Periodontal Disease and Detection of Human Herpesviruses in Saliva and Gingival Crevicular Fluid of Chronic Kidney Disease Patients

Debora Pallos[*], Gilson F. Ruivo[†], Sabino H. Ferrari-Junior[*], Claudio S. Pannuti[‡], Caroline Perozini[†], Dmitry J. S. Sarmento[**], Michelle Palmieri[**], Ana C. M. F. Souza[‡], Tania R. Tozetto-Mendoza[‡], Alain Doglio[§], Paulo H. Braz-Silva[‡,**]

[*] Department of Dentistry, University of Santo Amaro, São Paulo, Brazil

[†] Department of Medicine, University of Taubate, Taubate, Brazil

[‡] Laboratory of Virology, Institute of Tropical Medicine of São Paulo, School of Medicine, University of São Paulo, São Paulo, Brazil

[§] Laboratory MICORALIS (Microbiologie Orale, Immunité et Santé) School of Dentistry, University of Côte d'Azur, Nice, France

[**] Department of Stomatology, School of Dentistry, University of São Paulo, São Paulo, Brazil

Corresponding author:

Paulo H. Braz-Silva

Faculdade de Odontologia da Universidade de São Paulo

Departamento de Estomatologia

Av. Prof. Lineu Prestes, 2227 São Paulo – SP

05508-000

+5511 30917902

pbraz@usp.br

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Summary sentence: The present study verified the presence of human herpesviruses mainly HSV-1 and HCMV in saliva and gingival crevicular fluid samples from patients with periodontitis and CKD, demonstrating the importance of these viruses in the oral disbiosis in both conditions.

Authors Contribution: All authors have made substantial contributions to conception and design of the study. DP, GFR, SHF, DJSS, MP, and CP, have been involved in clinical data collection and analysis. CSP, ACMFS, TRT and PHB have been involved in laboratorial and molecular data collection and analysis. DP, GFR, CSP, AD, and PHB have been involved in data interpretation, drafting the manuscript and revising it critically. All authors have given final approval of the version to be published.

Abstract

Background: Patients with chronic kidney disease (CKD) have inability to maintain the normal levels of protein metabolism products, blood pressure and haematocrit. Periodontal disease (PD) involves an inflammatory destructive process. Identification of opportunistic viruses is extremely important as they are associated with co-morbidities. The objective of this study was to analyse the presence of human herpesviruses in saliva and gingival crevicular fluid (GCF) from patients with CKD. **Methods**: 131 individuals were divided depending on the stage of CKD: Group 1 (clearance of creatinine > 75 mL/min) patients with no renal disease (n=24); Group 2 (clearance of creatinine of 11-75 mL/min) patients with renal disease (n=67); Group 3 (clearance of creatinine < 10 mL/min) patients on haemodialysis (n=40). The parameters of PD were evaluated. The viral detection was assessed by PCR. **Results**: considering the 3 groups, the prevalence of HSV-1 were 9% in saliva and 5% in GCF; EBV 36% in saliva and 39% in GCF; HCMV 11% in GCF; VZV 6% in saliva and 3% in GCF; of HHV-6 6% in saliva and 2% in GCF; and HHV-7 44% in saliva and 8% in GCF. Of these patients, 46.48% presented with severe periodontitis. A statistically significant association between HSV-1 and HCMV was found in haemodialysis patients and severe periodontitis was also more frequent among them. **Conclusion**: These findings show the importance of evaluating the PD and detecting herpesviruses in patients with CKD as the inflammatory process observed in these clinical conditions may worsen the course of both PD and CKD.

Key-words: Chronic Renal Insufficiency, Periodontal Diseases; Saliva; Herpesviridae; Gingival Crevicular Fluid.

Introduction

Chronic kidney disease (CKD) is nowadays a major public healthcare problem. In 2010, 2.62 million individuals underwent dialysis worldwide and it is estimated that this figure will double by 2030[1].

CKD begins silently and evolves insidiously, impairing the renal function as the diseases progresses with increase in blood pressure (BP) and accumulation of nitrogenous substances, such as urea and creatinine[1,2]. For this reason, patients are monitored for serum levels of these markers and serial quantification of protein excretion[1,2]. CKD is classified into five stages according to glomerular filtration rates based on clearance of creatinine and excretion of urinary albumin[2]. Patients with values below 15 mL/min/1.73m^2 are regarded to be in the fifth or terminal stage, which is characterised by total loss of renal function[2].

