2)	0.1 (0–0.2)	>0.9999
API (%)	20	23	>0.9999

Notes: Data are presented as the median (minimum – maximum). Bold indicates statistically significant results.

Abbreviations: ACEI, angiotensin converting enzyme inhibitors; API, Approximal Plaque Index; CCB, calcium channel blockers; dmft, decayed, missing, filled teeth index for deciduous teeth; DMFT, decayed, missing, filled teeth index for permanent teeth; eGFR, estimated glomerular filtration rate using Schwartz formula; Cr, creatinine; GI, gingival index; PBI, papilla bleeding index; SA, salivary amylase; SFR, salivary flow rate; TP, total protein.

oral hygiene indicators indicate good oral health and the absence of periodontal disease. Importantly, none of the children had active caries.

Salivary Inflammatory and Anti-Inflammatory Profile

Pro-inflammatory cytokines are synthesized by granulocytes (especially lymphocytes and monocytes) and NK cells. Their secretion is a cascade process, which initiates the inflammatory response. Of the pro-inflammatory cytokines tested, TNF-α level was significantly higher, while IL-7 content – significantly lower in the saliva of children with CKD compared to healthy subjects. The concentration of salivary IL-1β did not differ between the groups (Figure 2).

Anti-inflammatory cytokines inhibit the synthesis of pro-inflammatory cytokines. They are responsible for inhibiting the activity of macrophages, monocytes and lymphocytes. The concentration of anti-inflammatory IL-10 was considerably higher in the saliva of children with CKD compared to the controls, and the concentration of IL-1RA and IL-13 did not differ between the groups (Figure 2).

The main role of Th1 lymphocytes is to participate in cell-type reactions, while Th2 lymphocytes are involved in humoral-type responses. Among Th1 cytokines, the content of IFN-γ and IL-15 was significantly higher in the saliva of children with CKD compared to healthy ones. Salivary IL-12 and IL-13 concentrations were not statistically different between the groups (Figure 2). The levels of all Th2 cytokines assayed (IL-4, IL-5, IL-6 and IL-9) were significantly elevated in the saliva of children from the study group compared to healthy controls (Figure 2).

The concentration of Th17 cytokines (IL-17) was not statistically different between the groups (Figure 2).

Chemokines are small-molecule peptides that are classified as cytokines with chemotactic properties. Of the chemokines tested, the levels of salivary MCP-1/CCL-2, MIP-1α/CCL-3 and EOTAXIN/CCL11 were significantly higher, while the content of MIP-1β/CCL4 – considerably lower in children with CKD compared to healthy subjects. Statistically significant changes were not observed for salivary IP-10/CXCL10 or IL-8/CXCL8 (Figure 3).

The concentration of G-CSF and FGF was notedly higher in the saliva of children with CKD compared to the controls. The content of other growth factors (GM-CSF, VEGF, PDGF) did not differ significantly between the groups (Figure 3).

Correlations

In children with CKD, salivary concentrations of most cytokines, chemokines and growth factors correlated negatively with saliva minute flow (except IL-13, IL-15 and VEGF). In addition, salivary levels of TNF-α and IL-1RA correlated inversely with total protein content, and MIP-1β/CCL4 level – with salivary amylase activity. We found no such correlations in healthy children (except IL-9, IL-12 and MIP-1β/CCL4) (Table S1).

The levels of salivary IL-1β, IL-10, INF-γ, IL-2, GM-CSF, FGF, and PDGF correlated positively with serum creatinine level in children with CKD. A positive correlation was noted between serum urea concentration and IL-10, IL-2 and IL-5 levels, while the content of IL-1β, IL-10, INF-γ, IL-17, FGF and PDGF correlated inversely with eGFR. Such correlations were not found in healthy children (IL-10 being the exception) (Table S2).

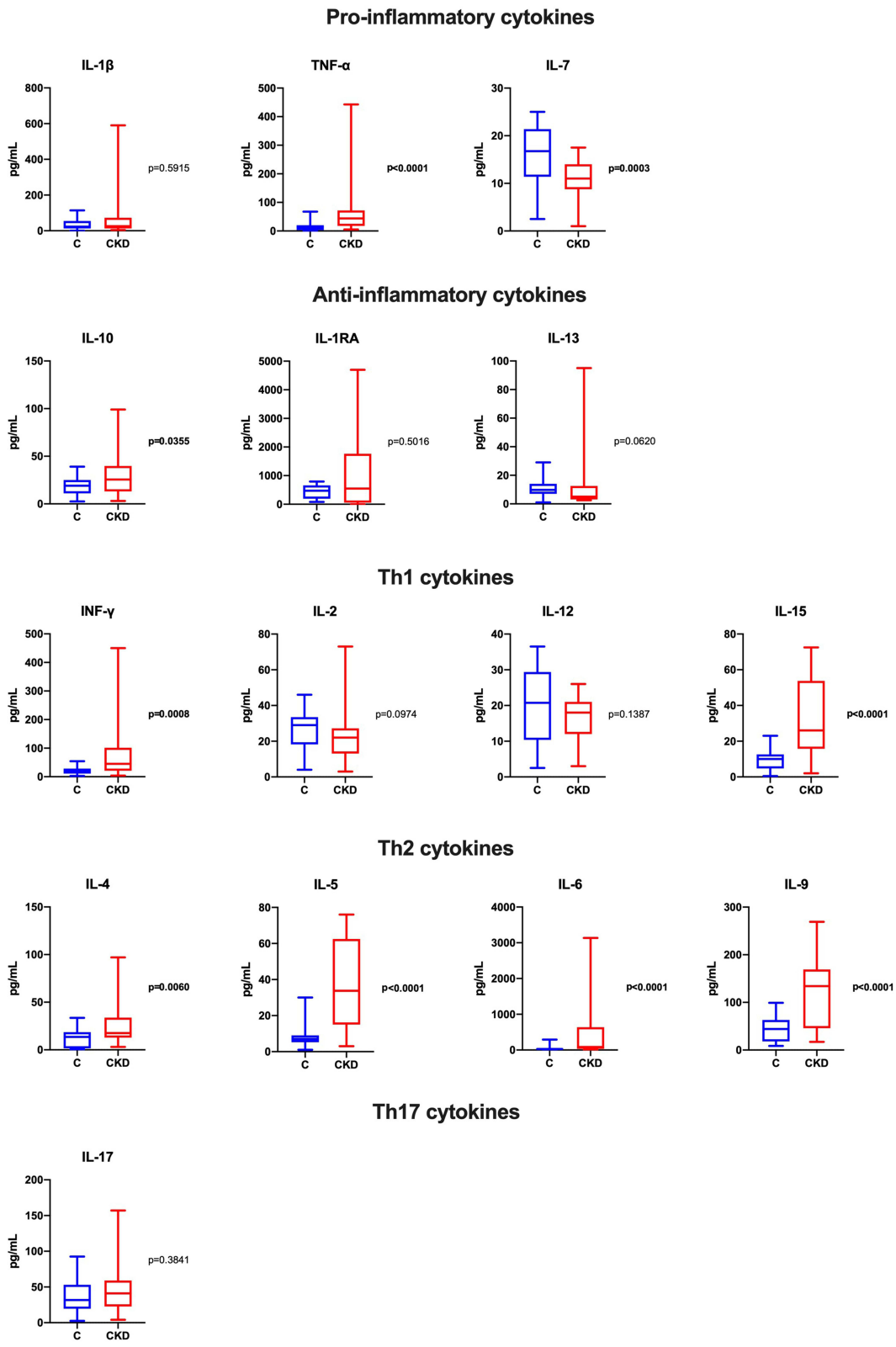

Figure 2 Concentration of salivary pro-inflammatory and anti-inflammatory cytokines as well as Th1, Th2 and Th17 in the saliva of children with chronic kidney disease (CKD) compared to healthy children (C).

