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Antioxidant Status and Dialysis: Plasma and Saliva Antioxidant Activity in Patients with Fluctuating Urate Levels

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The present study is concerned with the influence of processes occurring during dialysis on the antioxidant capacity of plasma and saliva. The biological fluids were also tested for uric acid and total protein content. Before hemodialysis, plasma antioxidant status of hemodialyzed patients appears slightly higher than the corresponding status in normal subjects; after hemodialysis it is found unchanged. The result can be explained by a balance between a reduction in uric acid plasma content, due to the dialytic procedure, and an increase in protein content, possibly due to a dialysis-related hemoconcentration. Moreover, pre-dialysis total antioxidant capacity of whole saliva samples is higher than in healthy individuals and drastically decreases towards normal values following dialytic procedure. Our data indicate a certain concentration of the uric acid in the saliva of hemodialyzed patients and evidence that both total protein concentration and uric acid level show a good correlation with saliva total antioxidant capacity, suggesting that proteins are major antioxidants of this fluid. Further observations are needed to assess whether this improved saliva antioxidant ability has any consequence on the periodontal conditions of hemodialyzed subjects.

Keywords: Antioxidant capacity, dialysis, saliva, uric acid, plasma proteins, periodontal status

Abbreviations: ABTS, 2,2′-azinobis (3-ethylbenzothiazoline 6-sulfonate); H_2O_2, hydrogen peroxide; Trolox, 2-carboxy-2,5,7,8-tetramethyl-6-chromanol

INTRODUCTION

Many reports have focused on the possibility that chronic renal failure and hemodialysis are associated with increased oxidative stress, a condition which occurs when there is excessive free-radical production[1] or low antioxidant levels.[2] In fact it is probable that granulocytes are activated at dialyzer membranes generating reactive oxygen species,[3] which can damage proteins, lipids, carbohydrates and nucleic acids.[4] In addition blood is exposed to artificial surfaces and daylight

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during dialysis which may cause further oxidative stress.[5] Free-radicals level is normally kept under control by an array of biochemical defense mechanisms which include enzymatic scavengers and non-enzymatic compounds.[6]

Even though many papers demonstrated increased level of peroxidation in patients undergoing hemodialysis,[7,8] some authors seriously questioned an increase of peroxidative insult in these patients.[9] Some data also indicated that susceptibility of LDL (low density lipoproteins) to oxidation is enhanced in hemodialysis patients,[10] even though a decrease of lipophylic antioxidants was not found in other studies.[9] Furthermore a recent study pointed out that a clear, inverse relationship between peroxidation extent and antioxidant capacity is not always present in critically ill patients.[11]

Several conflicting evidences exist on the total plasma antioxidant capacity of patients with chronic renal failure.[12–14] Saliva is also endowed with antioxidant capacity,[15] which might have some physiological consequence on the periodontal status. The aim of the present report was to determine the effects of hemodialysis itself on plasma antioxidant activity and to try to estimate whether processes occurring during hemodialysis could influence the antioxidant capacity of the saliva.

MATERIALS AND METHODS

Trolox (2-carboxy-2,5,7,8-tetramethyl-6-chromanol) and hydrogen peroxide, 30%, were purchased from Aldrich; 2,2′-azinobis (3-ethylbenzothiazoline 6-sulfonate) (ABTS) from Fluka; myoglobin from horse heart (95–100%) from Sigma.

All other chemicals used were of reagent or spectrophotometric grade.

Highly purified water (resistivity = 18 Mohm · cm) obtained through a Milli-Q water purification system (Millipore) was used for all solutions.

Blood Collection and Plasma Isolation

Blood from 15 healthy subjects recruited among blood donors of Gemelli Hospital Blood Bank (10 males and 5 females – age range 20–55 years) was obtained by venipuncture after an overnight fast (10–12 h), collected over heparin and immediately centrifuged ($1500 \times g$ for 10 min at 4°C); the supernatant (plasma) was recentrifuged at $11{,}000 \times g$ for 5 min to remove contaminating erythrocytes and platelets, stored at −30°C and analyzed at a later date (within 2 months). Repeated assays on a reference plasma sample showed that storage of samples in this condition did not significantly influence the values of total antioxidant capacity.