Periodontal disease is considered an inflammatory and infectious process affecting the gingiva and tooth supporting tissues in which the causative factors are biofilm accumulation and dental calculus on a chronic aspect[3]. Therefore, periodontal disease can be classified as either gingivitis or periodontitis. The disease has a slow progression and is characterised by an important inflammatory response with destruction of the periodontal tissues[3]. Periodontitis can be associated with increased systemic inflammation, perhaps because of the acute phase of the inflammatory process, thus elevating the levels of systemic inflammatory markers[4]. An important aspect is that periodontal disease not only causes oral impairment, but also has a negative impact on general health conditions, particularly in the more severe cases[5].

According to a recent review[6], periodontal disease has a significant direct effect and an indirect effect (diabetes) on the incidence of CKD. In addition,

periodontitis was also found to be an intermediate variable in the causal pathway of diabetes in CKD patients[6].

Human oral cavity presents a complex microbiome consisting of bacteria, archaea, protozoa, fungi and viruses living in a biofilm community with a large number of interactions (e.g., biochemical, immunological) which can be both beneficial and antagonist[7,8].

Human herpesviruses are the most studied virus related to periodontal disease[7]. The hypothesis of herpesviruses participation in periodontitis proposes that an active herpetic infection triggers the destruction of the periodontal tissue while the immune responses against this infection act as an important component in the aetiopathology of the disease[9]. Infection by herpesvirus unleashes the release of pro-inflammatory cytokines, which can potentially activate osteoclasts and matrix metalloproteinases, thus impairing the immune anti-bacterial mechanism and increasing the amount of periodontal pathogenic bacteria[9]. The association between herpesviruses and bacteria seems to be capable of explaining several physio-pathological characteristics of the periodontitis[9].

Herpesviruses comprise the most prevalent viral family in human saliva and are major periodontal pathogens. Eight species of herpesviruses with different biological and clinical characteristics can infect human beings, namely: herpes simplex viruses 1 and 2 (HSV-1 and HSV-2), varicella zoster virus (VZV), Epstein-Barr virus (EBV), human herpesvirus-6 (HHV-6), human cytomegalovirus (HCMV), and human herpesviruses 7 and 8 (HHV-7 and HHV-8 or Kaposi sarcoma virus)[10]. Herpesviruses establish a persistent infection over time and some species of herpesvirus infect about 90% of the adult population[10-11]. The clinical result of an infection by herpesvirus ranges from a mild disease to encephalitis, pneumonia and

several types of cancer, especially in immunosuppressed patients[12]. The salivary transmission of some herpesviruses is very well-established, as is the case of EBV, which is particularly present in renal transplant individuals[13-15]. Nevertheless, in some members of the viral family, the role played by saliva in the transmission of viruses is still little understood[10, 16-18]. The composition of saliva and other oral fluids can reflect the levels of infectious pathogens and is a valuable source for evaluation and monitoring of systemic diseases. Thus, saliva can be used as a practical assessment tool for development of increasingly more sensitive assays[19].

CKD patients have immune alterations, which is an important factor for occurrence of infections and consequently for mortality[20]. One can observe a greater proportion of neutrophils than lymphocytes and a normal total amount of leukocytes. Lymphopenia can occur in dialysis patients and results from malnutrition and membranes used in haemodialysis[20].

Saliva is a biological fluid of high diagnostic value. One great advantage of salivary testing is that saliva can be easily and safely collected without pain compared to venous puncture, especially when patients present with haemorrhagic or infectious complications. Salivary bio-molecules are used as an auxiliary diagnostic means for identification of a variety of tumours, illicit and prescription drug use, hereditary disorders and hormonal irregularities. Salivary testing can also identify infections such as human immunodeficiency virus (HIV), herpesvirus family members, hepatitis virus, measles virus and other pathogenic viruses and bacteria[7,21,22].

Therefore, the objective of this study was to assess the presence of human herpesvirus in saliva and gingival fluid by comparing the findings to clinical and laboratory features of chronic renal disease and periodontal disease.

Patients & Methods

This study was approved by the local research ethics committee (CEP-UNITAU) according to protocol number 165997 (2012). All the patients read and signed an informed consent form. All procedures in the study were performed in accordance with national human research ethics standards and with the 1964 Helsinki Declaration, including later amendments or comparable ethical standards. Patients were recruited from May 2014 to April 2016.

