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TNF-α, IL-1β, MMP-8 Crevicular Profile in Patients with Chronic Kidney Disease and Periodontitis

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Abstract: Increasing evidence sustains the potential of periodontitis as a risk factor for chronic kidney disease (CKD). Our study aimed to analyze several periodontal specific inflammatory biomarkers within the gingival crevicular fluid (GCF) of patients with CKD, compared to patients with normal kidney function, providing an inflammatory profile of the dialysis patient. The study comprised 79 patients divided into: group 1 (59 subjects with periodontitis and CKD) and group 2 (20 patients with periodontitis, without other systemic conditions). Clinical diagnosis was performed via dental and periodontal examination. GCF samples were collected from each patient, and the levels of TNF-α, IL-1β and MMP-8 were determined by using ELISA assay. In group 1, the average values were: 22.85 ± 5.87 pg/mL for TNF-α, 33.00 ± 39.68 pg/mL for IL-1β and 18.80 ± 27.75 ng/mL for MMP-8. In group 2, the mean values were: 2.10 ± 1.34 pg/mL for TNF-α, 0.71 ± 2.42 pg/mL for IL-1β and 5.35 ± 0.37 ng/mL for MMP-8. Statistical analysis revealed significant differences between groups as referring to all three biomarkers and, TNF-α and MMP-8, in certain stages of periodontitis. The level of TNF-α, IL-1β and MMP-8 points out the increased inflammatory status of the dialysis patient with PD, supporting the mutual connection of the two pathologies.

Keywords: periodontitis; chronic kidney disease; biomarkers; TNF-α; IL-1β; MMP-8

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1. Introduction

Chronic kidney disease (CKD), defined by a decline in glomerular filtration rate, an increased urinary albumin excretion or both, represents a critical public health issue [1,2]. Worldwide, the estimated prevalence ranges between 11.7 and 15.1 percent [2]. CKD complications include an increase in cardiovascular caused death, the progression loss of kidney function, acute renal damage, cognitive decline, anemia, mineral and bone metabolism impairment [3].

At the end of the 90s, solid scientific proof confirmed the role of inflammation in CKD pathogeny [4], with an exponential increase in concern towards this disease mechanism, and major changes in the perception of the importance of this etiopathogenetic factor. The inflammatory status in CKD is triggered by the activity of certain soluble factors from

the cytokine and chemokine family, that control leucocytes and monocytes chemotaxis towards the kidney damaged site [5,6]. On the other hand, CKD is associated with a poor immune status, determined either by the diseases that cause kidney failure (for example, diabetes mellitus and cardiovascular disorders), or uremia, or as a consequence of specific therapy (dialysis or transplant). This immuno-incompetence is the result of the process of non-compliant antigen recognition and antibody production, but also of impaired neutrophil function. By affecting the cellular or humoral mediated immune response, the host organism becomes ineffective in responding to bacterial aggressions [7]. Hence, CKD patients, especially those undergoing dialysis, develop an inadequate immune inflammatory response, that subsequently triggers infectious and thrombotic events which in turn create and maintain an added inflammatory burden [8].

Periodontal disease or periodontitis is defined as a multifactorial disorder, characterized by the disfunction of inflammation resolution pathways, which, in turn, leads to healing failure and a dominant chronic, progressive, destructive and irreversible inflammation upon periodontal level, with the consequent destruction of the attachment tissue and alveolar bone resorption [9,10]. Gram-negative subgingival microorganisms (i.e., *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia*) play an essential role in inflammatory response, that subsequently determines the progression of periodontal injuries [11]. After the recognition and presentation of microbial antigens to immune-inflammatory cells, the cytokines of the innate immune response-tumor necrosis factor alpha (TNF-α), interleukin IL-1β, IL-6 are the first to be generated during the development of periodontal impairment [12]. Periodontitis is frequently diagnosed in patients with CKD [13–16].

Based on the common inflammatory status, the relationship between periodontitis and CKD is an interesting avenue for the research of kidney disease progression. Literature indicates that periodontitis could be a potential risk factor for CKD [17,18], through interferences in the immune response control, but no cause-effect has yet been proven.