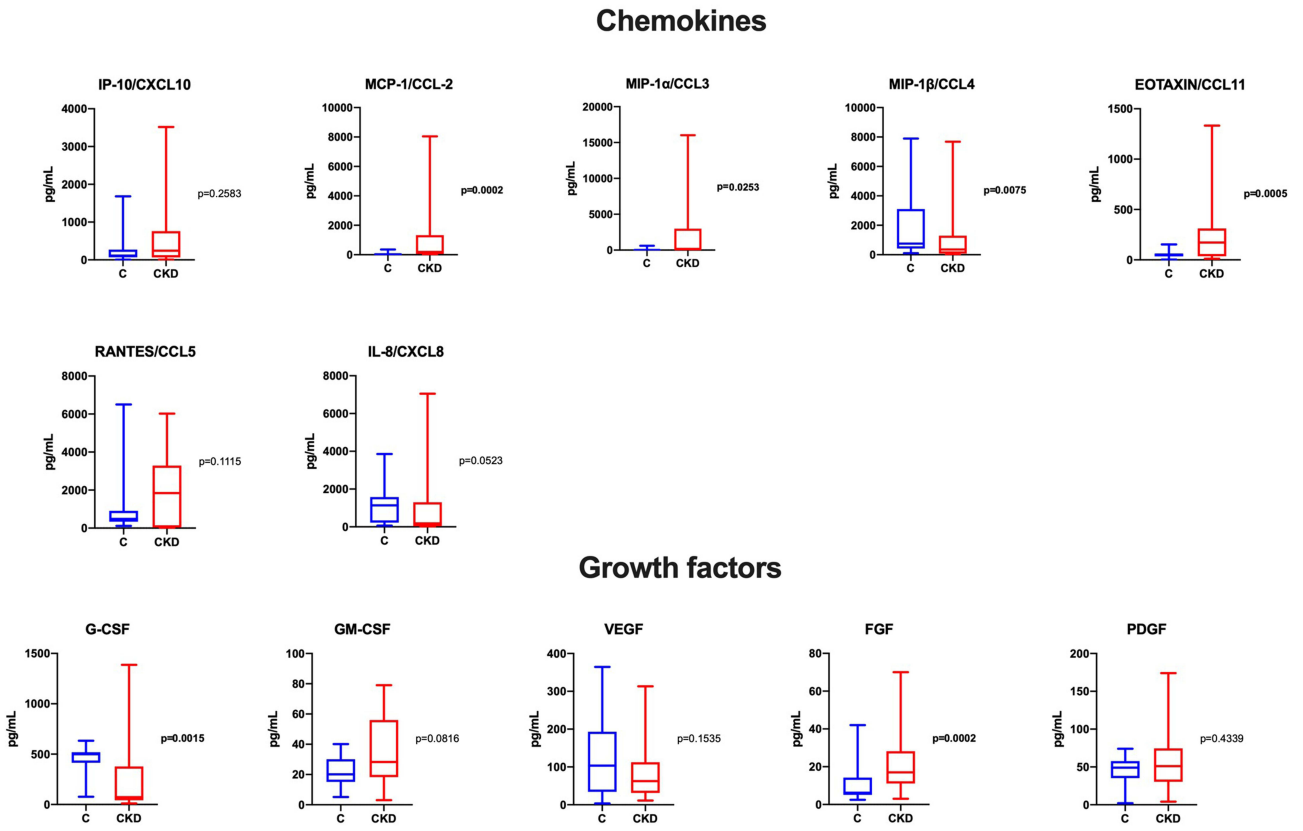

Figure 3 Concentration of salivary chemokines and growth factors in the saliva of children with chronic kidney disease (CKD) compared to healthy children (C).

Multivariate Regression Analysis

The relationship between the secretory function of the salivary glands and salivary inflammatory and anti-inflammatory profiles in children with CKD vs the controls was assessed by means of the multivariate linear regression analysis in which SFR, TP and SA were considered independent variables. We demonstrated that salivary TNF-α concentration is negatively correlated with SFR, similar to the content of IL-10, IL-1RA, INF-γ, IL-15, IL-4, IL-5, IL-6, IL-9, MIP-1α/CCL3, EOTAXIN/CCL11, GM-CSF and PDGF. The levels of salivary IL-12 and G-CSF depend positively on SA activity (Table 2).

By means of the multivariate regression analysis, also the correlation between renal function and salivary inflammatory and anti-inflammatory profiles was evaluated in children with CKD and the controls. The concentrations of creatinine and urea in serum as well as the content of eGFR were considered independent variables. We demonstrated that salivary TNF-α concentration depends positively on serum Cr, as does the content of IL-4, IL-6, MCP-1/CCL-2, MIP-1α/CCL3, G-CSF and FGF. The levels of salivary TNF-α, IL-6 and IL-9 depend negatively on serum urea and eGFR content (Table 3).

ROC Analysis

Since MIP-1α/CCL3 is most dependent on the secretory function of the salivary glands in the multivariate linear regression, we used ROC analysis to evaluate the diagnostic utility of this biomarker in differentiating children with CKD and hyposalivation from patients with normal saliva secretion. We observed high sensitivity (78.57%; 95% CI = 52.41% to 92.43%) and specificity (93.75%; 95% CI = 71.67% to 99.68%) of this parameter in differentiating children with CKD and reduced (< 0.2 mL/min) SFR from patients with normal SFR (> 0.2 mL/min) (AUC 0.8549; 95% CI 0.7051 to 1.000) (Figure 4).