This is a cross-sectional study with 131 individuals who met the inclusion criteria and were being followed up at the Nephrology Outpatient Clinic of the University of Taubate Hospital, at the Nephrology Institute of Taubate (NEFRO), at the State Centre for Renal Disease Treatment of the Vale do Paraiba and at the Nefrovale Clinic of Pindamonhangaba. The inclusion criteria were to present at least 15 teeth in the oral cavity and provide time availability for work execution. For exclusion criteria: have received periodontal treatment in the last 6 months; antibiotic therapy in the last 72 hours before periodontal examination, seropositivity for HIV, hepatitis C virus (HCV) or hepatitis B (HBV).

Clinical and laboratory data were collected, and the patients were referred to the University of Taubate Dental Clinic for evaluation, which included clinical analysis, periodontal examination and collection of saliva and gingival crevicular fluid.

The patients were divided into three groups according to the stage of the renal disease[2] based on glomerular filtration rate as follows

Group 1 (clearance of creatinine > 75 mL/min): patients with no renal

disease

Group 2 (clearance of creatinine of 11-75 mL/min): patients with mild-moderate renal disease

Group 3 (clearance of creatinine < 10 mL/min): patients with terminal renal disease - on haemodialysis

Periodontal Clinic Examination

Patients were examined to determine the presence of periodontal disease and the following parameters were assessed: probing depth (PD), clinical attachment loss (AL), gingival index[23] and plaque index[24].

The patients were classified according to periodontal parameters used by Eke et al. (2012)[25]. Mild periodontitis was defined as two or more interproximal sites with AL ≥ 3 mm and two or more interproximal sites with PD ≥ 4 mm (not on the same tooth) or one interproximal site with PD ≥ 5 mm; moderate periodontitis was defined as two or more interproximal sites with AL ≥ 4 mm (not on the same tooth) or two or more interproximal sites with PD ≥ 5 mm (not on the same tooth); and advanced periodontitis was defined as two or more interproximal sites with AL ≥ 6 mm (not on the same tooth) and one or more interproximal sites with PD ≥ 5 mm.

Saliva Collection

Saliva was collected with the patient seated and head slightly inclined forwards and downwards. The patient was also instructed not to talk in order to avoid salivation and not to swallow the saliva naturally secreted by the salivary glands. The technique of unstimulated salivary flow was used for saliva collection, which lasted

five minutes. The patient spitted the saliva into a disposable container, which was labelled and stored in a freezer at -80°C.

Gingival Crevicular Fluid (GCF) Collection

The dental surfaces were washed with water spray, dried with air jet and isolated with cotton rolls for selection of the sites in each patient. Paper strips[††] were inserted into the sulcus and/or periodontal pocket and left in position for 30 seconds until there was a certain resistance. Two sites with greater probing depth were selected for the GCF collection. The strips were removed and placed into 1.5 ml separated centrifugation tubes, which were labelled before being immediately frozen in dry ice and stored at -80°C for later laboratory analysis. All samples were used in the molecular analysis.

Laboratory Procedures

a. DNA Extraction from Saliva and Gingival Fluid

Extraction of DNA from saliva and gingival fluid was performed by using the Blood kit[‡‡] according to the manufacturer's instructions. The samples were quantified by using a spectrophotometer[§§] following parameters of purity and quality, as suggested by the manufacturer.

b. Human Herpesvirus, PCR and Restriction Enzyme Digestion

Two sets of primers (i.e. HSVP1/P2 and VZVP1/P2) were designed to target a well-conserved region of the DNA polymerase gene based on the alignment of DNA sequences from the eight known human herpesviruses, as described by Johnson et

[††] PerioPaper (Oralflow Inc) Hewlett, New York- USA
[‡‡] QIAamp DNA Mini Kit - Qiagen, Valencia, California, USA
[§§] NanoDrop ND-1000 Spectrophotometer v.3.0.1, Labtrade

al. (2000)[26]. The positive samples were submitted to enzymatic digestion with the restriction enzymes BamHi and BstUI[***] for specific determination of each one of the eight herpesviruses.