In undialyzed patients with hyperuricemia (11.2 ± 1.6 mg/dl) (3 males and 3 females – age range 35–65 years) blood was collected in an identical manner to that of healthy subjects. Three of them had a certain degree of nitrogen retention as shown by an average creatinine level of 2.1 ± 0.9 mg/dl. They were in the early phase of post-kidney-transplant. The remaining 3, for whom creatinine level was 0.9 ± 0.3 mg/dl, had elevated uric acid values because of enhanced nucleic acid catabolism due to antiblastic therapy. All these patients had normal or subnormal protein levels (5.8 ± 0.8 g/dl) and their hematocrit was 29 ± 4%. None of them, just like the patients in the dialyzed group, were taking drugs for hyperuricemia. For this small group it was not possible to make a clinical assessment of oral cavity.

In hemodialyzed patients (15 males and 10 females – age range 21–79 years, mean age 53 ± 14 years) the underlying disease was represented by chronic glomerulonephritis in 43% of the cases, 19% had polycistic kidney, for the remaining 38% the cause which led to hemodialysis was nephrolithiasis or interstitial nephritis or nephrosclerosis or vasculitis. Blood was drawn from the fistula needle just before and at the end of the same hemodialysis session. The effect of recirculation on the results obtained is to be considered as not

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relevant, since recirculation extent was not higher than 5%. In fact blood flow rate was around 300 ml/min. Patients were receiving dialysis for a mean period of 11 ± 8 years (1–30 years range). Hemodialysis was performed three times a week, 4 h daily, 8 patients receiving hemodiafiltration, 4 acetate free-biofiltration and 13 hollow-fiber hemodialysis, using dialyzers equipped with polysulfone membranes (F5, F6 and F8, Fresenius AG, Germany), cellulose acetate membranes (Althin Medical, Inc.) or polyacrilonitrile AN-69 membranes (Hospal, Meyzieu, France). Reuse is never performed in our hemodialysis procedures. Following centrifugation, plasma was stored until use.

All subjects gave informed consent before participating in this study.

Saliva Collection

In subjects with apparently healthy gingivae (9 males, 8 females – age range 20–26 years, all dental students) saliva collection was made at a standardized time (10 a.m.), due to the circadian rhythm displayed by resting saliva secretion. Whole saliva (approximately 0.5 ml) was collected by spitting without stimulation directly into an ice-cooled Eppendorf centrifugal vial. Samples were immediately centrifuged after collection ($13{,}000 \times g$ for 3 min) and the supernatant removed and stored at −30°C for no longer than 1 month. No significant variation of total antioxidant capacity could be shown when saliva was stored up to 2 months in these conditions.

The subjects were instructed not to eat, drink, smoke or take physical exercise for 2 h prior to collection. Furthermore none was currently taking vitamin supplements or drugs.

Parotid, as well as submandibular/sublingual saliva samples (approximately 0.2 ml) were collected from the same subjects by catheterization of the duct with micropipettes of the type commonly used for endocanalar therapy.

Reference values for saliva were obtained on this group of dental students for practical reasons. Furthermore they represented a rather homogeneous group of young people with healthy gingivae.

In hemodialyzed patients saliva samples were collected in an identical manner to those of healthy subjects just before and at the end of the same hemodialysis session. Following centrifugation, the saliva was stored at −30°C until use.

Evaluation of Periodontal Status

Hemodialyzed patients underwent periodontal clinical examination with recording of the following parameters on 4 dental surfaces (mesial, buccal, distal, lingual): probing depth (PD), plaque index (PLI),[16] full mouth plaque score (FMPS), gingival index (GI),[17] full mouth bleeding score (FMBS). The measurements were performed after collecting saliva samples, in order not to contaminate saliva with blood nor to modify secretion rate.

Antioxidant Capacity

Plasma and salivary antioxidant capacity was determined according to Rice-Evans and Miller [18] with a method based on the inhibition by antioxidants of the absorbance of the radical cation $ABTS\bullet^{+}$ formed by the interaction of ABTS (150 μM) with the ferrylmyoglobin radical species, generated at 30°C by the activation of metmyoglobin (2.5 μM) with H_2O_2 (75 μM). Final volume was 1 ml, with reagents prepared in 20 mM phosphate-buffered saline, pH 7.4, except H_2O_2 which was diluted in water. The reaction was started directly in cuvette with H_2O_2 addition after 1 min equilibration of all other reagents (temperature control by a thermocouple probe, model 1408 K thermometer, Digitron Instrumentation Ltd.) and followed by continuous stirring, monitoring the absorbance at 734 nm. Absorbance reading at 10 min was used to calculate antioxidant capacity as percentage inhibition of the reaction, i.e. the blank (without plasma or saliva) absorbance minus the test (with plasma,