The concept of CKD patient predisposition towards periodontitis has also been taken into consideration, with both pros and cons. Conflicting data are due, most likely to method disparities in study design (no control population, differences in periodontitis and CKD definition) and bias. Some results do not confirm the differences in periodontitis prevalence in the general population and in patients with CKD, respectively [19]. On the other hand, numerous studies indicate a strong correlation between the two entities, supported by the increased prevalence and risk for periodontitis in CKD patients, compared to control populations [15,20–22]. Using mathematical models, it was confirmed the fact that some complementary pathologies (diabetes mellitus, systemic hypertension) are mediators of periodontitis effects on CKD and that there is a bidirectional relationship between periodontitis and CKD [22].

It is worth mentioning a recent study [18] that brings powerful evidence in favor of the association between periodontitis, diabetes mellitus and CKD. Based on a non-CKD representative cohort (2635 subjects) followed-up for a period of 10 years for non-communicable disease risk factors [23] and by the use of mediation analysis with bootstraping, the cause-effect link between periodontitis and CKD has been confirmed through the study design. The results point out that periodontitis has significant direct and indirect (through diabetes) impact on the incidence of CKD [18].

In patients with CKD, in general, and in hemodialysis patients in particular, chronic periodontal inflammation is associated with a systemic increase of inflammatory biomarkers (ILs, TNF-α, vascular endothelial growth factor-VEGF, matrix metalloproteinases-MMP) who directly influence the kidney inflammatory status and negatively alter the kidney function [6,13,24–29]. However, the existing information concerning the levels of inflammatory biomarkers in the gingival crevicular fluid (GCF) related to the periodontitis-CKD association is rather limited.

On this basis, our study pursued for local analysis of certain periodontal specific inflammatory biomarkers within the GCF of patients with CKD, compared to patients

with normal kidney function, aiming to define a periodontal inflammatory profile that characterizes the dialysis patient and substantiate the bidirectional relationship between the two conditions. More specific, our objective was to determine the level of TNF-α, IL-1β and MMP-8 in GCF of hemodialysis periodontal patients compared to healthy subjects with periodontitis, in order to substantiate the more advanced level of these three proinflammatory cytokines in CKD. To the best of our knowledge, this is the first study to asses in concert the GCF load of these biomarkers, in hemodialysis subjects.

2. Materials and Methods

2.1. Study Design

We developed an observational case-control study on two groups of patients (total number = 79), the first group including subjects with periodontitis and end-stage CKD (stage 5) undergoing dialysis, the second group with subjects with periodontitis and normal kidney function. The study has been approved by the Ethics Committee of "Grigore T. Popa" University of Medicine and Pharmacy Iasi, complying with the ethical standards of Helsinki declaration (approval no. 1/29 May 2016).

The first step of our study targeted the assessment of the dental and periodontal status via clinical examination, staging the periodontitis according to case definitions provided by AAP/EFP in 2018 [30]. The second step consisted in GCF sampling and testing for three inflammatory biomarkers: TNF-α, IL-1β and MMP-8. The obtained data has been statistically analyzed.

2.2. Patients

Group 1 comprised 59 subjects selected from the patients attending the Fresenius Nephrocare Dialysis Centers in Iasi, Romania. The subjects were included in the study after obtaining the informed consent. Patients who refused to take part in the study, fully edentulous subjects and those with current conditions that didn't allow their examination, were excluded.

Out of the group 1 subjects, 28 patients (47.4%) were female and 31 patients (52.6%) were male, with a mean age of 58.56 $\pm$ 14 years; 47 (79.6%) patients were from a rural area, while 12 (30.4%) resided in urban area.

The second group, considered the control group, embodied 20 patients with periodontitis, but without other systemic conditions, selected among the patients addressed for periodontal treatment in private dental offices from Iasi county. All patients had given their written informed consent to take part in the research. In group 2, 16 patients (80%) were female and 4 patients (20%) male, with a mean age of 35.25 $\pm$ 9.28 years; 19 of them (95%) were habitant from urban area and 1 (5%) of rural area.

2.3. Clinical Examination

The information corresponding to each dental unit was filled in a dento-periodontal file, using the ISO system of the World Health Organization. Missing teeth and the presence of fixed and/or removable prosthetics were identified and registered. The complete DMF index (decayed, missing, filled teeth) [31] couldn't be evaluated, as the clinical examination of study group patients during dialysis treatment was difficult. Consecutively, by identifying the missing teeth, only the M component of the DMF index was determined.