Table 2 Multivariate Linear Regression Analysis Between the Secretory Function of the Salivary Glands and Salivary Inflammatory and Anti-Inflammatory Profile in Children with CKD and the Control

	β1 (SFR)			β2 (TP)			β3 (SA)		
	Estimate	**95% Confidence Interval**	***P* value**	**Estimate**	**95% Confidence Interval**	***P* value**	**Estimate**	**95% Confidence Interval**	***P* value**
IL-1β	−187.4	−395.5 to 20.78	0.0765	0.002668	−0.07026 to 0.07560	0.9415	−30.22	−445.6 to 385.2	0.884
TNF-α	−202.4	−363.4 to −41.46	**0.0147**	−0.04764	−0.1018 to 0.006485	0.0833	−34.86	−324.7 to 255.0	0.8104
IL-7	−0.4545	−11.61 to 10.70	0.9353	0.002207	−0.001570 to 0.005985	0.2467	12.25	−8.109 to 32.61	0.2332
IL-10	−40.53	−72.47 to −8.594	**0.0139**	0.0008253	−0.009683 to 0.01133	0.8753	−16.59	−81.73 to 48.55	0.6113
IL-1RA	−2139	−3771 to −507.0	**0.0111**	−0.375	−0.9275 to 0.1775	0.1794	101.6	−2876 to 3079	0.9457
IL-13	33.38	−1.523 to 68.28	0.0605	−0.001509	−0.01320 to 0.01019	0.7966	−62.22	−126.5 to 2.048	0.0575
INF-γ	−204.3	−340.5 to −68.01	**0.004**	−0.002708	−0.05011 to 0.04470	0.9092	−90.16	−333.1 to 152.8	0.4599
IL-2	−24.33	−52.92 to 4.255	0.0934	0.0005636	−0.009597 to 0.01072	0.9116	42.09	−9.277 to 93.46	0.1059
IL-12	−15.29	−30.90 to 0.3228	0.0548	−0.001702	−0.007039 to 0.003634	0.5252	52.12	22.10 to 82.14	**0.001**
IL-15	−40.46	−71.95 to −8.961	**0.0128**	−0.003247	−0.01391 to 0.007416	0.5443	−40.65	−98.12 to 16.81	0.1619
IL-4	−41.32	−72.37 to −10.26	**0.0101**	−0.002877	−72.37 to −10.27	0.5689	−22.69	−72.37 to −10.28	0.5689
IL-5	−48	−85.56 to −10.45	**0.0133**	−0.003634	−0.01594 to 0.008670	0.5558	−62.77	−136.0 to 10.45	0.0913
IL-6	−1835	−3077 to −593.9	**0.0045**	−0.1437	−0.5640 to 0.2766	0.4962	−597.8	−2863 to 1667	0.5991
IL-9	−192	−295.8 to −88.22	**0.0005**	−0.003858	−0.003858	0.8263	−189.5	−381.4 to 2.362	0.0528
IL-17	−43.39	−99.50 to 12.72	0.1269	0.002034	−0.01654 to 0.02061	0.827	−13.11	−117.6 to 91.43	0.8024
IP-10/CXCL10	−995.2	−2543 to 552.3	0.2013	−0.3874	−0.9356 to 0.1608	0.1611	1212	−1476 to 3900	0.3678
MCP-1/CCL-2	−2192	−4536 to 152.9	0.0663	0.05866	−0.7662 to 0.8835	0.887	−1893	−6111 to 2324	0.3714
MIP-1α/CCL3	−8914	−15,135 to −2692	**0.0058**	−0.4349	−2.611 to 1.741	0.6901	−2573	−13,717 to 8572	0.6901
MIP-1β/CCL4	−2672	−6862 to 1518	0.2063	−0.7304	−2.076 to 0.6155	0.2811	5787	−1816 to 13,390	0.1327
EOTAXIN/CCL11	−465.6	−826.2 to −104.9	**0.0123**	0.006195	−826.2 to −104.9	0.9194	−425.7	−1084 to 232.2	0.2002
RANTES/CCL5	−2953	−5982 to 76.61	0.0559	0.004459	−1.021 to 1.030	0.9931	−2837	−8364 to 2690	0.3082
IL-8/CXCL8	−2341	−5093 to 411.1	0.0939	−0.3955	−1.344 to 0.5530	0.407	4007	−918.1 to 8933	0.1087

(*Continued*)

https://doi.org/10.2147/JIR.S399786 DovePress

Table 2 (Continued).

	β1 (SFR)			β2 (TP)			β3 (SA)		
	Estimate	**95% Confidence Interval**	***P* value**	**Estimate**	**95% Confidence Interval**	***P* value**	**Estimate**	**95% Confidence Interval**	***P* value**
G-CSF	−650.8	−1319 to 17.66	0.0561	−0.01748	−0.2259 to 0.1910	0.8669	1219	1.654 to 2436	**0.0497**
GM-CSF	−39.61	−74.71 to −4.510	**0.0278**	−0.004322	−0.01636 to 0.007717	0.4739	−6.46	−69.63 to 56.71	0.838
VEGF	63.72	−108.6 to 236.0	0.4609	−0.003759	−0.06296 to 0.05544	0.899	−0.06296 to 0.05544	−99.88 to 524.2	0.1781
FGF	−31.4	−57.07 to −5.732	0.0174	−0.002081	−0.01078 to 0.006619	0.6335	−6.97	−55.06 to 41.12	0.7725
PDGF	−74.97	−133.9 to −16.01	**0.0138**	−0.002557	−0.02245 to 0.01733	0.7973	31.58	−72.98 to 136.1	0.5468

Note: Bold indicates statistically significant results.

Abbreviations: IL-1β, interleukin 1β; TNF-α, tumor necrosis factor α; IL-7, interleukin 7; IL-10, interleukin 10; IL-1RA, interleukin 1RA; IL-13, interleukin 13; INF-γ, interferon γ; IL-12, interleukin 12; IL-2, interleukin 2; IL-15, interleukin 15; IL-4, interleukin 4; IL-5, interleukin 5; IL-6, interleukin 6; IL-9, interleukin 9; IL-17, interleukin 17; IP-10/CXCL10, chemokine (C-X-C motif) ligand 10/interferon gamma-induced protein 10; MCP-1/CCL2, monocyte chemoattractant protein-1; MIP-1α/CCL3, chemokine ligands 3/macrophage inflammatory protein 1α; MIP-1β/ CCL4, chemokine ligands 4/macrophage inflammatory protein 1β; CCL11/eotaxin, chemokine ligand 11/eotaxin; CCL5/RANTES, chemokine ligand 5/regulated on activation, normal T cell expressed and secreted; IL-8/CXCL8, interleukin 8; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; VEGF, vascular endothelial growth factor; FGF basic, fibroblast growth factor; PDFG-BB, platelet-derived growth factor.