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5 μl, or saliva, 10 μl) absorbance, divided by the blank absorbance (× 100). This value defines the response of the system and is proportional to the antioxidant capacity of the sample. Standard curves were obtained by using fixed volumes of increasing concentrations of Trolox. Results are expressed as the initial concentration of a Trolox solution having the same antioxidant capacity of the sample, i.e.: as μM Trolox which, when added to our testing system in the same volume as for the sample, yields the same percent of inhibition. All plasma total antioxidant capacity values were obtained using 5 μl of sample and all saliva values using 10 μl. These volumes were chosen on the basis of a linear dose response curve, since they were capable of inducing an inhibition of about 50% for plasma and about 35% for saliva.

Absorbance was measured with a Hewlett-Packard 8450A UV/Vis spectrophotometer equipped with a cuvette stirring apparatus and a constant temperature cell holder.

Measurements of pH were made with a PHM84 Research pHmeter (Radiometer), the electrode response was corrected for temperature.

Uric Acid and Total Protein Determination

Uric acid was measured by Trinder method modified with uricase, automated on Hitachi 717 (Boehringer Mannheim, France) both in plasma and saliva.

Total proteins were measured by the biuret method in plasma and in saliva by a turbidimetric method employing solfosalicilic acid, automated on Hitachi 717, usually employed to determine protein concentration in cerebro-spinal fluid.

Statistical Analysis

Statistical difference was tested using Student's *t*-test, paired or unpaired as appropriate. The Student's unpaired *t*-test was used to evaluate the significance of differences between plasma and saliva samples from healthy subjects and from patients. The Student's paired *t*-test was used to evaluate the significance of differences between plasma and saliva samples in pre-dialysis and post-dialysis conditions and between the several kinds of saliva in the same subjects.

Simple regression analysis was used to determine the relationships between antioxidant capacity and uric acid or total protein levels.

A p value of 0.05 or less was considered statistically significant.

Unless stated differently, experiments were repeated two to three times; qualitatively similar results were obtained for each set of experiments with individual values varying < 8%.

Data are presented as mean ± SD.

RESULTS AND DISCUSSION

Total plasma antioxidant capacity, uric acid and proteins (the major contributors to the plasma antioxidant status)[19,20] of normal subjects are shown in Table I. These parameters are within normal range as regards uric acid and total protein content. Total plasma antioxidant capacity appears greater than the corresponding value reported by Miller and Rice-Evans;[21] in our opinion the finding can be due to the different assay conditions explained in Materials and Methods and possibly also to the different individuals included in our study, i.e. a population on a mediterranean diet.

Before hemodialysis, total plasma antioxidant status in hemodialyzed patients appears slightly, although significantly, higher than the corresponding status in normal subjects. This can be explained by the normal level of proteins and by the small, but significant, increase of uric acid present in these patients (Table I). After hemodialysis, total plasma antioxidant capacity appears unchanged. In our opinion this experimental observation can be explained by a balance between a reduction in uric acid plasma content, due to the dialytic procedure, and an increase in protein content, likely due to a dialysis-related

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TABLE I Total antioxidant capacity, protein content and uric acid level in plasma of healthy donors and hemodialyzed patients

Subjects	Total antioxidant capacity (μM)		Total proteins (g/dl)		Uric acid (mg/dl)	
Healthy donors ($n=15$)	2692 ± 298		6.9 ± 0.4		4.8 ± 1.0	
Hemodialyzed patients	Pre	Post	Pre	Post	Pre	Post
($n=16$)	2932 ± 178^{a}	$2876 \pm 290^{a,b}$	7.1 ± 0.5^{c}	$8.9 \pm 0.6^{a,d}$	6.9 ± 1.5^{a}	$2.2 \pm 0.6^{a,d}$

Total antioxidant capacity is expressed as μM Trolox (initial concentration) which, when added to our testing system in the same volume as for the sample (0.5%), yielded the same percent of inhibition. Results are reported as means ±SD. $^{a}p < 0.05$ compared with control group. [b]Not significantly different compared with pre-dialysis values. [c]Not significantly different compared with control group. $^{d}p < 0.05$ compared with pre-dialysis values.