Statistical Analysis

The results were statistically analysed by using the SPSS software version 17.0 [†††]. The quantitative variables in our study did not have a normal distribution (Kolmogorov-Smirnov, $P < 0.05$), thus Kruskal-Wallis' test was used to compare the periodontal scores (i.e. PD, AL, plaque index and gingival index). Pearson's chi-square test was used to analyse the qualitative variables (i.e. periodontal disease and CKD classification). Kappa test was used to assess the inter-rater reliability between virus excretion through saliva and that through gingival fluid. All statistical analyses were performed at significance level of 5%.

Results

A total of 131 individuals were divided into three different groups as follows: Group 1, consisting of patients with no renal disease (24/131; 18.3%); Group 2, consisting of patients with mild-moderate renal disease (67/131; 51.2%); and Group 3, consisting of haemodialysis patients with severe renal disease (40/131; 30.5%). The majority of the patients were female (69/131; 52.7%) and 13.7% (18/131) were smokers.

With regard to periodontal condition, of the 131 patients evaluated, 17.6% (23/131) were edentulous, 3.8% (5/131) were healthy, 26.7% (35/131) had moderate

[***] New England Biolabs - UK
[†††] SPSS, Inc., Chicago, IL, USA

chronic periodontitis and the majority (51.9%; 68/131) had severe periodontitis. Table 1 shows periodontal parameters assessed in patients, with differences being observed between the groups, edentulous patients were excluded.

Table 2 shows the distribution of the groups according to stage of renal disease and periodontal disease, with all the three groups having similar results and severe periodontitis being the most observed in each of them. No statistically significant differences were observed.

It was observed that the values of periodontal parameters (i.e. PD, AL, plaque index and gingival index) increased as the renal disease worsened, being statistically significant for CAL (P = 0.023) (Table 3). The edentulous were excluded of the analysis.

With regard to the presence of virus in saliva, there was identification of HHV-7 (46.6%), EBV (36.6%), HSV-1 (10.7%), VZV (6.9%) and HHV-6 (6.9%), whereas in GCF there was identification of EBV (39%), HCMV (11.5%), HHV-7 (6.1%), HSV-1 (4.6%), VZV (3.1%) and HHV-6 (2.3%). The presence of HSV-2 and HHV-8 was not detected in saliva or GCF. Inter-rater reliability analysis of virus excretion in saliva and GCF revealed that HSV-1 had moderate reliability coefficient (Kappa = 0.466), whereas the other herpesviruses had low (EBV) or even insignificant ones (VZV, HHV-6 and HHV-7) (Table 4).

In the association between stage of renal disease and presence of herpesviruses in saliva, it was found that HSV-1 and EBV were more prevalent in Group 3 (haemodialysis patients) compared to Groups 1 and 2. The same association for GCF showed an increased presence of HSV-1 and HCMV in the group of patients on haemodialysis. The results for these associations were statistically significant (Table 5). No statistical associations (P > 0.05) were found

between periodontal disease classification and excretion of herpesvirus in saliva and/or GCF.

Discussion

The present analysis of viruses HSV-1, HSV-2, EBV, HCMV, VZV, HHV-6, HHV-7 and HHV-8 in patients with chronic kidney disease associated with periodontal disease seems to be novel in the literature, as we have found no study associating all these viruses. Among these eight viruses studied, we have found neither HSV-2 nor HHV-8 in the samples of saliva or GCF. On the other hand, Al-Otaibi et al. 2009[17] and 2012[18] investigated the presence of HHV-8 in renal transplant and in haemodialysis patients and found DNA copies of this herpesvirus both before and after kidney transplantation, associating this finding with Kaposi's sarcoma. This different result can be related to the geographical regions where their studies were performed, that is, there is a higher incidence of non-HIV related Kaposi's sarcoma in Mediterranean and Middle-Eastern populations. In Brazil, the salivary excretion of HHV-8 is low even in HIV-positive individuals[27].

From these results, one can observe that the presence of HSV-1 in saliva and GCF among haemodialysis patients was the most relevant finding. A higher frequency of HCMV in GCF was also observed in these same patients. Interestingly, no HCMV was found in any sample of saliva, suggesting a certain gap between periodontal space and oral cavity.

A study by Nikoobaht et al. (2011)[15] found the presence of EBV in the saliva of patients after kidney transplantation, a finding also observed in the same patients during haemodialysis. The presence of EBV may be associated with post-transplant

lymphoproliferative alterations, resulting in a worse prognosis. Our results demonstrated the presence of EBV in the saliva (36.6%) and gingival fluid (35.9%) of all three groups, with statistical difference for saliva and no significant difference for GCF[16, 28].