Each of the present teeth was evaluated through periodontal probing with a Columbia periodontal probe, targeting 6 parameters: dental mobility (DM), bleeding on probing, gingival edema, gingival recession (GR), pocket probing depth (PPD), and clinical attachment loss (CAL).

Periodontitis diagnosis was established according to the 2018 EFP/AAP case definition, based on the presence of interdental CAL $\geq$ 2 mm in non-adjacent teeth, or buccal/oral CAL $\geq$ 3 mm with PPD > 3 mm present at $\geq$2 teeth [30]. The staging was determined taking into account the site of greatest loss, as follows: mild periodontitis (stage 1) for

interdental CAL of 1–2 mm; moderate periodontitis (stage 2) for interdental CAL 3–4 mm and severe periodontitis (stage 3 and 4) for interdental CAL ≥ 5 mm, respectively [30].

The difference between stage 3 and stage 4 of severe periodontitis is that stage 4 includes additional dysfunction, occlusal trauma, defects, bite collapse, and requires further periodontal assessment [30]. Due to this, we grouped both 3 and 4 stages into the severe periodontitis form, as to differentiate between them, especially for the study group, would have been unmanageable outside a dental office setting.

For each patient, using the performed measurements for each individual tooth, we calculated mean values for the following parameters: DM, GR, PPD and CAL. Based on the mean individual values of these parameters, we calculated mean values per each group (study and control, respectively); within groups we also calculated mean values for each periodontitis stage category. The other two parameters, namely bleeding on probing and gingival edema, were assessed in a dichotomous way (present/absent) and reported as percentages per group and within groups, for each periodontitis stage.

2.4. Assessment of TNF-α, IL-1β, MMP-8 in the Crevicular Fluid

Standard protection and hygiene measures had been used during collection of the samples, examination gloves and sterile dental kits being used. GCF was sampled from the sulcus of the Ramfjord teeth (1.6, 2.1, 2.4, 3.6, 4.1, 4.4) of each patient, using sterile paper points. The site with the deepest periodontal pocket has been identified and cleaned individually before collection with sterile gauze, to avoid biofilm contamination. When the respective Ramfjord tooth was missing, we used the next tooth, except in cases of extended or subtotal edentulousness, when only the existing teeth were used, even when their number was lower than 6. The paper points were inserted immediately into single use Eppendorf® tubes, sealed. Each tube was marked with an identifying number and samples were eluted into 250 µL phosphate buffered saline (PBS) and kept frozen at −80 °C until assayed.

The concentrations of the biomarkers were determined by commercially available ELISA kits: human IL-1β ELISA Kit (Sigma-Aldrich, St. Louis, MO, USA), human TNF-α ELISA Kit (MyBioSource, San Diego, CA, USA) and human MMP-8 ELISA Kit (Sigma-Aldrich, St. Louis, MO, USA), all suitable for serum, plasma and cell cultures.

2.5. Statistical Analysis

The results obtained were included in a standard, Excel data base. Statistical processing of data was carried out using SPSS Statistics for Windows, version 17.0 (SPSS Inc., Chicago, IL, USA). The numeric values were expressed as mean and standard deviation (SD). *t*-test was used for the analysis of the relationship between variables. Due to the unequal series of values, with different variances, we tested these differences beforehand using Fisher's exact test.

3. Results

3.1. Periodontal Profile in the Study Groups

The general and periodontal characteristics of the two analyzed groups are presented by comparison in Table 1.

In group 1, over 50% of the patients displayed inflammation symptoms: bleeding—83.05% (n = 49) and gingival edema—52.54% (n = 31). Mean DM was 1.37. Mean GR was 1.68 mm. Mean PPD was 2.37 mm, with a mean CAL of 3.56 mm. Of all teeth, the most frequently missing was the upper first permanent molar (35%, n = 83), followed by the lower first permanent molar (31%, n = 73). Through the diagnosis based on the 2018 case definition for periodontitis, our first batch included: 3 patients (5.08%) with stage 1 (mild form), 10 (16.94%) with stage 2 (moderate form) and 46 (77.96%) with a stage 3–4 (severe form). The mean values of the measurable parameters in each periodontitis stage subgroup are summarized in Table 2.

Table 1. General and periodontal characteristics within the study and control groups.

