

DOI: 10.5455/msm.2018.30.246-250

Received: October 29 2018; Accepted: December 18, 2018

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ORIGINAL PAPER

Mater Sociomed. 2018 Dec; 30(4): 246-250

Significance of Nitric Oxide Saliva Concentration of the Patients with Renal Failure on Hemodialysis

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ABSTRACT

Introduction: An increase in nitric oxide (NO) synthesis concentration could help alleviate some pathological changes directly related to uremia. **Aim:** To investigate the concentration of nitric oxide in saliva of patients with terminal stage of chronic renal failure on hemodialysis and to investigate the effect of hemodialysis on concentration of nitric oxide in saliva of the patients with terminal stage of chronic renal failure on hemodialysis. **Material and Methods:** The study had a prospective character and included 60 respondents of both sexes, at age between 20 and 60 years. The control group included 30 healthy volunteers of both sexes (15 men and 15 women) of the same age, who were based on subjective and objective health status without any manifestations of pathophysiological changes. The group of patients with terminal stage of chronic renal failure on hemodialysis involved 30 patients of both sexes (15 men and 15 women). **Inclusion criteria:** hemodialysis duration more than 6 months and age between 20 and 60 years. **Results:** The amount of non-stimulated saliva collected during 15 minutes in patients with chronic renal failure was lower by 31.3% compared to the control group of healthy subjects ($p < 0.0001$). Concentration of NO in saliva in patients with chronic renal failure was higher by 121% than in the control group ($p < 0.001$). Concentration of NO in saliva in patients with chronic renal failure after hemodialysis was 121% lower than in the same subjects prior to hemodialysis ($p < 0.001$). A statistically significant negative correlation ($r = -0.381$, $p < 0.05$) was found between the hemodialysis duration in months and the level of NO in saliva of the patients with chronic renal failure prior to hemodialysis. There was no statistically significant correlation ($r = -0.167$, NS) between the hemodialysis duration in

months and NO levels in saliva of the patients with chronic renal failure after hemodialysis treatment. **Conclusion:** Concentration of NO in saliva of the patients on hemodialysis was statistically significantly higher in relation to NO concentration in the saliva of healthy subjects and after hemodialysis was statistically significantly lower in relation to NO values prior to hemodialysis. Monitoring of changes in NO concentration dynamics in saliva of hemodialysis patients will probably be helpful in monitoring hemodialysis efficacy.

Keywords: nitric oxide, saliva, renal failure.

1. INTRODUCTION

Xerostomy is a subjective feeling of dry mouth and is a relatively common in dialysis patients. In patients undergoing hemodialysis, xerostomy causes disturbances in chewing and swallowing, taste and speech disorders, and lead to increased risk of oral diseases such as gingivitis and tongue lesions, bacterial and fungal infections. It was found that changes in nitric oxide (NO) concentration both in physiological and pathological conditions were observed with xerostomia. Significantly low salivary NO levels have been demonstrated in patients with Parkinson's disease compared to the control group (1). The authors consider that the decline in salivary NO synthesis in these patients may be associated with the dopamine concentration deficiency that is in basis of this disease, resulting in the absence of stimulation of NO production from acinus cells (1). Significant concentrations of NO in saliva of patients with diabetes mellitus have been demonstrated (2). Changes in NO concentration in saliva were also observed in smokers and in subjects after physical exer-

cise as well as in other diseases where xerostomy appears as one of the symptoms. It is still not sufficiently studied, although there are indications that the NO synthesis pathway could play a key role in mediating hemodynamic and hemostatic disorders mediating the progression of renal disease. It remains unclear whether in patients with chronic renal failure exist increased or decreased endogenous nitric oxide production. Matavulj et al. have shown that the concentration of nitric oxide in the serum of patients with chronic renal failure was statistically significantly higher than in the control group (3). The authors suggest that the change (increase) in the NO synthesis concentration may help to relieve some pathological changes that can be seen in uremia such as: predisposition to bleeding, well-known uremia complications and hemodialysis hypotension (3). Dejanova et al. have demonstrated higher levels of NO in patients on hemodialysis than those in the control group (4). The authors believe that the increase in NO in patients on hemodialysis is caused by hemodialysis membrane and/or lack of renal excretion. Blichard et al. have reported that nitric oxide is a significant biomarker in monitoring hemodialysis effects on salivary NO concentrations (5). They found that the concentration of NO in saliva of the patients on hemodialysis decreased with the duration of hemodialysis (5).