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hemoconcentration (Table I). The average hematocrit value was in fact 34 ± 4% before, and 39 ± 5% after dialysis.

Even though hemodialysis was performed by means of different types of membranes, results indicate that variability in absolute values of total antioxidant capacity is very low; therefore we did not attempt to calculate results for subgroups. This fact is confirmed by the lack of change in total antioxidant capacity induced by dialysis, which was common to all patients independently from the type of dialyzing membrane, therefore suggesting that the trend is absolutely constant.

Previous reports have provided conflicting evidence on the effect of hemodialysis in patients with chronic renal failure with both increased and decreased total plasma antioxidant capacity described.[12–14] Toborek *et al.*[12] found a decreased serum antioxidant activity in uremic patients, compared with controls, before hemodialysis and a lack of any change after hemodialysis. These authors do not explain the finding, but only comment that their results agree with the observations of Kuroda *et al.*[22] Jackson *et al.*[13] reported that total antioxidant capacity of serum was increased in dialysis patients, but there was a marked reduction after hemodialysis. The increase in total plasma antioxidant capacity before hemodialysis was almost entirely due to relatively high serum urate. The decrease of antioxidant content after hemodialysis was ascribed to the substantial fall in urate and ascorbate. Protein thiol groups were found increased after hemodialysis, although albumin remained unchanged.

We must necessarily assume that the total antioxidant capacity method employed in that study was particularly dependent on the uric acid concentration. In our case the substantial fall in uric acid induced by the hemodialytic procedure was somewhat efficiently counteracted by an increase in albumin concentration, capable of maintaining antioxidant activity values similar to the pre-dialysis levels. The relative importance of proteins will also appear from analysis of data obtained on saliva of these patients.

Very recently Nagase *et al.*[14] reported a decreased serum antioxidant activity in hemodialysis patients, demonstrated by electron spin resonance, evidencing a favorable effect of hemodialysis treatment, i.e. no significant difference in the signals between the reaction mixture containing post-hemodialysis and healthy sera.

It is possible that these findings differ from ours because of dietary restriction, severity of the disease, type of dialytic procedure, frequency of maintenance hemodialysis, assay employed to measure the antioxidant capacity and so on. However, our results appear consistent with the protein and uric acid behavior in our patients.

To estimate whether processes occurring during hemodialysis could influence the antioxidant capacity of the saliva with consequent potential significance in dentistry, we also determined the

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antioxidant capacity of the saliva of the same hemodialyzed patients.

Total antioxidant capacity in whole saliva, parotid saliva and submandibular/sublingual saliva in normal subjects is reported in Table II. In healthy individuals the highest values belong to parotid saliva ($p = 0.0025$ vs. submandibular/sublingual saliva), the lowest values to submandibular/sublingual saliva. Whole saliva shows intermediate values closer to the parotid ones, presumably due to a remarkable contribution of parotid origin. Currently, these are the first data regarding pure glandular salivary secretions. In fact, the only paper investigating saliva antioxidant activity is concerned with whole saliva under resting and stimulated conditions.[15] Our data show a higher whole saliva antioxidant capacity explained in part by the slightly different experimental conditions, in part possibly due to the type of population taken into account (see also plasma data).

The results obtained in hemodialyzed patients are also reported in Table II. In these subjects pre-dialysis samples for each kind of saliva show values of antioxidant capacity higher than the corresponding values in normal individuals, even if, with regard to the mean values, the difference is statistically significant for whole saliva only. At the end of the dialytic session a remarkable decrease of antioxidant activity was found for all three kinds of saliva. The reduction is particularly significant for whole saliva and for parotid saliva.

Therefore, patients with elevated plasma levels of uric acid show a large rise in whole saliva total antioxidant activity. This observation was confirmed by the results obtained on a small group of 6 undialyzed patients with a mean plasma content of uric acid equal to 11.2 ± 1.6 mg/dl (range from 9.2 to 13.2 mg/dl), whose saliva total antioxidant capacity was 2253 ± 539 µM Trolox, ranging from 1381 to 2787 µM. The common finding for these patients really was hyperuricemia, as their clinical conditions were different as specified in the Materials and Methods section. It is important to notice that the lowest value of antioxidant capacity showed in this group was much more elevated than the highest value obtained in the whole saliva of normal subjects. Furthermore, the mean value of this group is remarkably more elevated than the corresponding average obtained in hemodialyzed patients in pre-dialysis condition; similarly even their plasma uric acid level was remarkably higher compared with the uric acid levels in the pre-dialysis status.