Characteristics	Group 1 (n = 59)	Group 2 (n = 20)
Age (mean ± SD)	58.56 ± 14.00	35.25 ± 9.28
Gender (#, %)		
Female	28 (47.40)	16 (80.00)
Male	31 (52.60)	4 (20.00)
Environment (#, %)		
Urban	12 (30.40)	19 (95.00)
Rural	47 (79.60)	1 (5.00)
DM (mean)	1.37	0.47
Bleeding on probing (#, %)	49 (83.05)	15 (75.00)
Gingival edema (#, %)	31 (52.54)	6 (30.00)
GR (mean, mm)	1.68	0.52
PPD (mean, mm)	2.37	1.13
CAL (mean, mm)	3.56	1.28
Periodontitis stage (#, %)		
Mild stage	3 (5.08)	7 (35.00)
Moderate stage	10 (16.94)	5 (25.00)
Severe stage	46 (77.96)	8 (40.00)

#—number of patients, %—percentage.

Table 2. Periodontal parameters in the study group in correspondence to periodontitis staging.

Periodontitis Staging	DM (Mean Value)	GR (Mean Value, mm)	PPD (Mean Value, mm)	CAL (Mean Value, mm)
Stage 1 (mild) n = 3	1.51	0.98	1.21	0.20
Stage 2 (moderate) n = 10	1.06	1.45	2.08	1.33
Stage 3–4 (severe) n = 46	1.38	1.80	2.68	4.65

In group 2, the control group, gingival bleeding was identified in 75% (n = 15) of patients, and gingival edema was recorded in 30% of them (n = 6). Mean DM was 0.47. Mean GR was 0.52 mm, with a mean PPD of 1.13 mm and a CAL of 1.28 mm. The most frequently absent tooth was the upper first permanent molar (23%, n = 20).

According to the 2018 periodontal classification criteria, 7 patients (35%) had mild periodontitis (stage 1), 5 (25%) presented a moderate form (stage 2) and 8 (40%) a severe one (stage 3–4).

The mean values of the measurable parameters in each periodontitis stage subgroup are summarized in Table 3.

Table 3. Periodontal parameters in the control group in correspondence to periodontitis staging.

Periodontitis Staging	DM (Mean Value)	GR (Mean Value, mm)	PPD (Mean Value, mm)	CAL (Mean Value, mm)
Stage 1 (mild) n = 7	0.54	0.82	1.32	0.61
Stage 2 (moderate) n = 5	0.40	0.34	1.28	1.09
Stage 3–4 (severe) n = 8	0.44	0.38	0.93	1.78

The statistical analysis between the study and control groups showed no significant differences concerning gingival edema (p = 0.080) and bleeding on probing (p = 0.427) in patients with both CKD and periodontitis, compared to patients with periodontitis

and unaffected systemic condition. On the other hand, we registered highly statistically significant differences between the two groups concerning DM (p = 0.000), GR (p = 0.000), PPD (p = 0.000) and CAL (p = 0.000) (Figure 1).

