Oral Diseases (2007) 13, 198–205. doi:10.1111/j.1601-0825.2006.01267.x

http://www.blackwellmunksgaard.com

ORIGINAL ARTICLE

Periodontal and oral microbiological status of an adult population undergoing haemodialysis: a cross-sectional study

A Castillo[1], F Mesa[2], J Liébana[1], O García-Martinez[2], S Ruiz[2], J García-Valdecasas[3], F O'Valle[4]

[1]*Department of Microbiology, Schools of Medicine and Dentistry, University of Granada;* [2]*Department of Periodontics, School of Dentistry, University of Granada;* [3]*Department of Medicine, Section of Nephrology, "San Cecilio" University Hospital of Granada;* [4]*Department of Pathology, School of Medicine and IBIMER, University of Granada, Granada, Spain*

OBJECTIVES: The aim of this cross-sectional study was to evaluate the periodontal status and oral microbiological patterns of a population with end-stage renal disease (ESRD), undergoing haemodialysis (HD).

DESIGN: This was a cross-sectional study, involving 52 patients from the Nephrology Department and 52 matched control subjects.

MATERIALS AND METHODS: The subjects had a periodontal clinical examination; subgingival plaque samples were taken and analysed using a semiquantitative polymerase chain reaction (PCR) test to detect *Porphyromas gingivalis*, *Tannerella forsythia*, *Prevotella intermedia*, *Prevotella nigrescens* and *Actinobacillus actinomycetemcomitans*. Subgingival plaque and saliva samples were studied for *Candida* and Enterobacteriaceae.

MAIN OUTCOME MEASURES: Most of the 104 subjects had some degree of loss of periodontal attachment (LPA) ≥3 mm [11 (10.5%) had severe LPA; 16 (15.4%) moderate LPA; and 64 (61.5%) mild LPA]. Only 13 subjects (12.5%) presented good periodontal health.

RESULTS: No statistically significant differences were found between the HD patients and the control group regarding bleeding index, number of teeth, or percentage of LPA ≥3 mm. However, a statistically significant difference was seen in the degree of oral hygiene.

CONCLUSIONS: On the basis of the findings presented here, we cannot associate ESRD with more severe periodontal destruction. Although HD patients presented a higher number of periodontopathic microorganisms than the matched controls, a prolonged duration of HD did not bear a statistically significant relationship with the percentage of sites with LPA ≥3 mm, specific microbiota or composition of biofilm.

Correspondence: Dr Francisco Mesa, Facultad de Odontología, Campus de Cartuja s/n, Universidad de Granada, E-18071 Granada, Spain. Tel: +34-958240654, Fax: +34-958240908,
E-mail: fmesa@ugr.es
Received 14 October 2005; revised 16 February 2006; accepted 28 February 2006

Oral Diseases (2007) **13**, 198–205

Keywords: periodontal disease; microbiology; PCR; chronic renal failure; haemodialysis; ESRD

Introduction

Chronic renal failure (CRF) is a progressive and irreversible loss of functioning nephrons, resulting in a decline in the glomerular filtration rate. The mechanisms of progression to end-stage renal disease (ESRD) may involve glomerular hyperfiltration, high blood pressure with cytokine induction (mainly angiotensin II and transforming growth factor-β), sustained proteinuria or renal ischaemia. ESRD requires renal treatment with haemodialysis (HD), peritoneal dialysis, or transplant. The incidence of ESRD in Spain has stabilized over recent years at 885 cases per 1 million inhabitants. Half the mortalities among these patients is due to cardiovascular diseases, and a quarter is due to infectious diseases (López *et al*, 2001; Amenábar *et al*, 2002).

There is limited information in the scientific literature on the oral health status of patients receiving HD, and most studies report a high prevalence of dental and periodontal diseases (Klassen and Krasko, 2002; Al-Wahadni and Al-Omari, 2003; Marakoglu *et al*, 2003). Naugle *et al* (1998) reported that 100% of patients on dialysis presented some form of periodontal disease, most often severe gingivitis or mild periodontitis with a high index score of oral hygiene. These authors recommended comprehensive professional oral care and self-care instruction for the HD population, regardless of the length of treatment. Moreover, as plaque-related diseases can prove to be a source of active infection in these medically compromised individuals, Klassen and Krasko (2002) recommended active oral treatment.

Different alterations in the periodontal tissues have been documented in HD-treated patients. A relatively

pale tone is usually present because of the drop in haemoglobin levels (anaemia resulting from a deficit in erythropoietin) and the inflammatory response to bacterial plaque is consequently diminished or inadequate (Jaffe *et al*, 1986). The usual increase in parathyroid activity – secondary to altered serum calcium and phosphate levels – gives rise to craniofacial bone alterations attributable to secondary hyperparathyroidism (HPT) (Baylink *et al*, 1974; Kelly *et al*, 1980). One of the oral implications of HPT is the loss of the lamina dura (Bramley and Dwyer, 1970; Kennett and Pollick, 1971). Dental calculus may form at a greater speed because of an imbalance in the calcium phosphate in serum, favouring even ectopic calcifications. Other oral symptoms described in these patients are an enlargement of the tongue and a metallic taste before each dialysis session, related to anaemia and uraemia (Westbrook, 1978). Nonetheless, there are not sufficient data to establish a positive association between dental, periodontal or mucous membrane disease and ESRD requiring HD (Gavaldá *et al*, 1999; Marakoglu *et al*, 2003). Some populations of CRF patients undergoing HD show no difference in the gingival index, plaque index or probing depths when compared with the general population (Marakoglu *et al*, 2003), and a very small portion of the population undergoing renal dialysis is affected by severe forms of periodontitis, although all such patients should be given oral hygiene education as a priority (Duran and Erdemir, 2004).