2. AIM

The aim of the study was to investigate the concentration of nitric oxide in saliva of the patients with terminal stage of chronic renal failure on hemodialysis and to investigate the effect of hemodialysis on the concentration of nitric oxide in saliva of the patients with terminal stage of chronic renal failure on hemodialysis.

3. PATIENTS AND METHODS

The study was prospective and involved 60 respondents of both sexes, at age between 20 and 60 years. The control group included 30 healthy volunteers of both sexes (15 men and 15 women) of the same age, which, based on subjective and objective indicators of the general health condition was without any manifestation of pathophysiological changes. The mean age of the control group was 44 years. The group of patients with terminal stages of chronic hematoma renal failure was comprised of 30 patients of both sexes (15 men and 15 women) hospitalized at the Clinic for Hemodialysis at the Clinical Center of the University of Sarajevo. The mean age of the examined group was 47 years. The study is planned as a prospective, laboratory and manipulative research. Criteria for inclusion in the study: the hemodialysis period longer than 6 months and the age between 20 and 60 years. Exclusion criteria included patients suffering from diabetes mellitus, smokers, patients suffering from lumbar disease, patients suffering from malignant and infectious diseases, and lack of cooperation. The survey was conducted by a common approach to the respondent. Anamnestic data were taken from all subjects and underwent a physical examination and a laboratory test. Patients were on hemodialysis three times a week for 4 hours using bicarbonate hemodialysis.

When taking anamnestic data, a specially designed ques-

tionnaire was used that included general data (initials, sex, age), socio-epidemiological data (smoking), data on disease leading to terminal renal failure and duration of hemodialysis in months. These last data are taken from the patients' history of illness. Within the physical examination arterial blood pressure was measured immediately before and after hemodialysis. Blood pressure measurement was performed at rest after the patient was in the sitting position for five minutes and immediately before switching on the appliance and immediately after switching off the hemodialysis apparatus. The measurement was performed on the right-side arm, using a mercury manometer, and the values are expressed in millimeters of the mercury column (mmHg).

For laboratory tests, from subjects were taken blood samples from the cubic vein, and from which the value of urea and creatinine was determined immediately before and after hemodialysis. Laboratory processing was performed at the Institute for Clinical Chemistry and Biochemistry of the University Clinical Center in Sarajevo. Samples for determination of NO concentration in saliva were taken immediately before and immediately after hemodialysis. Previously, subjects rinse the mouth with water for 60 seconds to reduce bacterial contamination. Saliva samples were taken 30 seconds after rinsing the mouth and collected in a graduated tube with a funnel-covered filter paper for 15 minutes. After taking the filtered sample, the saliva was centrifuged for 5 minutes at 3000 g to remove the remaining microorganisms and debris. This procedure stabilized nitrates for later analysis. Saliva samples prior to storage were deproteinized by adding 0.05 ml of a 30% ZnSO₄ solution to 1 ml of the sample. After several minutes the sample was centrifuged for 10 minutes at 700 g and the separated supernatant was used to determine NO concentration. Samples of non-stimulated saliva secretion are harvested after a minimum of one-hour abstinence from food intake to avoid the influence of food on saliva production. The concentration of NO is determined indirectly by determining the concentration of nitrates and nitrite in saliva. Determination principle was the conversion of nitrate into nitrite using elemental zinc and then the nitrite concentration was measured by colorimetry using the prepared Griess reagents. After 10 minutes of stirring on a vibrator at room temperature, the absorption of light, optical density, was measured by a spectrophotometer at a filter of 546 nm. The concentration of nitrite was expressed from the standard curve with a known concentration of NaNO₂ of 1.56-100 nM. As a blank test, distilled water was added to which Griess reagent was added. The results are processed using standard statistical methods, using the computer program Excel (Microsoft Office Excel 2003) and the SPSS Statistical Package for Social Sciences package version 13.0. The results are expressed as mean (X) and standard error of mean (SEM). The Shapiro-Wilk test was used to test the significance of the difference in deviation from the normal distribution. For a comparative analysis between numeric variables, Student t-test was used. The degree of correlation was determined by the method by Pearson or Spearman. The value of $p < 0.05$ was taken as statistically significant.