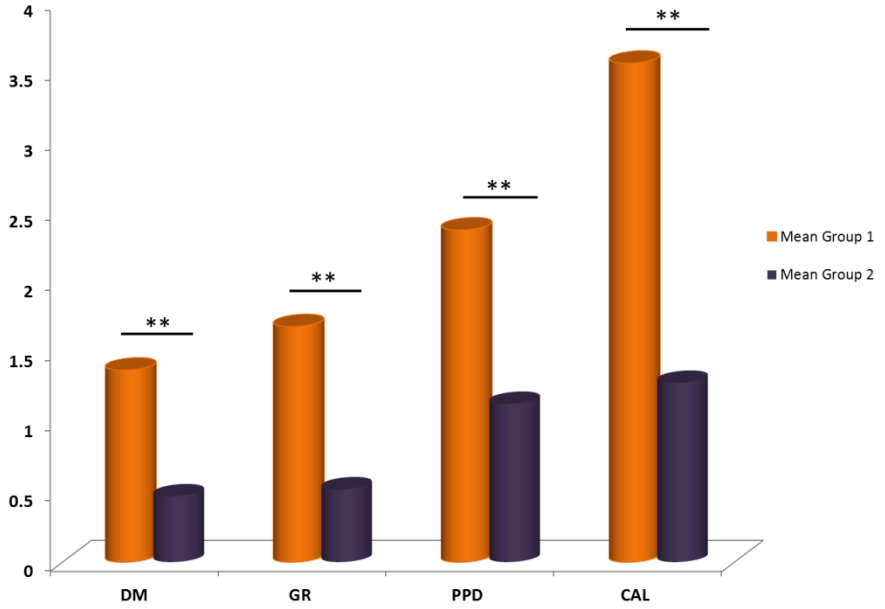

Figure 1. Comparison between periodontal parameters in group 1 vs. group 2 (DM—dental mobility, GR—gingival recession, PPD—pocket probing depth, CAL—clinical attachment loss, **—statistically significant difference).

Taking into consideration the periodontitis staging as well, the refined statistical analysis revealed significant differences between groups as follows: for DM, in stage 1 (p = 0.016) and stage 3–4 (p = 0.002); for GR, in stage 2 (p = 0.002) and stage 3 (p = 0.000); for PPD and CAL, only in stage 3–4 (p = 0.002 and p = 0.000, respectively).

3.2. Local Oral Expression of TNF-α, IL-1β and MMP-8 Inflammatory Biomarkers in Gingival Fluid

The representative values of inflammatory biomarkers for each patient are compiled in Table 4, for both the subjects with systemic and periodontal alteration (group 1) and the control group, without systemic alteration. Furthermore, Table 5 summarizes biomarker values according to periodontitis staging.

Table 4. GCF immune biochemical biomarkers levels in study group versus control group.

Values	TNF-α (pg/mL)		IL-1β (pg/mL)		MMP-8 (ng/mL)	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
Minimum	0.90	0.10	6.60	5.30	5.60	4.60
Mean	22.80	2.10	33.00	0.71	18.80	5.35
Maximum	35.90	5.50	201.30	60.80	121.50	6.10

In group 1, TNF-α recorded levels ranging between 0.90 pg/mL and 35.90 pg/mL, with an average of 22.85 ± 5.87 pg/mL. Cytokine IL-1β values varied between 6.60 pg/mL and 201.30 pg/mL, with an average of 33.00 ± 39.68 pg/mL. Nevertheless, the MMP-8 values in the double affected subjects recorded levels as low as 5.60 ng/mL and highest 121.50 ng/mL, with an average of 18.80 ± 27.75 ng/mL.

For group 2, TNF-α values varied less compared to those recorded in subjects with CKD (0.10 pg/mL and 5.50 pg/mL), with a mean of 2.10 ± 1.34 pg/mL. The same pattern regarding downsized levels have been recorded for cytokine IL-1β in this study group

compared to the patients in the first lot, with rather narrow fluctuations in the interval 5.30 pg/mL and 60.80 pg/mL and an average 0.71 ± 2.42 pg/mL, and for MMP-8 as well, the values varying between 4.60 ng/mL and 6.10 ng/mL, with an average of 5.35 ± 0.37 ng/mL.

The statistical analysis revealed significant differences between the mean values of groups as referring to both, cytokines TNF-α ($p < 0.000$), IL-1β ($p = 0.01$) and zinc-dependent endopeptidase MMP-8 ($p = 0.000$) crevicular levels (Figure 2).

Table 5. GCF immune biochemical biomarker levels in study group versus control group in correspondence to periodontitis staging.

Periodontitis Staging	TNF-α (pg/mL)		IL-1β (pg/mL)		MMP-8 (ng/mL)	
	Mean Values					
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
Stage 1 (mild)	27.40	2.10	30.30	10.70	7.70	5.50
Stage 2 (moderate)	23.50	2.00	39.15	14.40	32.65	5.70
Stage 3–4 (severe)	22.55	2.15	29.40	9.25	18.35	5.20