The aim of this cross-sectional study on a population with ESRD was to determine whether ESRD or the HD treatment is associated with periodontal disease or with specific or more complex subgingival or salivary microbiota when compared with a matched control group.

Materials and methods

A cross-sectional study was carried out on a population of patients suffering from ESRD who required HD at the Dialysis Unit of the Nephrology Department of the University Hospital 'San Cecilio', in Granada, Spain. The study was conducted according to the Declaration of Helsinki and approved by the Ethics Committee of the hospital. Subjects, not fulfilling the exclusion criteria, were included in this investigation after agreeing to participate and signing the appropriate consent forms.

We established a control group by enrolling trauma patients (mainly from mild traffic or work accidents) admitted to the Traumatology Department of our hospital (excluding outpatient visits) during the same period. The sampling procedure involved matching each HD patient with a control (age ± 3 years and sex matched).

The exclusion criteria were: having no teeth; having undergone periodontal treatment in the previous year or having received antibiotic treatment during the 72 h previous to the oral exploration; being seropositive for the human immunodeficiency virus (HIV), hepatitis C virus (HCV) or hepatitis B virus (HBV); or patients with maxillofacial trauma. Of the 110 HD patients with ESRD from the Dialysis Unit screened, 18 were edentulous, 22 were seropositive for HIV, HCV or HBV, eight were under antibiotic treatment and 10 chose not to participate in the study. The final sample consisted of 52 HD patients and 52 controls.

After gathering information regarding the number of months of receiving HD, the biochemical parameters (only HD group), age, sex and a periodontal examination, the subgingival plaque and saliva samples for microbiological analysis were collected from all the selected subjects.

Periodontal examination

Oral examination was carried out by a single calibrated dentist (OG). The calibration was carried out about 4 weeks before the start of the study in the Department of Periodontology of the Dental School. The diagnosis was compared with that of another author (FM) in 13 adult periodontal patients, obtaining intraclass correlation coefficients (for gingival retraction and pocket depth) of above 0.71, considered substantial on the scale of Landis and Koch (1977). Using the Michigan '0' periodontal probe (Hu-Friedy, Leimen, Germany) and a number 5 non-magnifying oral mirror (SE Plus, MFG Precision Dental Int., Inc., Canoga Park, CA, USA), the loss of periodontal attachment (LPA) was measured by adding the pocket depths and gingival recessions (in mm), evaluating six sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual and distolingual) for all the teeth present in the oral cavity. The degree of periodontitis was defined, after Arbes *et al* (1999) as the percentage of sites with a loss of attachment $\geq$3 mm, as follows: 0% = absent, 0–32% = mild, 33–66% = moderate and 67–100% = severe. We evaluated the periodontitis extension according to the methodology of these authors, and use the American Academy of Periodontology terminology throughout this paper.

We used the gingival bleeding index of Ainamo and Bay (1975) and the plaque index of Silness and Löe (1964) to assess the degree of gingival inflammation and oral hygiene, respectively.

Biochemical study

At the time of the oral examination, the clinical history was reviewed and the following serum biochemical parameters were selected for all HD patients: calcium, alkaline phosphatase (AP), glucose, albumin and parathyroid hormone (PTH).

Microbiological study

Different samples were processed for microbiological analysis. For polymerase chain reaction (PCR), subgingival plaque samples were taken from the deepest probing depth of each quadrant. Once the supragingival plaque had been removed with a cotton swab, a sterile paper point was introduced into the bottom of the pocket and left in place for 30 s (Maillefer Instruments, Ballaigues, Switzerland). Four samples from each subject were placed together in an Eppendorf tube containing 200 μl of distilled water (Sakamoto *et al*, 2001;

Takeuchi *et al*, 2001). Samples were taken immediately to the laboratory for processing.

PCR analysis

After agitating the vial containing the sample in a vortex for 10 s, the DNA was extracted following the manufacturer's instructions of the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). The DNA extracts were cryopreserved at −20°C until their use. Table 1 lists the PCR primers used in the study that have been described previously: *Porphyromonas gingivalis*, *Tannerella forsythia*, *Prevotella intermedia* and *Prevotella nigrescens* (Ashimoto *et al*, 1996), as well as *Actinobacillus actinomycetemcomitans* (García *et al*, 1998).

All primers were synthesized on Tib Molbiol (Berlin, Germany). We used the following reference strains as controls: *P. gingivalis* ATCC 33277, *T. forsythia* JCM 10827, *P. intermedia* ATCC 25611, *P. nigrescens* ATCC 33563 and *A. actinomycetemcomitans* CCUG 1210.