Table 3 Multivariate Linear Regression Analysis Between Renal Function and Salivary Inflammatory and Anti-Inflammatory Profile in Children with CKD and the Control

	β1 (Serum Cr)			β2 (Serum Urea)			β1 (eGFR)		
	Estimate	**95% Confidence Interval**	***P* value**	**Estimate**	**95% Confidence Interval**	***P* value**	**Estimate**	**95% Confidence Interval**	***P* value**
IL-1β	44,916	−14.05 to 56.29	0.2324	−0.1239	−1.640 to 1.392	0.8699	−0.1792	−1.081 to 0.7222	0.6905
TNF-α	52.04	24.16 to 79.93	**0.0004**	−1.935	−3.145 to −0.7244	**0.0023**	−0.8588	−1.562 to −0.1558	**0.0176**
IL-7	0.8119	−1.222 to 2.846	0.4273	0.0247	−0.06165 to 0.1110	0.5689	0.08248	0.03112 to 0.1338	**0.0022**
IL-10	2.909	−2.423 to 8.240	0.2785	0.1139	−0.1124 to 0.3403	0.317	0.03804	−0.09715 to 0.1732	0.5746
IL-1RA	96.21	−227.7 to 420.1	0.5542	1.584	−12.16 to 15.33	0.8183	−3.896	−12.07 to 4.282	0.344
IL-13	−4.296	−10.75 to 2.162	0.1876	−0.09585	−0.3711 to 0.1794	0.4875	−0.2638	−0.4483 to -0.07933	0.006
INF-γ	22.32	−2.810 to 47.44	0.0805	−0.4778	−1.544 to 0.5884	0.3727	−0.48	−1.118 to 0.1581	0.1372
IL-2	3.643	−1.253 to 8.538	0.141	0.01742	−0.1902 to 0.2251	0.141	0.103	−0.02489 to 0.2309	0.1118
IL-12	0.7658	−2.362 to 3.894	0.6255	0.07919	−0.05360 to 0.2120	0.2371	0.1001	0.02093 to 0.1792	0.0142
IL-15	1.884	−4.024 to 7.792	0.5256	0.1551	−0.09568 to 0.4059	0.2205	−0.01344	−0.1626 to 0.1357	0.8574
IL-4	7.024	1.428 to 12.62	**0.0149**	−0.1557	−0.3934 to 0.08203	0.1947	−0.06229	−0.2039 to 0.07933	0.3818
IL-5	0.6738	−5.746 to 7.094	0.834	0.2168	−0.05602 to 0.4896	0.1168	−0.07113	−0.2341 to 0.09181	0.3849
IL-6	354.1	139.3 to 568.8	**0.0017**	−10.69	−19.81 to −1.574	**0.0224**	−5.607	−11.03 to −0.1835	**0.043**
IL-9	20.62	−0.9466 to 42.18	0.0606	−0.8282	−1.743 to 0.08672	0.0751	−0.735	−1.281 to −0.1892	**0.0092**
IL-17	7.899	−2.035 to 17.83	0.1167	0.005244	−0.4165 to 0.4270	0.9802	0.08929	−0.1638 to 0.3423	0.4823
IP-10/CXCL10	107.5	−156.2 to 371.1	0.4151	−1.574	−12.75 to 9.605	0.7775	−1.396	−8.427 to 5.635	0.6905
MCP-1/CCL-2	1056	735.2 to 1377	**<0.0001**	−28.8	−43.07 to −14.52	**0.0002**	−0.1557	−9.174 to 8.863	0.9725
MIP-1α/CCL3	2042	1040 to 3044	**0.0001**	−59.6	−102.1 to −17.07	0.0069	−22.5	−47.93 to 2.940	0.0818
MIP-1β/CCL4	558.3	−213.2 to 1330	0.1524	−13.04	−45.78 to 19.71	0.4279	5.962	−13.62 to 25.55	0.5438
EOTAXIN/CCL11	47.22	−22.94 to 117.4	0.183	−0.5897	−3.568 to 2.389	0.6931	−0.9001	−2.672 to 0.8715	0.3132
RANTES/CCL5	708.3	123.3 to 1293	0.0185	−24.94	−49.77 to −0.1067	0.0491	−6.988	−21.76 to 7.783	0.3474
IL-8/CXCL8	390.6	−131.0 to 912.3	0.1391	−10.76	−32.91 to 11.38	0.3342	−2.281	−15.46 to 10.90	0.7301

(*Continued*)

https://doi.org/10.2147/JIR.S399786 DovePress

Table 3 (Continued).

	β1 (Serum Cr)			β2 (Serum Urea)			β1 (eGFR)		
	Estimate	**95% Confidence Interval**	***P* value**	**Estimate**	**95% Confidence Interval**	***P* value**	**Estimate**	**95% Confidence Interval**	***P* value**
G-CSF	186.2	80.76 to 291.7	**0.0009**	−5.799	−10.28 to −1.324	**0.0122**	0.8821	−1.821 to 3.585	0.5151
GM-CSF	4.503	−1.198 to 10.20	0.1188	0.06043	−0.1814 to 0.3023	0.6177	0.02139	−0.1261 to 0.1688	0.7718
VEGF	−7.344	−38.18 to 23.49	0.6343	0.862	−0.4460 to 2.170	0.1915	0.7562	−0.04027 to 1.553	0.0623
FGF	5.603	1.065 to 10.14	**0.0165**	−0.0919	−0.2845 to 0.1007	0.343	−0.0314	−0.1464 to 0.08365	0.5865
PDGF	34151	−2.252 to 18.11	0.1241	0.03751	−0.3949 to 0.4699	0.8624	0.07878	−0.1829 to 0.3404	0.5481

Abbreviations: IL-1β, interleukin 1β; TNF-α, tumor necrosis factor α; IL-7, interleukin 7; IL-10, interleukin 10; IL-1RA, interleukin 1RA; IL-13, interleukin 13; INF-γ, interferon γ; IL-12, interleukin 12; IL-2, interleukin 2; IL-15, interleukin 15; IL-4, interleukin 4; IL-5, interleukin 5; IL-6, interleukin 6; IL-9, interleukin 9; IL-17, interleukin 17; IP-10/CXCL10, chemokine (C-X-C motif) ligand 10/interferon gamma-induced protein 10; MCP-1/CCL2, monocyte chemoattractant protein-1; MIP-1α/CCL3, chemokine ligands 3/macrophage inflammatory protein 1α; MIP-1β/ CCL4, chemokine ligands 4/macrophage inflammatory protein 1β; CCL11/eotaxin, chemokine ligand 11/eotaxin; CCL5/RANTES, chemokine ligand 5/regulated on activation, normal T cell expressed and secreted; IL-8/CXCL8, interleukin 8; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; VEGF, vascular endothelial growth factor; FGF basic, fibroblast growth factor; PDFG-BB, platelet-derived growth factor. Bold indicates statistically significant results.

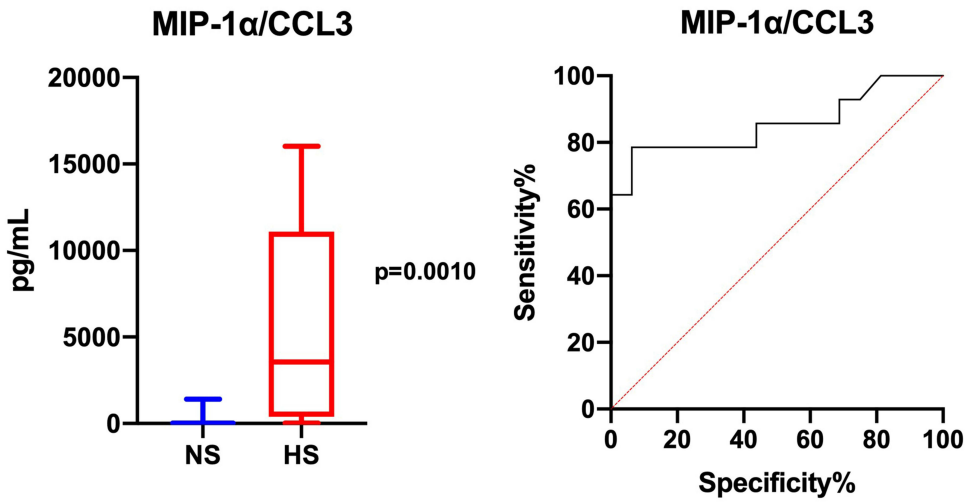

Figure 4 Analysis of the diagnostic utility of salivary MIP-1α/CCL3 in differentiating children with CKD and normal saliva secretion (NS) from patients with hyposalivation (HS).