4. RESULTS

The amount of non-stimulated saliva collected during 15 minutes in patients with chronic renal failure was lower by 31.3% compared to the control group of healthy subjects. This difference was statistically significant ($p < 0.0001$). Concentration of NO in saliva in patients with chronic renal failure was higher by 121% than the values determined in the control group of healthy subjects. This difference was statistically significant ($p < 0.001$). Concentration of NO in saliva in patients with chronic renal failure after hemodialysis was 121% lower than the values determined for the same subjects prior to hemodialysis. This difference was statistically significant ($p < 0.001$). The mean concentration of NO in saliva of male patients prior to hemodialysis ($65.39 \pm 18.28 \mu\text{mol/L}$) was statistically significantly higher in relation to NO concentration in saliva of the same subjects ($13.26 \pm 2.19 \mu\text{mol/L}$) after hemodialysis treatment ($p < 0.05$). The mean concentration of NO in saliva of female subjects prior to hemodialysis ($54.23 \pm 14.14 \mu\text{mol/L}$) was statistically significantly higher than that of NO in saliva of the same subjects ($21.94 \pm 6.90 \mu\text{mol/L}$) after hemodialysis treatment ($p < 0.05$) (Figure 1.).

The correlation between the NO concentration in saliva and the quantities of non-stimulated saliva collected over a 15-minute period is presented in Figure 2.

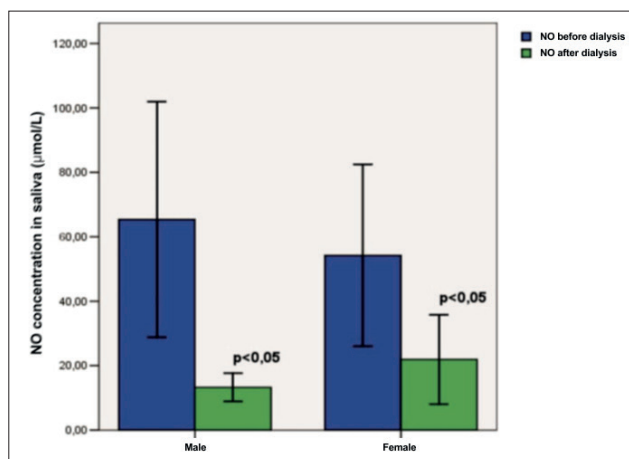


Figure 1. Concentration of NO in saliva before and after hemodialysis in patients with chronic renal insufficiency by sex (results are shown as mean ± standard error of mean ($x \pm \text{SEM}$))

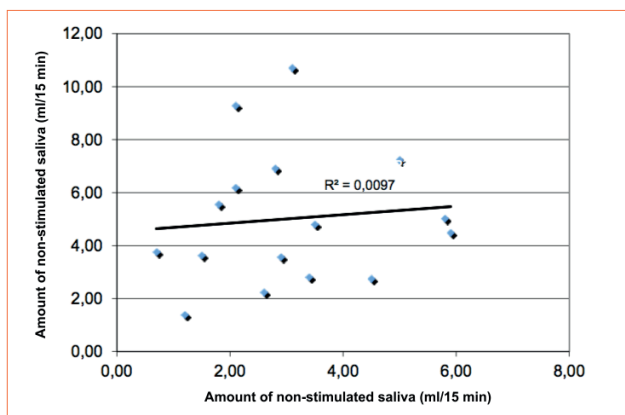


Figure 2. Correlation between the NO concentration in saliva and the amount of non-stimulated saliva tested groups (r -correlation coefficient).