The PCR mixtures for amplification were prepared following the steps proposed by the manufacturers of the primers, using Master Mix 2× (M7502, Promega) as the base and adjusting the final concentrations of each of the components. In all cases, 5 μl of the sample was added to 45 μl of the PCR Master Mix. The PCR temperature profile of *P. intermedia*, *P. nigrescens* and *A. actinomycetemcomitans* included an initial denaturation step at 95°C for 2 min, followed by 36 cycles of a denaturation step at 94°C for 30 s, a primer-annealing step at 55°C for 1 min and an extension step at 72°C for 2 min, with a final step of 72°C for 10 min. The temperature profile of *P. gingivalis* and *T. forsythia* included an initial step at 95°C for 2 min followed by 36 cycles of 95°C for 30 s, 60°C for 1 min and 72°C for 1 min, and a final step of 72°C for 2 min. The temperature profile of *A. actinomycetemcomitans* included an initial step at 94°C for 10 min followed by 30 cycles of 94°C for 1 min, 70°C for 1 min and 72°C for 1 min, and a final step of 72°C for 10 min.

Table 1 List of PCR primers used for the investigation of periodontal pathogens previously described by Ashimoto *et al* (1996) and García *et al* (1998)

Primer pairs (5′–3′)	*Amplicon length in bp*
P. gingivalis[a]	
AGG CAG CTT GCC ATA CTG CG	404
ACT GTT AGC AAC TAC CGA TGT	
T. forsythia[a]	
GCG TAT GTA ACC TGC CCG CA	641
TGC TTC AGT GTC AGT TAT ACC T	
P. intermedia[a]	
TTT GTT GGG GAG TAA AGC GGG	575
TCA ACA TCT CTG TAT CCT GCG T	
P. nigrescens[a]	
ATG AAA CAA AGG TTT TCC GGT AAG	804
CCC ACG TCT CTG TGG GCT GCG A	
A. actinomycetemcomitans[b]	
CGT GCC AGC AGC CGC GGT AAT ACG	253
CTT TGC ACA TCA GCG TCA	
GTA CAT CCC CAA GG	

[a]Ashimoto *et al* (1996) and [b]García *et al* (1998).

The PCR products were analysed by 3% agarose gel electrophoresis in TBE buffer 1x, with the addition of an ethidium bromide solution (46067 Fluka, BioChemika, Steinheim, Switzerland), until a final concentration of 0.5 μg ml^{-1} was obtained. In all cases, a molecular weight marker was included. After 45 min of electrophoresis at 80 V, the agarose gel was photographed with a Polaroid DS34 camera (Expotech^{TM} Houston, TX, USA) placed over an ultraviolet light source.

For the semi-quantitative assessment of the PCR products obtained from the plaque samples, we developed an assay based on the standard patterns obtained from reference strains following the methodology recommended by Ashimoto *et al* (1996). After cultivation of the five reference species, saline solution suspensions with turbidities of 1 and 0.5 on the MacFarland scale were prepared from the isolates, and a series of (150×10^6 ufc) 10-fold dilutions was prepared in the decreasing order (10^{-1} to 10^{-8}) to obtain a standardized number of bacteria (Tran and Rudney, 1999). These samples underwent DNA extraction, amplification with the corresponding primers, development with ethidium bromide, and visualization in agarose electrophoresis gel, as described. The gels obtained were photographed using a standard procedure to create an image pattern of PCR product from known numbers of bacteria. The photographs were digitalized using a scanner (Scanjet 5500c; Hewlett Packard, Palo Alto, CA, USA) on a 256-grey-level scale (black and white) and stored in TIFF format. The nucleic acid bands were then quantified by means of digital image analysis using Visilog 6.0 software (Noesis S.A., Courteboeuf, France) (Masseroli *et al*, 1993; Arrebola *et al*, 2001). We quantified the mean optic density (MOD) and the integrated mean optic density (IMOD) of the band, using the formula: IMOD = CMOD × area, where CMOD is the mean optic density corrected for the background optic density (BMOD); i.e. CMOD = MOD − BMOD.

The patterns obtained with the strains of reference were used to establish the quantity of DNA corresponding to the periodontopathogenic bacteria present in the samples.

Study of Candida spp. and Enterobacteriaceae

The analysis of these bacteria was performed after culturing the samples obtained from subgingival scrapings, introduced in Eppendorf tubes, in specific media, with 500 μl of phosphate-buffered solution (PBS), and from the saliva samples after rinsing with PBS. The media used were Saboureaud gentamicine agar for the study of *Candida* spp. and MacConkey agar for the study of Enterobacteriaceae. The micro-colonies obtained were studied quantitatively by the viable cell count method using dilutions up to 10^{-5}. Microbial identification was performed on the optimal dilution using the automated Vitek 2 system (Biomerieux, Lyon, France).

Statistical analysis

The design, coding and debugging of the database and its statistical analysis were carried out using the SPSS-PC/Windows version 12.0 software package (SPSS Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was used to assess the normality of the variables. After the descriptive analysis, the bivariate associations between the studied variables were analysed with the appropriate test for that type of variable (Student's *t*-test, Mann–Whitney *U*-test, chi-squared test or Pearson's correlation) to determine statistical significance. The confidence interval was 95% ($P < 0.05$).

Results

A total of 52 adult HD patients were evaluated: 24 men (46.2%) and 28 women (53.8%). The mean age of this sample was 61.5 years (s.d. 18.04). The HD time ranged from 4 to 142 months, with a mean of 43.4 months (s.d. 38.96), yet this time was much longer among the women of our study (56.4 months, s.d. 11.4) than among the men (26.8 months, s.d. 8.1) ($P = 0.046$, Student's *t*-test). When compared with the control group, there were no significant differences in age (59.8 years; s.d. 10.7, $t = 1.3$; $P = 0.1$) or gender.