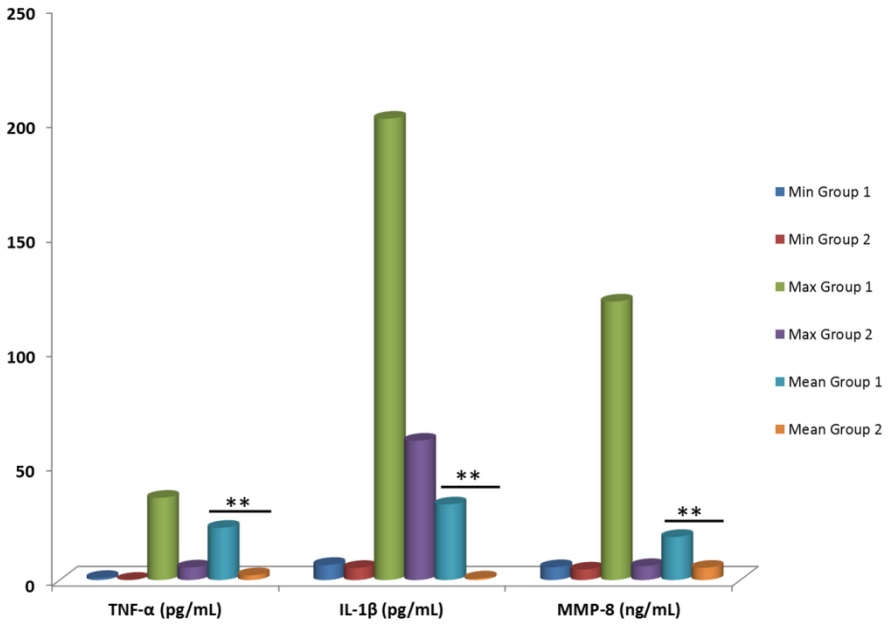

Figure 2. Comparison between inflammatory biomarkers in group 1 vs. group 2 (min—minimum value, max—maximum value, **—statistically significant difference).

Considering periodontitis staging, our results indicated statistically significant differences between groups in what concerns TNF-α values in all three stages, as follows: mild ($p = 0.000$), moderate and severe ($p < 0.000$). There were no statistically significant differences between groups for IL-1β, for any periodontitis stage. For MMP-8 the statistical analysis revealed significant differences in study group versus control group, in stage 1 ($p < 0.000$) and stage 3–4 ($p = 0.024$).

4. Discussion

Both periodontitis and CKD represent public health issues, with a compelling influence upon the life-quality of patients and their survival rate. Studies analyzing the interrelationship between these two entities are unsystematized, currently suggesting a bidirectional path [13–15,32]. This trajectory is primarily due to common inflammatory

mechanisms—bacteremia, production of cytokines and other proinflammatory molecules, oxidative stress and endothelial dysfunction [33].

Upon an exhaustive inspection of the literature data, one can realize the existence of narrow number of research aiming at the analysis of oral health status in patients with CKD, especially in the terminal stages. In subjects with CKD treated with hemodialysis or peritoneal dialysis and associated with untreated periodontitis, the risk of mortality is augmented significantly [34].

The inflammatory condition in CKD is characterized by the activation of soluble factors such as cytokines and chemokines (chemotactic cytokines). Moreover, the relationship between periodontitis and CKD, by reference to the common inflammatory status, is a topic of constant interest for specialists in the two fields. Several studies published in the main flow attest that periodontitis mediates the immune response in hemodialysis patients [13,28,29].

Among the cytokines involved in the systemic inflammatory mechanisms, TNF-α is part of the acute phase proteins produced by activated macrophages, but also by other cell types (i.e., CD4 T lymphocytes, NK cells, neutrophils, mast cells, eosinophils, neurons, fat and heart cells) [35–38].

IL-1 family members include 11 cytokines that regulate and initiate the inflammatory response through integrins expression modulation over leucocytes and endothelial cells and allowing immune competent cells to pass towards the infection site [39,40].

MMPs are a group of 24 endopeptidases with intracellular calcium ion-dependent action, who contain zinc in their structure [41]. These enzymes are capable of degrading all types of extracellular matrix proteins but can be implicated in the processing of bioactive molecules, as well. The most common classification of MMPs includes 5 main groups: collagenases, gelatinases, stromlysines, matrilysin and membrane type MMPs [41]. Collagenases are capable of degrading fibrillary collagen and comprise MMP-1, MMP-8, MMP-13, MMP-14 and MMP-18 [41].

The literature confirms the association between cytokines (TNF-α, IL-1, IL-6, IL-8, IL-17), periodontitis, and CKD [13,25,26,28].

In CKD, high serum levels of TNF-α are reported in relationship with the metabolic syndrome, cardiovascular disorders, congestive cardiac failure, progression and disease mortality [42–45], being considered a sensitive marker for periodontal inflammation [46].