To confirm our findings, the assays were repeated some months later in a greater number of patients only in plasma and whole saliva, due to the easier collection of this type of saliva. Moreover, as the major antioxidant in saliva is urate, with lesser contributions from albumin and ascorbate,[15] the biological fluids were also tested for uric acid and total protein content. The plasma parameters were found substantially unchanged (data not shown).

TABLE II Total antioxidant capacity in whole saliva, parotid saliva and submandibular/sublingual saliva of normal subjects and hemodialyzed patients

Subjects	Whole saliva (µM)		Parotid saliva (µM)		Submandibular/sublingual saliva (µM)	
Normal subjects ($n = 17$)	$711 \pm 198^{a,b}$		760 ± 271^{a}		589 ± 173	
Hemodialyzed patients	Pre	Post	Pre	Post	Pre	Post
($n = 16$)	1016 ± 398^{c}	$735 \pm 286^{d,e}$	904 ± 482^{d}	$651 \pm 342^{d,e}$	740 ± 290^{d}	$595 \pm 257^{d,f}$

Total antioxidant capacity is expressed as µM Trolox (initial concentration) which, when added to our testing system in the same volume as for the sample (1%), yielded the same percent of inhibition. Results are reported as means ±SD. [a]$p < 0.05$ compared with submandibular/sublingual saliva. [b]Not significantly different compared with parotid saliva. [c]$p < 0.05$ compared with control group. [d]Not significantly different compared with control group. [e]$p < 0.05$ compared with pre-dialysis values. [f]Not significantly different compared with pre-dialysis values.

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TABLE III Influence of a single dialytic session on whole saliva total antioxidant capacity, proteins and uric acid level

Total antioxidant capacity (μM)		Total proteins (mg/dl)		Uric acid (mg/dl)	
Pre	Post	Pre	Post	Pre	Post
1248 ± 442[a]	833 ± 418[b,c]	55 ± 62	33 ± 39[d]	8.3 ± 7.3	1.3 ± 1.4[c]

Whole saliva samples were analyzed as described in Materials and Methods in 25 hemodialyzed patients. Results are reported as means ±SD. [a]$p < 0.05$ compared with control group. [b]Not significantly different compared with control group. [c]$p < 0.05$ compared with pre-dialysis values. [d]Not significantly different compared with pre-dialysis values ($p = 0.056$).

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Whole saliva samples not only confirm, but also strengthen previous observations (Table III). Pre-dialysis total antioxidant capacity is statistically higher than in healthy individuals and drastically decreases towards normal values following dialytic procedure. Total proteins are present at a level comparable to that already reported as albumin amount,[15] even if there is a large dispersion of the data. On the contrary, uric acid content is much higher than in healthy subjects,[15] indicating a certain concentration of the antioxidant, which is present in saliva at levels greater than in plasma in all examined patients.

Both total protein concentration and uric acid level show a good correlation with total antioxidant capacity (Figures 1 and 2, respectively).

Post-dialysis protein content appears diminished, even if the decrease is only close to a statistical significance ($p = 0.056$), whereas uric acid falls at levels drastically lower than normal, so explaining the difference in antioxidant status (Table III).

Correlation between total protein concentration or uric acid level and total antioxidant capacity is clearly weaker in post-dialytic conditions ($r = 0.498$ and $r = 0.353$, respectively), being significant only as far as total protein is concerned ($p = 0.035$). It appears like the remarkable decrease in uric acid determined by dialytic procedure brings this metabolite to play a role of possible lesser importance compared to the protein level. In fact, uric acid levels drop to values which are close to one sixth of the initial ones, while proteins decrease less than 50%.