Table 2 gives the distributions, mean values, standard deviations and statistical significance of the periodontal variables in this study. No statistically significant differences were found in the bleeding index, number of teeth or percentage of LPA ≥3 mm between the HD patients and the control group. However, a noteworthy and statistically significant difference was seen in the degree of oral hygiene.

When the clinical variables of the HD group [age ($r = 0.259$), months of dialysis ($r = -0.227$), bleeding index ($r = 0.164$)] were studied with respect to the percentage of sites with LPA using the Pearson's test, no statistically significant correlations were obtained, except for the number of teeth ($r = -0.610$, $P = 0.002$). When these variables were categorized according to the time of dialysis treatment (either over or under 24 months) or by age interval, again there was no significant association with the LPA. More prolonged dialysis treatment did not have an effect on the extension of the periodontitis or on gingival bleeding to a statistically significant degree.

The biochemical profile obtained from the clinical history of HD patients included glucose, albumin, calcium, AP and PTH levels, assessed at the time of the oral examination. Eight patients (15.4%) presented with hypocalcaemia, with PTH values (mean 335.26 pg ml^{-1}, s.d. 270.4) five times the normal value, and in association with HPTs; the mean level of AP was 98.37 UI (s.d. 36.8; range 186–48). Six patients (11.5%) presented with moderate hypoalbuminaemia. In six patients for whom the cause of ESRD was diabetic nephropathy, glycaemia still gave a slightly high value. None of the analytical data showed a statistical relationship with periodontal status.

The microbiological PCR study (Figure 1) revealed only six HD patients (11.5%) to harbour all the five periodontal pathogens analysed. *Tannerella forsythia* was the most prevalent, isolated in 46 patients, followed by *P. nigrescens* (in 44 patients), *P. gingivalis* (in 40 patients) and *P. intermedia* and *A. actinomycetemcomitans* (in 18 patients). In two patients the PCR study did not reveal any of the species studied. In the control group, *P. nigrescens* was the most prevalent, isolated in 50 subjects, followed by *T. forsythia* (in 12 patients), *P. intermedia* (in 10 patients) and *P. gingivalis* and *A. actinomycetemcomitans* (in four patients), but no statistical differences were found between the composition of biofilm (the number of different periodontopathogenic species) in the HD patients and the control group ($\chi^2 = 0.333$, $P = 0.564$) (Table 3); there were, however, differences in the quantity of periodontopathogens ($P < 0.001$, Student's *t*-test) (Table 3). The highest concentration of the microorganisms studied corresponded to *T. forsythia*, and the lowest to *A. actinomycetemcomitans* in HD patients, whereas in controls the highest concentration corresponded to *P. nigrescens*, and the lowest to *A. actinomycetemcomitans* ($P < 0.001$, Student's *t*-test) (Table 3).

The LPA showed no statistical relationship with the number of periodontopathogenic species studied. In subjects with no LPA or <32% of sites, the mean total

Table 2 Comparison between oral status in haemodialysis patients and controls

	Index values			*Patients with sites LPA ≥3 mm*[c]			
	Plaque[a]	*Gingival bleeding*[b] *(%)*	*No. of teeth*	*0%*	*0–32%*	*33–66%*	*67–100%*
HD group ($n = 52$)	2.0 ± 0.7[d]	19.9 ± 6.2	19.8 ± 8.5	4	36	8	4
Control group ($n = 52$)	1.2 ± 0.6	18.2 ± 5.2	21.0 ± 7.6	9	28	8	7
	$t = 5.7$[e]	$t = 1.2$	$t = 1.1$		$\chi^2 = 3.74$, $P = 0.29$[f]		
	$P < 0.001$	$P = 0.2$	$P = 0.3$				

LPA, loss of periodontal attachment (gingival retraction + pocket depth); HD, haemodialysis group.
[a]Silness and Löe (1964).
[b]Ainamo and Bay (1975).
[c]Arbes *et al* (1999).
[d]Mean ± standard deviation.
[e]Student's *t*-test.
[f]Chi-squared test.