In periodontitis, the data about TNF-α serum and/or crevicular fluid dynamic are contradictory. Some studies show similar data for patients with periodontitis and patients without periodontal involvement [47,48], or minimal differences between the levels found in periodontal healthy sites and those with periodontitis [49,50]. Other studies point out considerable increase of crevicular TNF-α in periodontitis [51–53], supporting the fact that TNF-α concentration variations can be used to predict the progression of periodontal condition.

To the best of our knowledge, a single study has evaluated crevicular TNF-α (along with IL-8) in patients undergoing hemodialysis [54], resulting in a positive correlation between clinical indicators of periodontitis and CKD, and TNF-α and IL-8 levels, as well as statistically significant differences between the two biomarkers, in the CKD group compared to control [54]. In our study, TNF-α mean values in CGF in the study group ranged between 22.85 ± 5.87 pg/mL, and in the control group between 2.10 ± 1.34 pg/mL. Both values are comparable to the limited existing data (31.40 pg/mL, and 3.06 pg/mL, respectively) for crevicular analysis [54], confirming a ten-fold increase of TNF-α levels in periodontally affected patients on dialysis.

Moreover, our data showed that TNF-α differs significantly between groups, in each of the periodontitis stages. Thus, regardless of the poorly understood relationships between periodontitis, renal disease and chronic inflammation, our research provides additional evidence for TNF-α involvement in periodontitis and end stage kidney disorder interrelationship, hence supporting a potentiation of the inflammatory burden that aggravates kidney disease.

In subjects with end stage renal disease, plasma IL-1 and IL-1Ra levels may indicate a worse cardiovascular prognostic [55–57]. For the crevicular IL-1α levels in periodontitis, a mean value of 198.33 pg/mL ± 44.17 is reported [58]. In assessing the periodontal status, numerous studies claim the potential of IL-1β burden as a marker of differentiation between healthy and injured sites [50,59–62]. However, this differentiation is more evident in the case of advanced forms of periodontitis [60,63], compared to early stages or to gingivitis [64].

The literature review points only one study that analyses crevicular IL-1β in end stage kidney failure. This recent investigation [65] evaluates together with IL-1β also IL-6, IL-8 and C reactive protein (CRP) in serum and GCF, in patients with end stage CKD, compared to a healthy control group. The results indicate the absence of statistic significant differences between the levels of crevicular fluid IL-1β of both groups [65]. Mean values of crevicular IL-1β were 0.26 ng/mL in the CKD group and 0.34 ng/mL in the healthy control group [65]. However, the study was limited by the small study population.

In our study, the mean level of IL-1β in the study group was 33 ± 39.68 pg/mL, and in the control group—10.4 ± 15.95 pg/mL, with statistically significant differences between the two groups. Hence, our results acknowledge higher GCF mean levels of IL-1β in hemodialysis patients with periodontitis, compared to patients with periodontitis without kidney impairment. This pattern represents evidence suggesting the role of cytokines in the immune inflammatory mechanism that characterizes the two pathological entities, in contrast to the data from Ma et al., who report a higher mean IL-1β level in the control group [65]. In our opinion, these contradictory results may be explained by the small sized group used by Ma et al., in his study (15 subjects per group). On the other hand, when analyzing our data related to the periodontitis staging, there were no significant differences in IL-1β levels between the study and control groups, in any stage. Given the above, it seems reasonable to suggest that the understanding of IL-1β dynamic in crevicular fluid in the context of periodontitis -CKD relationship, is far from clear.

Our research targeted measurement of the oral gingival fluid levels of MMP-8 (neutrophilic collagenase) produced by activated neutrophils [66]. Biological functions of this collagenase are still not fully unfolded, although it is involved in numerous tissue remodeling processes associated to inflammatory or healing events [67–69].

MMPs and especially MMP-8 are involved in kidney pathology, through their tissue destruction ability [29], resulting in chronic remodeling and kidney fibrosis, found in diabetic nephropathy, polycystic kidney disorder and glomerulonephritis [70]. In addition, MMPs are part of the kidney inflammatory regulation network [70]. MMP-8 and MMP-9 have been identified in diabetic nephropathy patients' urine, supporting this molecule's involvement in its pathogeny [71].