No statistically significant correlation was found between the NO concentration in saliva and the amount of non-stimulated saliva in the tested group. The mean duration of hemodialysis expressed in months in the tested group was 62.83 ± 8.747 . A statistically significant negative correlation was established ($r = -0.381$, $p < 0.05$) between the months spent on hemodialysis and the level of NO in saliva of the patients with chronic renal failure before hemodialysis. There was no statistically significant correlation between the duration of hemodialysis in months and NO levels in saliva of the patients with chronic renal failure after hemodialysis treatment ($r = -0.167$, NS). There was no statistically significant correlation ($r = -0.131$, NS) between serum urea concentration and NO concentration in saliva of the patients with chronic renal failure before hemodialysis. There was no statistically significant correlation ($r = 0.007$, NS) between serum urea concentrations and NO concentration in saliva of the patients with chronic renal failure after hemodialysis treatment. There was no statistically significant correlation between serum creatinine concentration and NO concentration in saliva of the patients with chronic renal failure prior to hemodialysis ($r = -0.01$, NS). No statistically significant correlation ($r = 0.190$, NS) was found between serum creatinine concentrations and NO concentration in saliva of the patients with chronic renal failure after hemodialysis treatment.

5. DISCUSSION

Hemodialysis is the most commonly used method of replacing the renal function in case of permanent renal failure. The largest number of patients on hemodialysis is at the age group between 45-64 years (6). In our study, the average age of patients was 47 years, which is consistent with the results of other similar studies (6). The results showed that the concentration of non-stimulated saliva was lower in patients in the study group compared to healthy subjects. Kaushik et al. followed the changes in the amount of saliva and changes in the mouth of hemodialysis patients and attempted to correlate these changes with renal failure. In a study that lasted 15 months, 25 patients on hemodialysis were examined. It has been found that a lower amount of saliva is associated with hemodialysis compared to healthy subjects. Their result resembles ours. Investigations of NO concentration in biological fluids such as serum, plasma, urine and saliva in patients with chronic renal failure gave unconfirmed results (7). Mikulić et al. showed that the urinary and serum levels of NO were statistically significantly higher in patients with chronic renal disease compared to the control group. The same authors have shown that the level of endothelin-1 as well as large endothelin-1 (big endothelin-1) in serum and urine is significantly higher in patients with renal disease compared to healthy subjects. The authors suggest that NO and endothelin-1 concentrations in serum and urine can serve as potential biomarkers of chronic renal disease (8).

Many studies show that saliva specimen analysis is a suitable disease monitoring as well as disease severity estimation tool (1,2). It is a good indicator of plasma levels of various substances such as hormones and drugs, which can be used to follow the progression of the disease as

well as the therapeutic effect of the drug on the basis of its concentration. According to a group of Chinese authors, the effect of hemodialysis monitoring can be monitored through urea, creatinine and uric acid in saliva. They state that the effect of secretion of urea, creatinine and uric acid in saliva is the same as in serum. They expect the saliva to completely replace the serum for hemodialysis efficacy evaluation in these patients (8).

The results of our study showed that the concentration of NO in saliva of the patients on hemodialysis was statistically significantly higher than the NO concentration in the saliva of the control group subjects. In the available literature we did not find a study comparing the concentration of saliva NO concentration in patients on hemodialysis with respect to the control group so that we are not able to fully explain why patients with terminal chronic renal failure on hemodialysis have a significantly higher NO concentration in saliva compared to the control group. The NO and nitrite sources of these stable NO metabolites in the oral cavity may also be induced either by physiological reduction of nutritional nitrates or by the reaction of L arginine catalyzed by inducible nitric oxide synthase (iNOS) enzyme expressed in rat lymph nodes and cells of exogenous canals that may be stimulated by inflammatory stimuli (9). Our results also showed that the concentration of NO in saliva of the patients after hemodialysis was statistically significantly lower than the NO concentration in saliva of the same patients before hemodialysis. Our results are consistent with the results of Bryan et al. They have shown that hemodialysis significantly reduces NO concentration in saliva. They suggest that chronic and persistent depletion of plasma and salivary nitrates and nitrites is likely to reduce the bioavailability of NO. This can be associated with increased risk for cardiovascular mortality of hemodialysis patients (10).