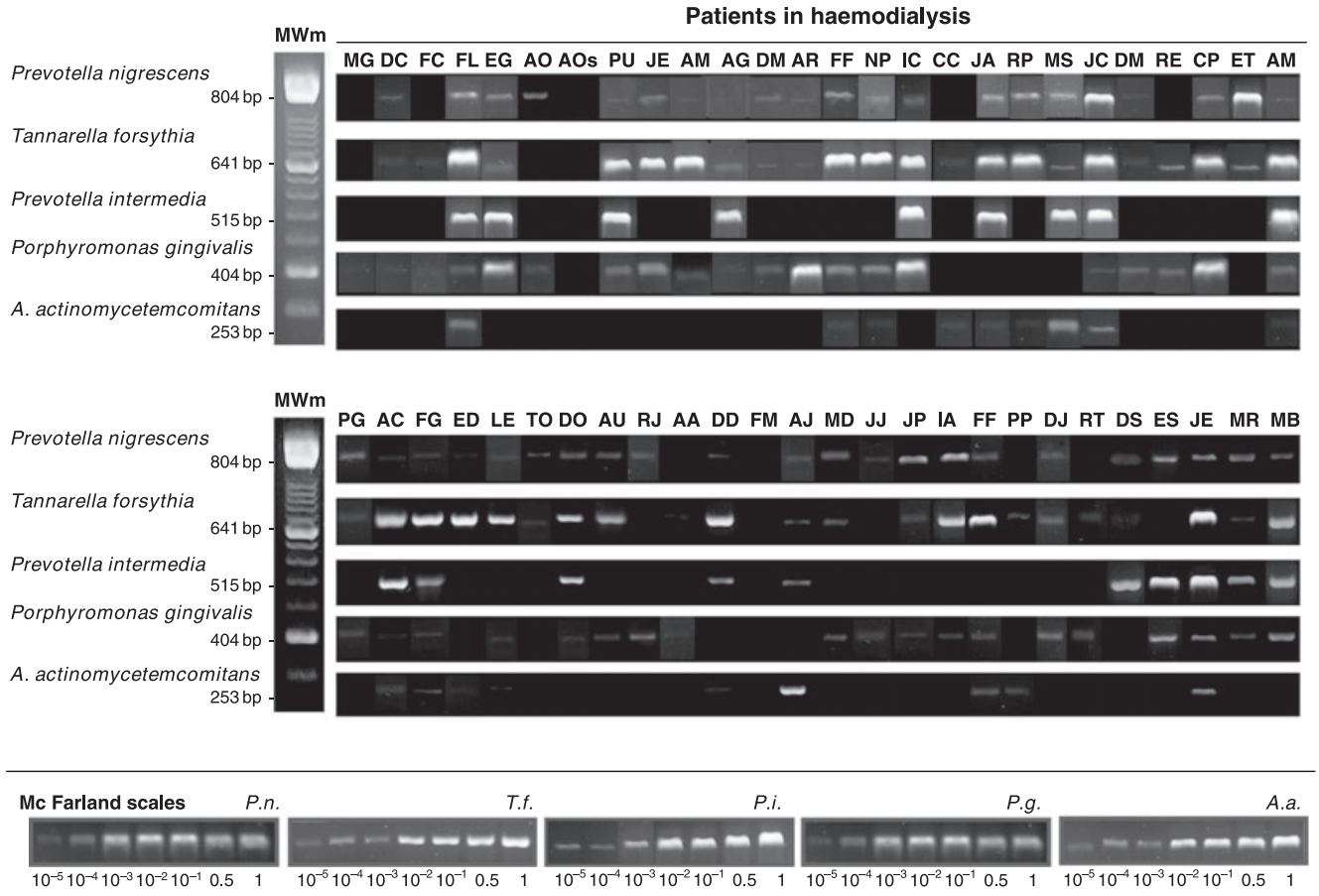

Figure 1 Electrophoresis in agarose gels of the PCR products used to detect the presence of the periodontopathogenic bacteria. Each column corresponds to a single patient. Note that only in four patients it was possible to demonstrate the presence of all five periodontopathogens. Below, different PCR patterns (MacFarland) obtained with the strains of reference to establish the quantity of DNA corresponding to the periodontopathogenic bacteria present in the samples

Pathogen	*Control group (n = 52)*	*HD group (n = 52)*	*t-value*[a]	*P-value*
Aa	3.3E+3 ± 1.4E+3	69E+3 ± 308E+3	1.53	0.127
Pg	17.9E+4 ± 1184E+3	204.5E+4 ± 3928E+3	3.27	0.001
Pn	3.4E+4 ± 373E+3	97E+4 ± 1094E+3	3.91	< 0.001
Tf	0.34E+8 ± 83223E+3	1.4E+8 ± 15962E+3	4.22	< 0.001
Pi	0.05E+7 ± 18982E+3	6.8E+7 ± 125038E+3	3.570	0.001

The values of the different periodontopathogens correspond to the extrapolation of data from strains of reference, expressed as mean ± standard deviation.
Aa, *A. actinomycetemcomitans*; *Pg*, *P. gingivalis*; *Pn*, *P. nigrescens*; *Tf*, *T. forsythia*; *Pi*, *P. intermedia*.
[a]Student's *t*-test.

Table 3 Comparison of the mean number of periodontopathogens, by digital image analysis of agarose gel electrophoresis DNA bands, of HD patients and controls

number of microorganisms was 3.1×10^6 (s.d. 0.25) as opposed to 3.5×10^6 (s.d. 0.56) in the cases of 33–100% of LPA ($P = 0.47$, Student's *t*-test) (Table 4).

In 14 HD patients (27%), the salivary samples showed strains of *Candida albicans*, as did 10 (20%) from the control group. This presence was significantly higher among males than females ($P = 0.038$, Mann–Whitney *U*-test). *Serratia marcescens* was isolated from saliva samples in two HD patients, while *Klebsiella oxytoca* was identified in another two. In the control group, two cases with *Escherichia coli* were identified. In no case Enterobacteriaceae were identified from the subgingival scraping of HD patients; and *C. albicans* was isolated in four patients, but in no control cases.