Discussion

The presented study is the first to evaluate the salivary inflammatory and anti-inflammatory profile in children with CKD. We demonstrated statistically significant changes in salivary levels of pro-inflammatory cytokines (↑TNF-α, ↓IL-7), anti-inflammatory cytokines (↑IL-10), Th1 (↑INF-γ, ↑IL-15), Th2 (↑IL-4, ↑IL-5, ↑IL-6, ↑IL-9) and Th17 (IL-17) cytokines as well as chemokines (↑MCP-1/CCL-2, ↑MIP-1α/CCL3, ↓MIP-1β/CCL4, ↓EOTAXIN/CCL11) and growth factors (↑G-CSF, ↑FGF) in unstimulated saliva of children with CKD compared to the controls.

Despite tremendous advances in nephrology, it is still a major problem to ensure long-term survival of children with CKD without developing organ complications.[3,4] The results of numerous epidemiological studies indicate that CKD is a direct cause of not only kidney and cardiovascular diseases, as children with CKD have an increased susceptibility to gum and periodontal disease, fungal infections, olfactory and taste disorders, halitosis and salivary gland dysfunction.[5–7,36] The most common oral complications of CKD include hyposalivation, the main consequence of which is progressive caries. This is caused by an impaired mechanism of self-cleaning of tooth surfaces by saliva flowing around them, as well as changes in the qualitative composition of the latter.[7,37] Indeed, patients with CKD have been found to have a deficiency of salivary buffering systems (carbonate and phosphate buffer), as well as changes in the rheological parameters of saliva (increased viscosity) and an increase in its pH.[6,7,9] However, the causes of salivary gland dysfunction in children with CKD are unknown. Although water-electrolyte imbalances undoubtedly play a role here,[27] it is speculated that inflammation may also contribute to hyposalivation and impaired protein secretion into saliva. The results of recent studies indicate the involvement of salivary cytokines, chemokines and growth factors in impairing the secretory function of the salivary glands in patients with chronic heart failure,[38] Hashimoto's disease,[39] psoriasis[40,41] or Sjogren's syndrome.[42,43] Since hyposalivation significantly reduces patients' quality of life,[44] determining its causes in children with CKD may be of great importance to both patients and physicians.

Substances in saliva can be synthesized in the salivary glands or transported from the blood by passive or specific transport, or ultrafiltration.[45,46] Although we did not evaluate the inflammatory profile in the plasma/serum of children with CKD, it is well known that most cytokines are not stored, but produced at the target site (salivary glands) in response to an internal or external stimulus.[46,47] Therefore, cytokines in the saliva are most likely of salivary-gland origin. This hypothesis can be confirmed by the negative correlation between the content of salivary cytokines, chemokines and growth factors and SFR in children with CKD (exception: IL-13, IL-15, VEGF). We did not reveal such a relationship in healthy children (except IL-9, IL-12, MIP-1β/CCL4). This may also indicate the involvement of inflammation in salivary gland dysfunction in children with CKD. The multivariate regression analysis also proved that

the content of salivary cytokines, chemokines and growth factors depends on SFR as well as TP and SA. It is suggested that salivary cytokines may originate from inflammatory cells infiltrating the salivary glands. It is commonly known that cytokines are released by stimulated macrophages and endothelial cells in the inflammatory microenvironment of the salivary glands.[48] In patients with Sjogren's syndrome, salivary level of IL-6 has been shown to correlate positively with the size of lymphocytic infiltration in the labial salivary glands.[38,49,50] IL-6 enhances the local inflammatory process by inducing T-lymphocyte proliferation and B-lymphocyte differentiation, and reducing the number of Treg lymphocytes.[38,51,52] It has been observed that increased secretion of IL-1, TNF-α and INF-γ inhibits acetylcholine release in the salivary glands, causing impairment of the response of acinar cell, and is thus responsible for hyposalivation.[38,53] In addition, INF-γ decreases mucin production in the salivary acinar cells,[54] which may be an explanation of the changes in saliva viscosity in patients with CKD.[55]

Although the evaluation of the salivary inflammatory profile does not indicate a particular dominance of any of the immune system branches, we observed a statistically significant increase in the concentration of all Th2 cytokines assayed (↑IL-4, ↑IL-5, ↑IL-6, ↑IL-9). Th2 cells are known to mainly produce anti-inflammatory cytokines and induce the humoral type responses. Indeed, Th2 lymphocytes, which produce IL-4 and IL-13, contribute to the development of M2 macrophages which demonstrate anti-inflammatory activity.[56,57] The increase in salivary Th2 (↑IL-4, ↑IL-5, ↑IL-6, ↑IL-9) and anti-inflammatory (↑IL-10) cytokines may indicate an adaptive response of the salivary glands to local inflammation in children with CKD. Moreover, MIP-1α/CCL3 is also noteworthy because, along with other chemoattractants, it is aimed at guiding leukocytes to the salivary gland inflammatory focus.[58] A linear regression analysis showed that the concentration of MIP-1α/CCL3 in saliva is most dependent on the secretory function of the salivary glands. Therefore, we used ROC analysis to evaluate the diagnostic utility of MIP-1α/CCL3 in differentiating children with CKD and hyposalivation (<0.2 mL/min) from patients with normal saliva secretion (>0.2 mL/min). We demonstrated that this parameter differentiated children with CKD according to SFR (AUC = 0.85) with high sensitivity (>78%) and specificity (>93%). Although our study was purely observational, the exclusion of oral and periodontal diseases (including active caries) as well as autoimmune (diabetes, Sjogren's syndrome, systemic scleroderma, rheumatoid arthritis, lupus, psoriasis), infectious (viral and bacterial) and other systemic diseases in patients may point to an inflammatory etiology of salivary gland hypofunction in the course of CKD. Since it is unethical to harvest salivary glands from children with CKD, further studies on in vitro and animal models are needed to mechanistically elucidate the role of cytokines, chemokines and growth factors in salivary gland hypofunction under conditions of renal failure.