Some studies showed an association between presence of virus and periodontal disease, such as the one by Saygun et al. (2008)[21], who found HCMV and EBV in the saliva of patients with periodontitis. These authors found HCMV (50%) and EBV (79%) in patients with periodontitis as well as EBV in patients with gingivitis (33%) and in edentulous patients (54%).

In the present study, we have found that haemodialysis patients had the highest values of AL compared to the other three parameters (Table 3). Kshirsagar et al. (2007)[29] observed that severe periodontal disease was associated with a glomerular filtration rate below 60 mL/min/1.73 m^2 (OR 2.00; 95% CI; 1.23 to 3.24), a finding also observed in other study in which patients with severe CKD had a worse periodontal condition compared to those with mild CKD[30].

The presence of HSV-1 in saliva and GCF was associated with haemodialysis patients who had moderate periodontitis. In the other groups of renal patients, no statistically significant differences were found regarding the periodontal disease.

In the literature, different classifications and diversity of methodologies of categorization in periodontal diseases were used. However, there were no consensus on which would be most appropriate[31]. In this study, we adopted the classification of Eke et al, 2012[25] because it was conducted before 2017. Given the new classification, patients with moderate and severe PD correspond to Stages III and IV [32].

The composition of saliva and other oral fluids can affect the levels of infectious pathogens, meaning that they can be valuable sources for evaluation and monitoring of systemic diseases. Therefore, saliva and other oral fluids can be used as a practical assessment tool for development of increasingly more sensitive assays. A previous study with the same group of patients found a higher frequency of salivary inflammatory markers in patients on haemodialysis[19].

We have found an association between presence of HSV-1 in saliva and that in GCF, as the likelihood of its detection in this fluid was 99 times higher when the virus was also detected in saliva. As for EBV, this virus was eight times more likely to be present in saliva when it was also present in GCF.

Inflammation is a multifactorial biological process whose mechanism involves innate immunity, which can be observed in different clinical conditions ranging from acute (i.e. immediate response) to chronic (i.e. late response) situations, consequently leading to inflammatory process[33-34]. There are several causes of inflammation in which infections resulting from viral agents, such as HIV, HSV and EBV, can be observed[35]. Despite the therapeutic activity promoted by the inflammatory process, there are several chronic diseases associated with chronic inflammation[36]. During the chronic inflammatory process one can observe the presence of free radicals, which induce the activation of signalling molecules and transcription factors, such as nuclear factors Kappa B (NF-kb) and STAT3, all involved in the genesis of several diseases[36,37]. Other bio-markers involved in chronic diseases include inflammatory cytokines, such as tumoral necrosis factor (TNF-α), interleukins (IL-1, 6 and 8), C-reactive protein (CRP), adhesion molecules, vascular endothelial growth factor (VEGF), among others[38-40]. Therefore, chronic

inflammatory process plays an important role in the development and progression of CKD.

CKD can present different clinical manifestations during progression of uraemia, including oral involvement[41-42]. Uraemic syndrome causes oral disorders with higher frequency than in individuals without renal disease, in which there is a wide range of lesions affecting oral mucosa, teeth, salivary glands and mandible bones[43]. Clinically, they manifest as xerostomia, uraemic stomatitis and periodontal disease. In addition, poor oral hygiene is also associated, especially in the presence of inflammatory markers[43].

The association between systemic inflammatory markers, periodontitis and malnutrition in dialysis patients is well known, being considered a major factor for hospitalisation and death in the future. It is important to highlight that the treatment of periodontal disease has a positive impact on inflammatory markers[44-46].

The inflammatory process observed in CKD systemically and in periodontal disease locally can modulate the immune response of these patients both directly and indirectly. This can promote changes in the systemic or local defence response, thus favouring a higher prevalence of pathogens such as bacteria and viruses, especially in the oral cavity[4,12].

It is important to emphasise that CKD has an insidious progression during its clinical course and, despite the non-correlation with traditional aggravating factors (e.g. persistently elevated blood pressure and glycemic levels) or other factors acting as sources of inflammation (e.g. periodontal disease), can decisively contribute to worsening the clinical course, which can accelerate the reduction of glomerular filtration rate and lead to the need for haemodialysis and kidney transplantation[47]. The progressive reduction of glomerular filtration rate causes uraemia – a process

contributing to compromising the immune response, which is a factor further aggravated by the malnutrition[18,47]. These situations can significantly contribute to a higher risk of infection by human herpesviruses[18,47].