Discussion

Many systemic diseases and conditions such as CRF can have direct oral manifestations or cause an indirect effect by modifying the host inflammatory or immune response and by changing the host–parasite interaction balance. This is crucial in the pathogenesis of the two

Table 4 Comparison of the densitometric values of the DNA bands of the different periodontopathogens according to LPA in haemodialysis patients

A: ≤33% with ≥3 mm of LPA (n = 40) *B: >33% with ≥3 mm of LPA (n = 12)*	*Optic density (mean ± s.d.)*[a]	*Integrated optic density (mean ± s.d.)*[a]	*Total bacteria (mean ± s.d.)*[b]	*95% Confidence interval*	*P-value (Student's t-test)*
P. gingivalis					
A	168.55 ± 114.55	564.58 ± 483.91	2.69 ± 4.59	−1.73 to 6.14	0.42
B	152.79 ± 102.89	319.88 ± 381.30	0.492 ± 0.76	−0.02 to 4.43	
A. actinomycetemcomitans					
A	45.62 ± 93.75	41.37 ± 133.62	0.0008 ± 0.01	−0.002 to 3	0.44
B	78.30 ± 121.30	88.40 ± 141.77	0.0003 ± 0.001	−0.001 to 2	
P. intermedia					
A	73.72 ± 115.56	150.18 ± 289.38	60.27 ± 114.95	−147.88 to 96.93	0.88
B	82.13 ± 127.27	210.92 ± 409.5	85.75 ± 166.47	−200.02 to 149.07	
P. nigrescens					
A	158.19 ± 119.27	370.71 ± 479.76	0.57 ± 0.94	−2.17 to (−0.16)	0.05
B	208.25 ± 102.11	1110.54 ± 845.58	1.73 ± 1.38	−2.61 to 0.28	
T. forsythia					
A	198.15 ± 101.88	751.46 ± 644.87	140.30 ± 169.4	–	0.9
B	244.99 ± 8.18	830.88 ± 633.15	149.18 ± 174.7	–	

[a]The values are expressed as arbitrary units of optic density obtained by digital image analysis.
[b]Number of bacteria $\times 10^6$.

most prevalent oral infections – caries and periodontal diseases (Kesavalu *et al*, 2002).

Previous authors have reported that the oral health of HD patients is worse than that of the general population in terms of caries, gingivitis, periodontitis, plaque buildup and general oral health status (Locsey *et al*, 1986; Naugle *et al*, 1998; Klassen and Krasko, 2002; Al-Wahadni and Al-Omari, 2003). A recent study that evaluated the periodontal status of 45 patients in HD demonstrated some degree of oral pathology in 100% of the patients, with noteworthy percentages of gingivitis and periodontitis (36% and 28% respectively) (Naugle *et al*, 1998). In our case, the mean value seen for gingival bleeding was 20% of the sites examined. We, however, believe that this finding may be biased. As Cohen (1994) explains, these patients may present bleeding and prolonged coagulation time, with episodes of spontaneous gingival haemorrhage. That would explain why the mean percentage of bleeding in our study bears no relation with the extension of the periodontitis: 19.64% (s.d. 3.21) in the cases of no or mild periodontitis, yet only 20.83% (s.d. 6.38) in the moderate and severe cases. When we compared this index with the number of sites with a periodontal lesion (clinical loss of attachment), we obtained a very low and non-significant correlation. Similar findings are reported elsewhere (Gavaldá *et al*, 1999; Klassen and Krasko, 2002; Rahman *et al*, 2002).

Many of the patients in our sample (45.5%) presented PTH levels over 450 pg ml^{-1}, indicative of high remodelled bone activity; 22% had values between 60 and 450 pg ml^{-1}; and 22.5% presented PTH values that would be normal for the general population. Contradictory results about the effect of HPTs and osteodystrophy on periodontal tissues have been published. Some authors describe an accelerated process of periodontal destruction (Spolnik *et al*, 1981; Bras *et al*, 1985; Carl, 1987; Grossi *et al*, 1995; Khocht, 1996), while others find no influence on periodontal attachment (Grossi *et al*, 1994; Frankenthal *et al*, 2002; Llodra *et al*, 2002). The fact that the PTH levels monitored in our patients were consistently within an acceptable range may explain the very low incidence of bone lesions in our study.

Possibly because of the low number of patients who smoked ($n = 8$) or had diabetes ($n = 6$), these factors were not correlatable in the statistical analysis with any of the periodontal variables. Likewise, the medication administered to the patients had no manifest effect on the statistical study (except the cases where it was the grounds for exclusion).

The oral cavity may harbour different microbial microenvironments of heterogeneous compositions. More than 300 different bacterial species have been described together with other microorganisms, such as viruses, fungi, mycoplasmas, etc. The subgingival biofilm is a complex bacterial community adhering to the root surface separated from the oral cavity by the soft tissue pocket wall. Under these conditions, many bacterial species with a high potential virulence, such as *A. actinomycetemcomitans* and *P. gingivalis*, are able to colonize, grow and cause periodontal tissue damage (Kesavalu *et al*, 2002). Systemic diseases, which may influence the microenvironment of periodontal pockets, could potentially affect the composition of this subgingival biofilm. However, there are no evaluations in the literature of the microbial composition of the subgingival microflora in the patients undergoing HD. Our study did not find any specific microbial pattern, although the five analysed periodontal pathogens assayed were found regularly in the pockets from these subjects when compared with the control group. When we attempted to correlate the composition and quantity of these pathogens in relation with the extension of disease, we observed a statistical difference only for *P. nigrescens*, the incidence and quantity of which significantly correlated with a higher LPA. Other authors similarly report a high prevalence of these pathogens in mild periodontitis cases (Fukui *et al*, 1999). Although some authors

have observed a high prevalence of species such as *P. nigrescens* and *T. forsythia* associated with oral health (Maeda *et al*, 1998; Choi *et al*, 2000; Tan *et al*, 2001), others report this association only with cases of different types of periodontitis (Colombo *et al*, 1998; Mullally *et al*, 2000). In this study we detected *P. nigrescens* in low amounts in comparison with other species (Table 3).