CKD is a serious clinical problem in children and adolescents, as evidenced by the high prevalence of CKD in the general population, as well as the asymptomatic course of the disease. Indeed, in this group of patients, CKD is often diagnosed at its end stage that already requires renal replacement therapy.[3,59] Moreover, the progression of kidney damage in children results in significantly increased risk of cardiovascular disease.[3,59] This can be counteracted by early diagnosis of the disease as well as early initiation of nephroprotective treatment. To determine renal function, it is common practice to assess serum creatinine concentration and calculate the eGFR index.[23] However, the use of this index in children is limited due to dependence of creatinine content on muscle mass and differences in tubular reabsorption.[60] Not surprisingly, the use of saliva to assess classical CKD biomarkers has been postulated. Of particular interest seem to be salivary urea and creatinine, although the results of previous studies are not convergent.[22,61] Therefore, new biomarkers are still being sought to provide information on the disruptions of the renal function already at the early stages of CKD.[62]

An additional aim of our study was to evaluate the diagnostic utility of salivary cytokines, chemokines and growth factors in children with CKD. We demonstrated that salivary concentration of IL-1β, IL-10, INF-γ, IL-2, GM-CSF, FGF and PDGF correlates positively with the level of serum creatinine in children with CKD. We noted a positive correlation with serum urea concentration for IL-10, IL-2 and IL-5, while the levels of IL-1β, IL-10, INF-γ, IL-17, FGF and PDGF correlated inversely with eGFR. We found no such correlations in healthy children. The multivariate linear regression analysis also confirmed the diagnostic utility of the biomarkers evaluated. It is well known that linear regression models are the best tool in biomedical sciences to determine the relationship between a quantitative trait and a fixed set of independent variables.[63] We showed that salivary TNF-α concentration depends positively on serum Cr, as do the levels

of IL-4, IL-6, MCP-1/CCL-2, MIP-1α/CCL3, G-CSF and FGF. In contrast, salivary levels of TNF-α, IL-6 and IL-9 correlate negatively with eGFR. However, these results should be interpreted with caution, as the biomarkers assessed are not specific to CKD alone. Assessment of the content of salivary cytokines, chemokines and growth factors may be of limited diagnostic value in children with salivary gland hypofunction or other comorbidities; therefore, further studies on a larger population of children with CKD are needed in this respect. Since the main sources of oral inflammation are periodontal disease and caries, it is also necessary to evaluate the salivary inflammatory profile in children with CKD and concomitant oral diseases.[22,64]

The limitations of our work should also be mentioned. One of them was undoubtedly the small number of children with CKD, although – according to the sample size calculation – it was sufficient for analysis. Only children without oral or general diseases (including autoimmune diseases) were included in the study, which enables reliable assessment of the salivary gland function in the course of CKD. Due to ethical restrictions of the study, we could not collect the salivary glands for examination, which meant that we only assessed changes in the circulating inflammatory biomarkers. However, we analyzed them in close relation to biological functions (secretory function of the salivary glands). Since the evaluation of inflammatory and anti-inflammatory factors in unstimulated saliva mainly reflects the secretory function of the submandibular glands,[65] further research is needed to evaluate the inflammatory profile in stimulated saliva as well as in blood (plasma/serum) and in vitro and in vivo models. Furthermore, we could not eliminate the effect of the taken medications on the evaluated salivary biomarkers.

Conclusions

1. Children with CKD experience salivary gland dysfunction manifested as hyposalivation and decreased secretion of salivary proteins.
2. Children with CKD demonstrate increased production of salivary cytokines, chemokines and growth factors, the concentration of which significantly depends on the secretory function of the salivary glands.
3. Among the biomarkers evaluated, salivary MIP-1α/CCL3 best differentiates children with CKD and hyposalivation from patients with normal saliva secretion.
4. The evaluation of the salivary inflammatory profile in children with CKD does not indicate a particular dominance of any of the immune system branches, although we observed an increase in salivary levels of all Th2 cytokines assayed.
5. Further studies on in vitro and animal models are needed to mechanistically elucidate the involvement of inflammatory processes in salivary-gland hypofunction under conditions of renal failure.

The study's conclusions are also presented graphically in Figure 5.

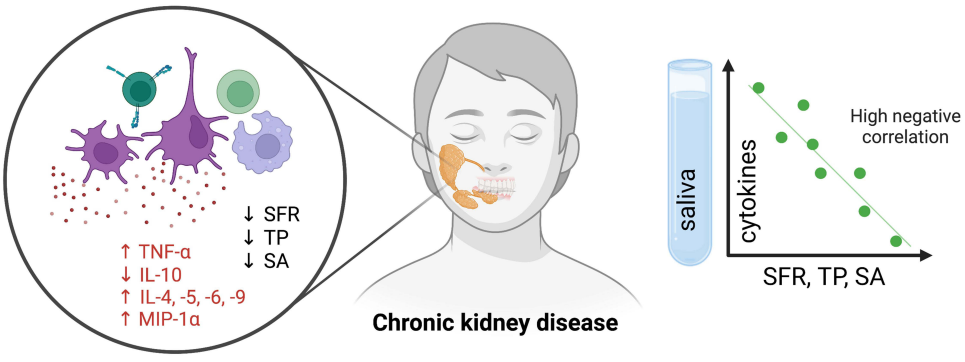

Figure 5 Graphical conclusions from the study. Created with BioRender.com.

Data Sharing Statement

The original contributions presented in the study are included in the paper. Further inquiries can be directed to the corresponding author on reasonable request.

Ethics Statement

The studies involving human participants were approved by the Bioethics Committee of the Medical University of Bialystok, Poland (permission number R-I-002/43/2018). The patients/their legal guardians provided written informed consent to participate in this study.