The herpesviruses present in the oral cavity of CKD patients can contribute to the occurrence of periodontal disease. On the other hand, in CKD patients undergoing kidney transplantation, the immunosuppressive therapy can allow a better modulation of the viral activity as there is a higher expression of herpesviruses, which contributes to or even determines a higher risk of severe infections or even acute and/or chronic rejection of the renal graft[12,29].

Another relevant aspect is that CKD patients have higher cardiovascular risk, since cardiovascular disease is the main cause of mortality in this population. Infection by HSV-1 and HSV-2 was associated with the presence of inflammatory process resulting from atherosclerosis, especially in haemodialysis patients with cardiovascular disease undergoing kidney transplantation[48].

Otero et at. (2011)[49] identified the presence of herpesviruses (i.e. HSV-1, HHV-6B, HHV-7 and HCMV) in the saliva of CKD patients aged 4-20 years old, the majority undergoing haemodialysis. In our study, we have assessed older patients and found a higher frequency of HSV-1, EBV and HHV-7 in the saliva and gingival fluid of CKD patients in Groups 2 and 3 as well as HCMV in Group 3.

In a recent study, Bruggeman (2019)[50] assessed the mechanisms by which viruses can promote renal lesions in which genomic data were also included, suggesting a future possibility of viral aetiologies for idiopathic CKD. This, therefore, highlights the importance of evaluating the occurrence of viral infections in this group of patients.

A potential bias in our study was the healthy periodontal group, which presented just 5 patients (3%). Periodontal clinic examination decreases this possible effect. Another limitation presented by was the molecular technique not being able to identify viral replication.

Since CKD patients present with compromised immune response, there is a higher likelihood that opportunistic pathogens will cause infections in this population. This suggests that measures to prevent and treat infections should be considered as an important aspect in the assessment and care of those patients who are undergoing conservative treatment or dialysis.

Conclusion

These findings show the importance of evaluating the periodontal disease and detecting herpesviruses in patients with CKD as the inflammatory process observed in these clinical conditions may worsen the course of both periodontal disease and CKD.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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Table 1. Association between periodontal classification and periodontal parameters assessed

Periodontium		**PD**		**AL**		**PI**		**GI**	
	N	**Mean rank**	**Mean±SD**	**Mean rank**	**Mean±SD**	**Mean rank**	**Mean±SD**	**Mean rank**	**Mean±SD**
Healthy	5	27.20	2.16±0.21	4.60	1.12±0.48	23.30	1.21±0.40	19.40	1.04±0.31
MCP	35	34.09	2.27±0.47	35.17	3.02±1.13	43.28	1.54±0.50	39.03	1.32±0.42
SCP	68	67.01	3.12±0.89	68.12	4.71±1.62	61.62	1.87±0.51	65.04	1.74±0.50
P value*		**< 0.001***		**< 0.001***		**0.001***		**< 0.001***	

Periodontium		**PD**		**AL**		**PI**		**GI**	
	N	**Mean rank**	**Mean±SD**	**Mean rank**	**Mean±SD**	**Mean rank**	**Mean±SD**	**Mean rank**	**Mean±SD**
Healthy	5	27.20	2.16±0.21	4.60	1.12±0.48	23.30	1.21±0.40	19.40	1.04±0.31
MCP	35	34.09	2.27±0.47	35.17	3.02±1.13	43.28	1.54±0.50	39.03	1.32±0.42
SCP	68	67.01	3.12±0.89	68.12	4.71±1.62	61.62	1.87±0.51	65.04	1.74±0.50
P value*		**< 0.001***		**< 0.001***		**0.001***		**< 0.001***	

Legends: MCP – moderate chronic periodontitis; SCP - severe chronic periodontitis; PD – probing depth; AL – clinical attachment loss; PI –plaque index; GI – gingival index; SD – Standart deviation.

[1]Kruskal Wallis test;

*****Statistical significance.

Table 2. Distribution of the groups according to stage of renal disease and periodontal disease.