A recent study of the prevalence and amount of six targeted species harboured by subjects with periodontitis, compared with using real-time PCR assay, gives the percentages of incidence in putative periodontopathogens similar to our results (Kuboniwa *et al*, 2004). These authors found high amounts of *P. intermedia* and low quantities of *A. actinomycetemcomitans*, with minor variation in the other three species we included in our study; yet they found the periodontal pathogens to be more prevalent and in a significantly greater proportion in diseased pockets. We observed no significant associations between the amount of periodontopathogen and the extension of periodontitis; this may be attributed to the fact that we studied only pockets with a loss of attachment greater than 3 mm (considered pathological). The two patients who had no DNA of the species studied were 75 and 77 years old with mild LPA, where the loss of attachment was mainly caused by gingival recession and not by true periodontal pockets.

In our sample, there was no significant difference in the presence of *C. albicans* in saliva of the two groups. Four of the HD patients also demonstrated *C. albicans* isolates in cultures from subgingival plaque, which might be due to the degree of immunocompetence of these patients. In the two cases presenting *K. oxytoca*, which metabolizes in urea, the explanation might be the levels of urea in the saliva of HD patients (Klassen and Krasko, 2002).

On the basis of the findings presented here, we cannot associate ESRD with more severe periodontal destruction. The prolonged duration of HD treatment did not bear a statistically significant relationship with the percentage of sites with LPA ≥3 mm, specific microbiota or composition of biofilm. However, the HD patients presented a higher number of periodontopathic microorganisms when compared with the matched control subjects.

Acknowledgements

This study was supported in part by the Andalusian Regional Government through the research project 'Microbiology, Immunology and Epidemiology of Oral Diseases', by National Project FIS No. PI 020997 and the European Regional Development Fund (ERDF). We thank Francisca Castillo for her key technical assistance and Jean Sanders for translating and editing the text.

References

Ainamo J, Bay I (1975). Problems and proposal for recording gingivitis and placa. *Dent J* **25:** 229–235.

Al-Wahadni A, Al-Omari MA (2003). Dental diseases in a Jordanian population on renal dialysis. *Quintessence Int* **34:** 343–347.

Amenábar JJ, García F, Robles NR *et al* (2002). Informe de diálisis y trasplante de la Sociedad Española de Nefrología y Registros Autonómicos, año 2000. *Nefrología* **22:** 310–317.

Arbes SJ, Slade GD, Beck, JD (1999). Association between extent of periodontal attachment loss and self-reported history of heart attack: an analysis of NHANES lll data. *J Dent Res* **78:** 1777–1782.

Arrebola F, Masseroli M, Reguero ME *et al* (2001). Image analysis procedure to quantify fluorescent bands of nucleic acids in two-dimensional gel electrophoresis. *Investigación Clinica* **4:** 1–5.

Ashimoto A, Chen C, Bakker I *et al* (1996). Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis lesions. *Oral Microbiol Immunol* **11:** 266–273.

Baylink DJ, Wergedal JE, Yamamoto K *et al* (1974). Systemic factors in alveolar bone loss. *J Prosthet Dent* **31:** 486–505.

Bramley P, Dwyer D (1970). Primary hyperparathyroidism. Its effect on a mother and her children. *Oral Surg Oral Med Oral Pathol* **30:** 464–471.

Bras J, Van Ooij CP, van den Akker HP (1985). Mandibular atrophy and metabolic bone loss. *Int J Oral Surg* **14:** 16–21.

Carl W (1987). Chronic renal disease and hyperparathyroidism: dental manifestations and management. *Compend Contin Educ Dent* **8:** 679–704.

Choi BK, Park SH, Yoo YJ *et al* (2000). Detection of major putative periodontopathogens in Korean advanced adult periodontitis patients using a nucleic acid-based approach. *J Periodontol* **71:** 1387–1394.

Cohen SG (1994). Renal disease. In: Lynch MA, Brightman VJ, Greenberg MS, eds. *Burket's oral medicine*, 9th edn. JB Lippincott Co, Philadelphia, PA, pp. 487–509.

Colombo AP, Haffajee AD, Dewhirst FE *et al* (1998). Clinical and microbiological features of refractory periodontitis subjects. *J Clin Periodontol* **25:** 169–180.

Duran I, Erdemir EO (2004). Periodontal treatment needs of patients with renal disease receiving haemodialysis. *Int Dent J* **54:** 274–278.

Frankenthal S, Nakhoul F, Machtei EE *et al* (2002). The effect of secondary hyperparathyroidism and hemodialysis therapy on alveolar bone and periodontium. *J Clin Periodontol* **29:** 479–483.

Fukui K, Kato N, Kato H *et al* (1999). Incidence of Prevotella intermedia and *Prevotella nigrescens* carriage among family members with subclinical periodontal disease. *J Clin Microbiol* **37:** 3141–3145.