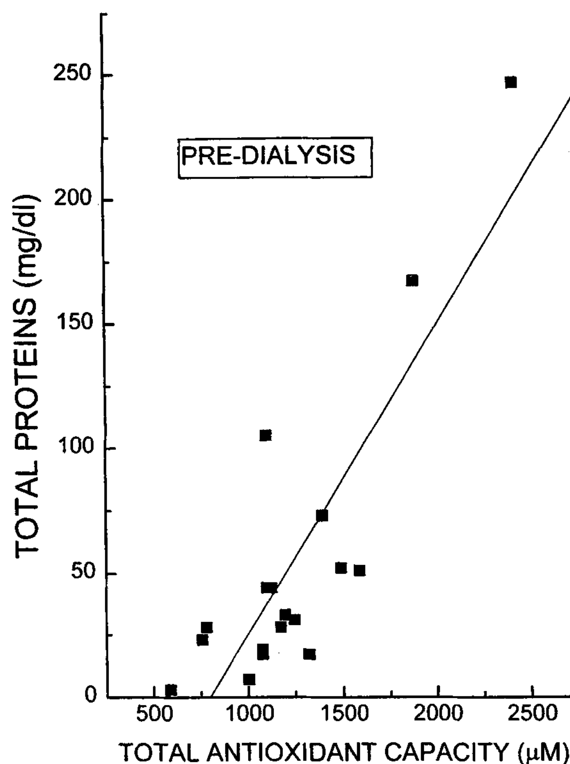

FIGURE 1 The relationship between total antioxidant capacity and total protein concentration in whole saliva. Whole saliva samples were collected and analyzed as described in Materials and Methods. Total antioxidant capacity is expressed as μM Trolox which, when added to our testing system in the same volume as for the sample (1%), yielded the same percent of inhibition. Simple regression analysis was used to determine the relationship ($r = 0.850$).

Variability among the single patients could mask the essence of some data. It is therefore worthwhile to analyze single data belonging to some of the patients whose behavior is representative of the influence of hemodialysis on the

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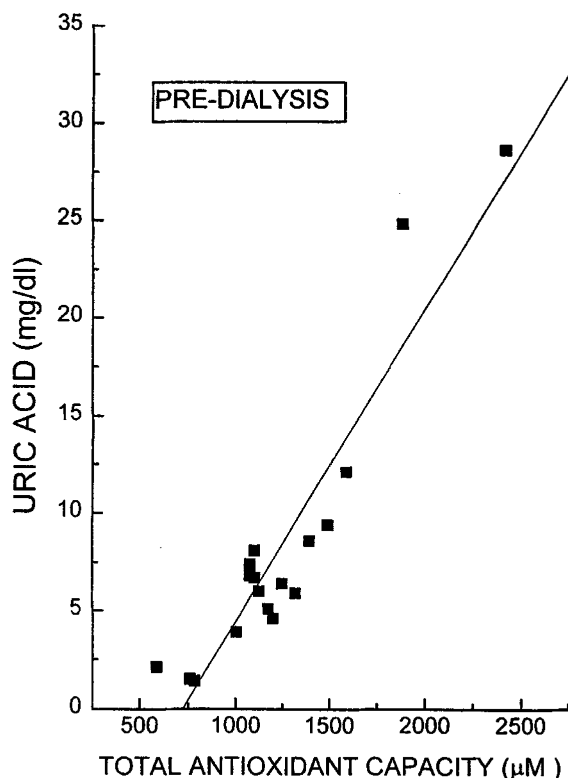

FIGURE 2 The relationship between total antioxidant capacity and uric acid concentration in whole saliva. Whole saliva samples were collected and analyzed as described in Materials and Methods. Total antioxidant capacity is expressed as μM Trolox which, when added to our testing system in the same volume as for the sample (1%), yielded the same percent of inhibition. Simple regression analysis was used to determine the relationship ($r = 0.932$).

antioxidant status. Table IV refers to a group of patients showing features which are remarkably different compared to normal subjects, especially as far as saliva is concerned.

It can easily be noticed that plasma antioxidant capacity is not affected by the hemodialytic procedure, presumably because the decrease in uric acid is always counterbalanced by an increase in total proteins, related to a certain degree of hemoconcentration. In fact, patients 3 and 4, in whom the increase in protein concentration related to hemodialysis is of lesser extent, even show a slight, although not significant, decrease in total antioxidant activity. Variation of these two metabolites in saliva is even more pronounced. In fact some of the patients show pre-dialysis values of uric acid highly increased compared to plasma, and this must necessarily be related to a process of uric acid concentration in the saliva of these patients. Also in the case of saliva, dialysis leads to a dramatic decrease in uric acid which usually results in a very evident decrease in total antioxidant capacity; this because total proteins also diminish in saliva following dialysis. Case 5 represents the exception, which confirms the rule: in fact it is the only case where antioxidant capacity of saliva is rather low at the beginning and increases after dialysis. This fact is not surprising: it should