Our results also correspond to the results of Blichard et al. (5). They found that NO is a significant biomarker in monitoring hemodialysis effects on salivary nitrate. It has been shown that the concentration of NO in saliva of the patients on hemodialysis decreases with the duration of hemodialysis in patients with chronic renal failure. The nitrate trend in saliva during hemodialysis was in a significant decline. Given that patients with insufficient kidney have high plasma NO concentration and salivary concentrations, salivary NO can serve as a good indicator of hemodialysis efficiency if its trend from the beginning to the end of hemodialysis itself is monitored. Matavulj et al. showed that the concentration of NO in serum patients with chronic renal failure on hemodialysis was statistically significantly higher than in the control group. There was no significant difference in the serum NO concentration between male and female patients, regardless of the treatment. The authors believe that increasing NO synthesis could help resolve some pathological changes that can be seen in uremic states such as bleeding predisposition (3).

Dejanova et al. have shown that patients on hemodialysis have significantly higher levels of NO compared to control group subjects (4).

Meyer et al. showed that during hemodialysis blood levels of free hemoglobin increase, which may exhibit in-

hibitory effects on NO production in these patients. The authors suggest that therapeutic effects that can stimulate free oxidation of free hemoglobin could reduce the bioavailability of NO and thus have positive effects on vascular function in this disease (11).

Given that the results of our study showed a statistically significant reduction of NO concentration in saliva after hemodialysis compared to its value prior to hemodialysis, we consider that the salivary NO concentration can be an indicator of hemodialysis efficiency. Although there are already known positive sides of NO, there are also its negative side, especially if it is present in excessive concentrations. Nitric oxide can be very harmful because it possesses an unopened electron so that it is subject to oxidation and becomes a labile free radical. As such, it reacts quickly with the superoxide radical by building an extremely reactive peroxynitrite anion and then a peroxynitrite acid. It is very dangerous because it results in: oxidation of thiol groups, tyrosine and phenylalanine nitrosylation, lipid oxidation, DNA chain breaking and nitriding and disamination of nucleic bases. This macromolecule damage can cause a number of undesirable changes that break down the function of the molecule and hence the cells, tissues and organs. If this fact is taken into consideration, hemodialysis can also manifest beneficial effects by eliminating the excess of accumulated NO. The results of our research have shown that serum concentrations of urea prior to hemodialysis were statistically significantly higher in relation to its concentration after hemodialysis. The results of our study showed that serum creatinine concentrations of hemodialysis patients after hemodialysis were statistically significantly lower than their concentration before hemodialysis. It can be rightly said that urea and creatinine are irreplaceable parameters that show the efficacy of hemodialysis in such patients (12-16).

6. CONCLUSION

Concentration of NO in saliva of the patients on hemodialysis was statistically significantly higher in relation to the NO concentration in saliva of the healthy subjects. Concentration of NO in saliva of the patients after hemodialysis was statistically significantly lower in relation to NO before hemodialysis.

Hemodialysis significantly reduces the values of urea and creatinine, so these are still sovereign parameters for evaluating the efficacy of the hemodialysis. Glomerulonephritis is the condition which most commonly leads to terminal renal failure and hemodialysis. Monitoring of changes in NO concentration dynamics in saliva of the hemodialysis patients will probably be helpful in monitoring hemodialysis efficacy.

- Author's Contribution: M.V. and J.H. gave substantial contribution to the conception or design of the work and in the acquisition, analysis and interpretation of data for the work, F.Z, A.D.N., E.J. and N.R. had role in drafting the work and revising it critically for important intellectual content. Each author gave final approval of the version to be published and they are agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

- **Financial support and sponsorship:** None.
- **Conflicts of interest:** There are no conflicts of interest.

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