García L, Tercero JC, Legido B *et al* (1998). Rapid detection of *Actinobacillus actinomycetemcomitans*, *Prevotella intermedia* and *Porphyromonas gingivalis* by multiplex PCR. *J Periodontal Res* **33:** 59–64.

Gavaldá C, Bagán JV, Scully C *et al* (1999). Renal hemodialysis patients: oral, salivary, dental and periodontal findings in 105 adult cases. *Oral Dis* **5:** 299–302.

Grossi SG, Zambon JJ, Ho AW *et al* (1994). Assessment of risk for periodontal disease. I. Risk indicators for attachment loss. *J Periodontol* **65:** 260–267.

Grossi SG, Genco RJ, Machtei EE *et al* (1995). Assessment of risk for periodontal disease. II. Risk indicators for alveolar bone loss. *J Periodontol* **66:** 23–29.

Jaffe E, Chantler C, Carter J (1986). Dental findings in chronic renal failure. *Br Dent J* **160:** 18–20.

Kelly WH, Mirahmadi MK, Simon JH *et al* (1980). Radiographic changes of the jawbones in end-stage renal disease. *Oral Surg Oral Med Oral Pathol* **50:** 372–381.

Kennett S, Pollick H (1971). Jaw lesions in familial hyperparathyroidism. *Oral Surg Oral Med Oral Pathol* **31:** 502–510.

Kesavalu L, Chandrasekar B, Ebersole JL (2002). In vivo induction of proinflammatory cytokines in mouse tissue by *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans*. *Oral Microbiol Immunol* **17:** 177–180.

Khocht A (1996). Periodontitis associated with chronic renal failure: a case report. *J Periodontol* **67:** 1206–1209.

Klassen JT, Krasko BM (2002). The dental health status of dialysis patients. *J Can Dent Assoc* **68:** 34–38.

Kuboniwa M, Amano A, Kimura RK *et al* (2004). Quantitative detection of periodontal pathogens using real-time polymerase chain reaction with TaqMan probes. *Oral Microbiol Immunol* **19:** 168–176.

Landis JR, Koch GG (1977). The measurement of observer agreement for categorical data. *Biometrics* **33:** 159–174

Llodra J, Bravo M, Cortés FJ (2002). Encuesta de salud oral en España (2000). *RCOE* **7:** 19–63.

Locsey L, Alberth M, Mauks G (1986). Dental management of chronic hemodialysis patients. *Int Urol Nephrol* **18:** 211–213.

López K, Saracho R, García F *et al*. (2001). Informe de diálisis y trasplante año 2001 de la Sociedad Española de Nefrología y Registros Autonómicos. http://www.senefro.org.

Maeda N, Okamoto M, Kondo K *et al* (1998). Incidence of *Prevotella intermedia* and *Prevotella nigrescens* in periodontal health and disease. *Microbiol Immunol* **42:** 583–589.

Marakoglu I, Gursoy UK, Demirer S *et al* (2003). Periodontal status of chronic renal failure patients receiving hemodialysis. *Yonsei Med J* **44:** 648–652.

Masseroli M, Messori A, Bendotti C *et al* (1993). Automatic quantitative evaluation of autoradiographic band films by computerized image analysis. *Life Sci* **53:** 331–336.

Mullally BH, Dace B, Shelburne CE *et al* (2000). Prevalence of periodontal pathogens in localized and generalized forms of early-onset periodontitis. *J Periodontal Res* **35:** 232–241.

Naugle K, Darby M, Bauman D *et al* (1998). The oral status of individuals on renal dialysis. *Ann Periodontol* **3:** 197–205.

Rahman M, Caglayan F, Rahman B (2002). Periodontal health parameters in patients with chronic renal failure and renal transplants receiving immunosuppressive therapy. *Blood Purif* **20:** 113–119.

Sakamoto M, Takeuchi Y, Umeda M *et al* (2001). Rapid detection and quantification of five periodontopathic bacteria by real-time PCR. *Microbiol Immunol* **45:** 39–44.

Silness P, Löe H (1964). Periodontal disease in pregnancy-II.Correlation in between oral hygiene and periodontal condition. *Acta Odontol Scan* **22:** 121–135.

Spolnik KJ, Patterson SS, Maxwell DR *et al* (1981). Dental radiographic manifestations of end-stage renal disease. *Dent Radiogr Photogr* **54:** 21–31.

Takeuchi Y, Umeda M, Sakamoto M *et al* (2001). *Treponema socranskii*, *Treponema denticola* and *Porphyromonas gingivalis* are associated with severity of periodontal tissue destruction. *J Periodontol* **72:** 1354–1363.

Tan KS, Song KP, Ong G (2001). *Bacteroides forsythus* prtH genotype in periodontitis patients: Occurrence and association with periodontal disease. *J Periodontal Res* **36:** 398–403.

Tran SD, Rudney JD (1999). Improved multiplex using conserved and species-specific 16S rRNA gene primers for simultaneous detection of *Actinobacillus actimycetemcomitans*, *Bacteroides forsythus* and *Porphyromonas gingivalis*. *J Clin Microbiol* **37:** 3504–3508.

Westbrook S (1978). Dental management of patients receiving hemodialysis and kidney transplants. *J Am Dent Assoc* **96:** 464–468.