TABLE IV Total protein and uric acid influence on plasma and whole saliva antioxidant status: some explanatory examples

Patient number	Sample	Total antioxidant capacity (μM)		Total proteins (g/dl in plasma, mg/dl in saliva)		Uric acid (mg/dl)	
		Pre	Post	Pre	Post	Pre	Post
1	Plasma	2594	2692	6.7	8.6	6.5	2.4
	Saliva	2347	1370	246	136	28.6	3.9
2	Plasma	2838	2887	7.5	10.8	7.4	1.8
	Saliva	1492	1126	52	35	9.4	0.3
3	Plasma	2496	2399	7.2	7.7	5.8	3.0
	Saliva	1883	418	167	14	24.8	1.6
4	Plasma	2741	2643	7.8	8.3	7.4	2.4
	Saliva	1077	344	17	3	7.4	0.9
5	Plasma	2789	2692	6.8	8.5	6.4	1.3
	Saliva	589	931	5	68	2.1	1.3

Plasma and whole saliva samples were collected and analyzed as described in Materials and Methods. Results are reported as mean of three separate determinations.

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rather be expected since this is also the only case where uric acid concentration is very low in pre-dialysis and therefore it only slightly decreases by dialysis. For some unknown reason, protein content increases considerably in saliva of this patient and this leads to the expected increased antioxidant capacity.

Even if patients reported in Table IV show a broad range of absolute values, it is remarkable that, except the case 5, there is no variability in the trend of the change of antioxidant capacity following dialysis. Of course we do not exclude that other plasma or saliva low molecular weight components, also influenced by dialysis, could contribute to total antioxidant capacity fluctuation. Nevertheless it was shown that two of them, namely urea and creatinine, are avoided of antioxidant capacity.[23]

One last consideration might arise, i.e., whether other kinds of antioxidants, namely the lipophylic ones, play a role in determining total antioxidant capacity in the saliva of our patients. On a basis of a recent report by Hirayama *et al.*[24] the contribution of lipid soluble components to antioxidant capacity of human saliva is about 10%. In our case it was practically impossible to extract lipid soluble components, since sample volumes, especially for parotid and for submandibular saliva, were insufficient for such extraction. Lipid soluble antioxidants are carried by lipoproteins, the presence of which is known to be very low in saliva. Uric acid and proteins seem to play a far more important quantitative role at this regard.

Saliva of these patients is therefore endowed with a better antioxidant capacity compared to normal subjects, especially in pre-dialysis conditions. One logical question arises from a clinical point of view on whether this improved antioxidant ability has any consequence on the periodontal conditions of these patients. Clinical assessment of the periodontal status in these patients showed that none of our 25 patients had oral conditions typical of severe periodontitis. In fact, probing depth (3.57 ± 1.55) and plaque index (0.94 ± 0.48) are very similar to those found in healthy subjects of comparable mean age (53 years).[25,26] Full mouth plaque score value of hemodialyzed patients (64.8 ± 29.3) suggests that this group has poor oral hygiene also taking into account that measurements were often performed in the morning and in unhospitalized patients;[27] on the other hand, it is worth of notice that gingival index (0.55 ± 0.34) is not as high as one could expect in patients with relatively poor oral hygiene. Also full mouth bleeding score is not very high (20.3 ± 12.1), mainly considering that high scores were obtained in food impaction sites. In conclusion, the reported parameters, taken as a whole, reveal that hemodialyzed patients, in spite of poor oral hygiene, do not show severe periodontal lesions. Furthermore, they had a fairly good percentage of residual elements for each dental arcade (65–70%) and from anamnestic data it was evident that the missing teeth had been lost not as a result of periodontal problems.

Previous studies by Obry *et al.*[28] showed a low caries activity together with higher salivary pH in youngsters dialyzed for chronic renal failure: this could be considered as a clinical consequence of a biochemical change determined by dialysis.

On the basis of our data we cannot conclude that higher total antioxidant capacity leads to improved periodontal status in our patients. Nevertheless, these results suggest that this hypothesis should be more thoroughly inquired.

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