Renal classification	Periodontal disease					
	Edentulous	Healthy	Moderate periodontitis	Severe periodontitis	Total	P value[1]
Group 1	2 (8.3)	1 (4.2)	10 (41.7)	11 (45.8)	24 (100.0)	
Group 2	14 (20.9)	2 (3.0)	17 (25.4)	34 (50.7)	67 (100.0)	0.541
Group 3	7 (17.5)	2 (5.0)	8 (20.0)	23 (57.5)	40 (100.0)	
Total	**23 (17.6)**	**5 (3.8)**	**35 (26.7)**	**68 (51.9)**	**131 (100.0)**	

[1]Pearson's chi-square test.

Table 3. Association between renal classification and periodontal parameters assessed.

Renal classification	N	PD		AL		PI		GI	
		Mean rank	Mean±SD	Mean rank	Mean±SD	Mean rank	Mean±SD	Mean rank	Mean±SD
Group 1	23	49.43	2.62±0.71	39.67	3.21±1.33	46.95	1.62±0.58	44.39	1.44±0.60
Group 2	53	54.66	2.80±0.90	56.81	3.99±1.59	54.28	1.74±0.50	54.44	1.55±0.50
Group 3	33	59.42	2.88±0.91	62.77	4.49±2.10	58.26	1.81±0.55	61.33	1.68±0.49
P value*		0.505		**0.023***		0.415		0.145	

Legends: PD – probing depth; AL – clinical attachment loss; PI –plaque index; GI – gingival index; SD – Standart deviation

Kruskal Wallis test; *Statistical significance.

Table 4. Prevalence of herpesviruses in saliva and gingival fluid and Kappa values.

Virus	Presence	Saliva		Gingival fluid		Kappa value	*P*-value
		N	%	N	%		

HSV 1	No	117	89.3	125	95.4	0.466	**<0.001***
	Yes	14	10.7	6	4.6		
EBV	No	83	63.4	84	64.1	0.323	**<0.001***
	Yes	48	36.6	47	35.9		
HCMV	No	131	100	116	88.5	-	-
	Yes	0	0	15	11.5		
VZV	No	122	93.1	127	96.9	-0.044	0.581
	Yes	9	6.9	4	3.1		
HHV 6	No	122	93.1	128	97.7	0.137	0.067
	Yes	9	6.9	3	2.3		
HHV 7	No	70	53.4	123	93.9	0.106	**0.017***
	Yes	61	46.6	8	6.1		

* Statistical significance

Table 5. Association between herpesviruses in saliva and gingival fluid according to stage of renal disease.

Variable	**Presence**	**Renal classification**			***P* -value**[1]
		Group 1	**Group 2**	**Group 3**	
		SALIVA			
HSV 1	No	23 (95.8)	63 (94.0)	31 (77.5)	**0.014***
	Yes	1 (4.2)	4 (6.0)	9 (22.5)	
EBV	No	19 (79.1)	45 (67.1)	19 (47.5)	**0.026***
	Yes	5 (20.9)	22 (32.9)	21 (52.5)	
VZV	No	24 (100)	62 (92.5)	36 (90.0)	0.298
	Yes	0 (0)	5 (7.5)	4 (10.0)	
HHV-6	No	23 (95.8)	62 (92.5)	37 (92.5)	0.845
	Yes	1 (4.2)	5 (7.5)	3 (7.5)	
HHV-7	No	15 (62.5)	35 (52.2)	20 (50.0)	0.600
	Yes	9 (37.5)	32 (47.8)	20 (50.0)	

		GINGIVAL CREVIVULAR FLUID			
HSV-1	No	24 (100)	66 (98.5)	35 (87.5)	**0.015***
	Yes	0 (0)	1 (1.5)	5 (12.5)	
EBV	No	19 (79.1)	43 (64.1)	22 (55.0)	0.149
	Yes	5 (20.9)	24 (35.9)	18 (45.0)	
HCMV	No	24 (100)	60 (89.5)	32 (80.0)	**0.048***
	Yes	0 (0)	7 (10.4)	8 (20.0)	
VZV	No	23 (95.8)	65 (97.0)	39 (97.5)	0.931
	Yes	1 (4.2)	2 (3.0)	1 (2.5)	
HHV-6	No	24 (100)	65 (97.0)	39 (97.5)	0.699
	Yes	0 (0)	2 (3.0)	1 (2.5)	
HHV-7	No	24 (100)	61 (91.0)	32 (80.0)	0.273
	Yes	0 (0)	6 (9.0)	8 (20.0)	
TOTAL		**24(100)**	**67(100)**	**40(100)**	

[1]Pearson's chi-square test. * Statistic significance