Funding

This work was supported by the Medical University of Bialystok, Poland (Grant No. SUB/1/DN/22/002/3330) and National Science Center, Poland (Grant No. 2021/05/X/NZ5/01644).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Kovesdy CP. Epidemiology of chronic kidney disease: an update 2022. *Kidney Int Suppl*. 2022;12(1):7–11. doi:10.1016/j.kisu.2021.11.003
2. López-Novoa JM, Martínez-Salgado C, Rodríguez-Peña AB, Hernández FJL. Common pathophysiological mechanisms of chronic kidney disease: therapeutic perspectives. *Pharmacol Ther*. 2010. doi:10.1016/j.pharmthera.2010.05.006
3. Harada R, Hamasaki Y, Okuda Y, Hamada R, Ishikura K. Epidemiology of pediatric chronic kidney disease/kidney failure: learning from registries and cohort studies. *Pediatr Nephrol*. 2022;37(6):1215–1229. doi:10.1007/s00467-021-05145-1
4. Masalskienė J, Rudaitis Š, Vitkevič R, Čerkauskienė R, Dobilienė D, Jankauskienė A. Epidemiology of chronic kidney disease in children: a report from Lithuania. *Medicina*. 2021;57:2. doi:10.3390/medicina57020112
5. Oyetola EO, Owotade FJ, Agbelusi GA, Fatusi OA, Sanusi AA. Oral findings in chronic kidney disease: implications for management in developing countries. *BMC Oral Health*. 2015;15(1):24. doi:10.1186/s12903-015-0004-z
6. Anuradha B, Katta S, Kode V, et al. Oral and salivary changes in patients with chronic kidney disease: a clinical and biochemical study. *J Indian Soc Periodontol*. 2015. doi:10.4103/0972-124x.154178
7. Velan E, Sheller B. Oral health in children with chronic kidney disease. *Pediatr Nephrol*. 2021;36(10):3067–3075. doi:10.1007/s00467-020-04913-9
8. Deschamps-Lenhardt S, Martin-Cabezas R, Hannedouche T, Huck O. Association between periodontitis and chronic kidney disease: systematic review and meta-analysis. *Oral Dis*. 2019;25(2):385–402. doi:10.1111/odi.12834
9. Maciejczyk M, Szulimowska J, Taranta-Janusz K, Wasilewska A, Zalewska A. Salivary gland dysfunction, protein glycooxidation and nitrosative stress in children with chronic kidney disease. *J Clin Med*. 2020;9:5. doi:10.3390/jcm9051285
10. Roblegg E, Coughran A, Sirjani D. Saliva: an all-rounder of our body. *Eur J Pharm Biopharm*. 2019;142:133–141. doi:10.1016/j.ejpb.2019.06.016
11. Pedersen AML, Sørensen CE, Proctor GB, Carpenter GH, Ekström J. Salivary secretion in health and disease. *J Oral Rehabil*. 2018. doi:10.1111/joor.12664
12. Dhande IS, Braun MC, Doris PA. Emerging insights into chronic renal disease pathogenesis in hypertension from human and animal genomic studies. *Hypertension*. 2021;78(6):1689–1700. doi:10.1161/HYPERTENSIONAHA.121.18112
13. Tomino Y. Pathogenesis and treatment of chronic kidney disease: a review of our recent basic and clinical data. *Kidney Blood Press Res*. 2014. doi:10.1159/000368458
14. Stenvinkel P, Chertow GM, Devarajan P, et al. Chronic inflammation in chronic kidney disease progression: role of Nrf2. *Kidney Int Rep*. 2021;6(7):1775–1787. doi:10.1016/j.ekir.2021.04.023
15. Mihai S, Codrici E, Popescu ID, et al. Inflammation-related mechanisms in chronic kidney disease prediction, progression, and outcome. *J Immunol Res*. 2018;2018:2180373. doi:10.1155/2018/2180373
16. Akchurin OM, Kaskel F. Update on Inflammation in Chronic Kidney Disease. *Blood Purif*. 2015;39(1–3):84–92. doi:10.1159/000368940
17. Maciejczyk M, Szulimowska J, Skutnik A, et al. Salivary biomarkers of oxidative stress in children with chronic kidney disease. *J Clin Med*. 2018;7:8. doi:10.3390/jcm7080209
18. Andrade-Oliveira V, Foresto-Neto O, Watanabe IKM, Zatz R, Câmara NOS. Inflammation in renal diseases: new and old players. *Front Pharmacol*. 2019;10:1. doi:10.3389/fphar.2019.01192
19. Nonaka T, Wong DTW. Saliva Diagnostics. *Annu Rev Anal Chem*. 2022;15(1):107–121. doi:10.1146/annurev-anchem-061020-123959
20. Alam BF, Nayab T, Ali S, et al. Current scientific research trends on salivary biomarkers: a bibliometric analysis. *Diagnostics*. 2022;12:5. doi:10.3390/diagnostics12051171
21. Maciejczyk M, Nesterowicz M, Szulimowska J, Zalewska A. Oxidation, glycation, and carbamylation of salivary biomolecules in healthy children, adults, and the elderly: can saliva be used in the assessment of aging? *J Inflamm Res*. 2022;15:2051–2073. doi:10.2147/JIR.S356029
22. Celec P, Tóthová Ľ, Šebeková K, Podracká Ľ, Boor P. Salivary markers of kidney function - Potentials and limitations. *Clin Chim Acta*. 2016. doi:10.1016/j.cca.2015.11.028
23. Willis K, Cheung M, Slifer S. KDIGO 2012 clinical practice guideline for evaluation & management of CKD. *Kidney Int Suppl*. 2013. doi:10.1038/kisup.2012.76
24. Schwartz GJ, Muñoz A, Schneider MF, Mak RH, Kaskel F, Warady BAFS. New equations to estimate GFR in children with CKD. *J Am Soc Nephrol*. 2009;20:629–637. doi:10.1681/ASN.2008030287

25. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis*. 2002;39(2 Suppl 1):S1–266.
26. Toczewska J, Zalewska A, Konopka T, Maciejczyk M. Enzymatic antioxidants activity in gingival crevicular fluid and saliva in advanced periodontitis. *Oral Dis*. 2022;2022:5. doi:10.1111/odi.14287
27. Saleh J, Figueiredo MAZ, Cherubini K, Salum FG. Salivary hypofunction: an update on aetiology, diagnosis and therapeutics. *Arch Oral Biol*. 2015;60(2):242–255. doi:10.1016/j.archoralbio.2014.10.004
28. Walker JM. The bicinchoninic acid (BCA) assay for protein quantitation. In: *Basic Protein and Peptide Protocols*. Vol. 32. New Jersey: Humana Press; 1994:5–8.
29. Bendelow VM. Modified procedure for the determination of diastatic activity and α-amylase activity. *J Inst Brew*. 1963;69(6):467–472. doi:10.1002/j.2050-0416.1963.tb01954.x
30. Kang J-H, Kho H-S. Blood contamination in salivary diagnostics: current methods and their limitations. *Clin Chem Lab Med*. 2019;57(8):1115–1124. doi:10.1515/cclm-2018-0739
31. World Health Organization. *Oral Health Surveys: Basic Methods - World Health Organization*. World Health Organization; 2018.
32. Mühlemann HR, Son S. Gingival sulcus bleeding--a leading symptom in initial gingivitis. *Helv Odontol Acta*. 1971;15(2):107–113.
33. Silness J, Löe H. Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand*. 1964;22(1):121–135. doi:10.3109/00016356408993968
34. Lange DE, Plagmann HC, Eenboom A, Promesberger A. Klinische Bewertungsverahren zur Objektivierung der Mundhygiene [Clinical methods for the objective evaluation of oral hygiene]. *Dtsch Zahnarztl Z*. 1977;32(1):44–47. German.
35. Binnicker MJ, Jespersen DJ, Rollins LO. Evaluation of the bio-rad bioplex measles, mumps, rubella, and varicella-zoster virus IgG multiplex bead immunoassay. *Clin Vaccine Immunol*. 2011;18(9):1524–1526. doi:10.1128/CVI.05207-11
36. Freitas-Fernandes LB, Fidalgo TKS, de Almeida PA, Souza IPR, Valente AP. Salivary metabolome of children and adolescents under peritoneal dialysis. *Clin Oral Investig*. 2020;2020:47.
37. Tadakamadla J, Kumar S, Mamatha GP. Comparative evaluation of oral health status of chronic kidney disease (CKD) patients in various stages and healthy controls. *Spec Care Dent*. 2014;34(3):122–126. doi:10.1111/scd.12040
38. Klimiuk A, Zalewska A, Knapp M, Skutnik-Radziszewska A, Maciejczyk M. Could inflammation contribute to salivary gland dysfunction in patients with chronic heart failure? *Front Immunol*. 2022;2022:1. doi:10.3389/fimmu.2022.1005981
39. Morawska K, Maciejczyk M, Zięba S, et al. Cytokine/chemokine/growth factor profiles contribute to understanding the pathogenesis of the salivary gland dysfunction in euthyroid hashimoto's thyroiditis patients. *Mediators Inflamm*. 2021;2021:3192409. doi:10.1155/2021/3192409
40. Skutnik-Radziszewska A, Maciejczyk M, Flisiak I, et al. Enhanced inflammation and nitrosative stress in the saliva and plasma of patients with plaque psoriasis. *J Clin Med*. 2020;2020:67. doi:10.3390/jcm9030745
41. Belstrøm D, Eiberg JM, Enevold C, et al. Salivary microbiota and inflammation-related proteins in patients with psoriasis. *Oral Dis*. 2020;26(3):677–687. doi:10.1111/odi.13277
42. Roescher N, Tak PP, Illei GG. Cytokines in sjögren's syndrome. *Oral Dis*. 2009;15(8):519–526. doi:10.1111/j.1601-0825.2009.01582.x
43. Sakai A, Sugawara Y, Kuroishi T, Sasano T, Sugawara S. Identification of IL-18 and Th17 cells in salivary glands of patients with sjögren's syndrome, and amplification of IL-17-mediated secretion of inflammatory cytokines from salivary gland cells by IL-18. *J Immunol*. 2008;181(4):2898–2906. doi:10.4049/jimmunol.181.4.2898
44. Laheij A, Rooijers W, Bidar L, et al. Oral health in patients with end-stage renal disease: a scoping review. *Clin Exp Dent Res*. 2022;8(1):54–67. doi:10.1002/cre2.479
45. Pfaffe T, Cooper-White J, Beyerlein P, Kostner K, Punyadeera C. Diagnostic potential of saliva: current state and future applications. *Clin Chem*. 2011;2011:79. doi:10.1373/clinchem.2010.153767
46. Maciejczyk M, Mil KM, Gerreth P, Hojan K, Zalewska A, Gerreth K. Salivary cytokine profile in patients with ischemic stroke. *Sci Rep*. 2021;11(1):17185. doi:10.1038/s41598-021-96739-0
47. Diesch T, Filippi C, Fritschi N, Filippi A, Ritz N. Cytokines in saliva as biomarkers of oral and systemic oncological or infectious diseases: a systematic review. *Cytokine*. 2021;143:155506. doi:10.1016/j.cyto.2021.155506
48. Baker OJ, Camden JM, Redman RS, et al. Proinflammatory cytokines tumor necrosis factor-α and interferon-γ alter tight junction structure and function in the rat parotid gland Par-C10 cell line. *Am J Physiol Physiol*. 2008;295(5):C1191–C1201. doi:10.1152/ajpcell.00144.2008
49. Benchabane S, Boudjelida A, Toumi R, Belguendouz H, Youinou P, Touil-Boukoffa C. A case for IL-6, IL-17A, and nitric oxide in the pathophysiology of Sjögren's syndrome. *Int J Immunopathol Pharmacol*. 2016;29(3):386–397. doi:10.1177/0394632016651273
50. Moriyama M, Hayashida J-N, Toyoshima T, et al. Cytokine/chemokine profiles contribute to understanding the pathogenesis and diagnosis of primary Sjögren's syndrome. *Clin Exp Immunol*. 2012;169(1):17–26. doi:10.1111/j.1365-2249.2012.04587.x
51. Favalli EG. Understanding the Role of Interleukin-6 (IL-6) in the joint and beyond: a comprehensive review of IL-6 inhibition for the management of rheumatoid arthritis. *Rheumatol Ther*. 2020;7(3):473–516. doi:10.1007/s40744-020-00219-2
52. McGeachy MJ, Bak-Jensen KS, Chen Y, et al. TGF-β and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain TH-17 cell–mediated pathology. *Nat Immunol*. 2007;8(12):1390–1397. doi:10.1038/ni1539
53. Tzioufas AG, Tsonis J, Moutsopoulos HM. Neuroendocrine dysfunction in Sjögren's syndrome. *Neuroimmunomodulation*. 2008;15(1):37–45. doi:10.1159/000135622
54. Pflugfelder SC, De Paiva CS, Moore QL, et al. Aqueous tear deficiency increases conjunctival interferon-γ (IFN-γ) expression and goblet cell loss. *Invest Ophthalmol Vis Sci*. 2015;56(12):7545–7550. doi:10.1167/iovs.15-17627
55. Trzcionka A, Twardawa H, Mocny-Pachońska K, Korkosz R, Tanasiewicz M. Oral mucosa status and saliva parameters of multimorbid adult patients diagnosed with end-stage chronic kidney disease. *Int J Environ Res Public Health*. 2021;18:23. doi:10.3390/ijerph182312515
56. Nakayama T, Hirahara K, Onodera A, et al. Th2 cells in health and disease. *Annu Rev Immunol*. 2017;35(1):53–84. doi:10.1146/annurev-immunol-051116-052350
57. Li Z, Zhang Y, Sun B. Current understanding of Th2 cell differentiation and function. *Protein Cell*. 2011;2(8):604–611. doi:10.1007/s13238-011-1083-5
58. Bhavsar I, Miller CS, Al-Sabbagh M. Macrophage inflammatory protein-1 alpha (MIP-1 alpha)/CCL3: as a biomarker. In: Preedy VR, Patel VB, editors. *General Methods in Biomarker Research and Their Applications*. Dordrecht: Netherlands: Springer; 2015:223–249.

59. Harambat J, Van Stralen KJ, Kim JJ, Tizard EJ. Epidemiology of chronic kidney disease in children. *Pediatr Nephrol*. 2012;27(3):363–373. doi:10.1007/s00467-011-1939-1
60. Mirna M, Topf A, Wernly B, et al. Novel biomarkers in patients with chronic kidney disease: an analysis of patients enrolled in the GCKD-study. *J Clin Med*. 2020;9:3. doi:10.3390/jcm9030886
61. Maciejczyk M, Żukowski P, Zalewska A. Salivary biomarkers in kidney diseases. In: Tvarijonaviciute A, Martínez-Subiela S, López-Jornet P, Lamy E, editors. *Saliva in Health and Disease*. Springer International Publishing: Cham; 2020:193–219.
62. Al-Aly Z, Bowe B. Biomarkers of CKD in children. *J Am Soc Nephrol*. 2020;31(5):894–896. doi:10.1681/ASN.2020020212
63. Schober P, Vetter TR. Linear regression in medical research. *Anesth Analg*. 2021;132(1):1.
64. Hassaneen M, Maron JL. Salivary diagnostics in pediatrics: applicability, translatability, and limitations. *Front Public Heal*. 2017;5:45. doi:10.3389/fpubh.2017.00083
65. Carpenter GH. The secretion, components, and properties of saliva. *Annu Rev Food Sci Technol*. 2013;2013:34. doi:10.1146/annurev-food-030212-182700