Several studies have linked MMPs to periodontitis [29], MMP-8 being the main collagenase (80%) identified and analyzed in the gingival tissue and GCF [72]. Periodontal bacteria stimulate immune cells, which, in turn, produce and release MMP-8 into the blood stream, so that serum MMP-8 records higher levels in generalized and invasive periodontitis patients [73]. Current data support the relationship between periodontal bacteria (*F. nucleatum*, *P.s gingivalis*, *T. denticola*, *Aggregatibacter actinomycetemcomitans*) and MMP production, either directly or through neutrophil activation [74].

In periodontitis, high crevicular levels of MMP-8 are reported [75–77], the recognized variations emphasizing further that MMP-8 may be an indicator of lesion induction and progression [78]. However, keeping in mind the variability of the methods used for GCF collection, it should not be surprising the result that support the absence of statistic significant differences between crevicular MMP-8 levels in periodontitis versus control groups [79,80]. MMP-8 crevicular values reported in the literature vary: 95.20 ng/mL in healthy subjects [77], 240.24 ng/mL [79,80] and 428.60 ng/mL [77], respectively, in patients with periodontitis.

A seminal article for the study of the CKD and periodontitis relationship, that analyses the salivary expression of MMP-8, suggests that periodontal inflammation may trigger

systemic conditions, including CKD [29] Despite the numerous proof that suggest MMPs connection with periodontitis and CKD etiopathogeny, this potential route is far to be confirmed. More so, the current literature does not include data on the crevicular expression of MMP-8 in CKD.

Our results registered distinct elevation of mean GCF MMP-8 level—18.80 ± 27.75 ng/mL in patients with declined renal filtration rate, in contrast with control group values of 5.35 ± 0.37 ng/mL. As well as for the other investigated markers, the mean MMP-8 levels were higher in hemodialysis patients with periodontitis, compared to patients with periodontitis without kidney involvement, with statistically significant differences between the two groups ($p < 0.001$). Moreover, through the significant differences in MMP-8 values, our data show a correlation of this biomarker to periodontitis aggressiveness indicated by its staging.

Accordingly, our data suggest that MMP-8 play a pivotal role in the immune-inflammatory blueprint of periodontitis-associated end-stage renal disorder, compared with periodontitis without systemic damage. In the same register with the previous comments, we consider that this information, regarding the MMP-8 expression at crevicular level in the context of the periodontitis—CKD association emphasize higher GCF collagenolytic MMP-8 activity, GCF constituents being thus candidate biomarkers that might reflect the interplay between local periodontal and systemic inflammation, respectively.

Hemodialysis patients are characterized by an immune deficiency that contributes greatly to the increased incidence and rapid evolution of periodontitis [81]. In parallel, this deficiency enhances the spread of bacteria from the periodontal territory toward the bloodstream, contributing to the systemic inflammation associated with hemodialysis [33]. However, the current level of knowledge is not able to cover the image of the existing interrelationships between the individual and common pathogenic mechanisms of the two pathological conditions. For this reason, extensive studies involving larger groups of patients are needed, for an in-depth understanding of the pathological features of periodontitis in hemodialysis patients.

Dental and periodontal status in hemodialysis patients is negatively influenced by increasing the amount of biofilm and dental calculus, salivary concentration of urea and salivary pH [81,82]. Poor oral hygiene of hemodialysis patients (including gingival bleeding and decreased salivary secretion) compared to healthy individuals is directly correlated with periodontal tissue-specific inflammation reflected in inflammatory molecules [26,81,83–85]. Objectively, periodontal inflammation can be assessed through several methods, both direct (clinical examination) and indirect (histological exam, serum, salivary and GCF immunoassays, optical coherence tomography) [25,60,72,86–88]. Immunoassays are a safe and accessible method, especially in the context of hemodialysis patients.

The presence of inflammatory biomarkers is one of the solid evidences supporting the mutual potential of the two pathologies. However, in research targeting the common inflammatory substrate of CKD and periodontitis there are a number of limitations that make it difficult to construct a study protocol and validate the commented results, and which are worthwhile.

Understanding the complex interplay between the immune-inflammatory biomarkers and the mechanism triggering the inflammatory environment that contributes to disease progression will lay the foundation for development of novel therapeutic approaches to improve periodontally-affected hemodialyzed patient status.

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