Significantly Reduced Salivary Nitric Oxide Synthesis in Patients with Parkinson's Disease

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Abstract

In order to study concentration of nitric oxide (NO) in the saliva of patients with Parkinson's disease (PD), we measured the concentration of its stable metabolite nitrite (NO_2^-) in the saliva of these patients and healthy subjects. We analyzed saliva flow rate and salivary NO concentrations in 16 subjects with Parkinson's disease and in 16 healthy subjects. Concentration of nitrite was determined by colorimetric method using Griess reaction. Saliva flow rate was significantly lower in patients with Parkinson's disease (0,2±0,03 mL/min; X±SEM) than in healthy subjects. Salivary NO_2^- concen¬tration was significantly lower (5,02±0,64) than in healthy individuals (22,39±1,24; $p<0,0001$).

KEY WORDS: nitric oxide; Parkinson's disease; saliva

Introduction

Nitric oxide (NO) is a prod¬uct of nitrogen metabolism with short life. NO is synthesized from L-arginine by NO synthase (NOS) which is made up of at least three isoforms (1). Although initially investigated and characterized as endogenous vasodilator, NO is now known to perform a wide range of physiological and pathophysiologic functions. (2). Previous studies have shown that NO is present in human saliva. However, the physiological role of NO in saliva is not clear. Clinical investigations of salivary NO concentration have shown that the production of this free radical changes in various diseases. It was reported that salivary NO production is significantly lower in smokers than in non smokers (3). Furthermore, some authors found that salivary NO levels were significantly decreased in healthy subjects after a 2 day course of the broad spectrum antibiotics then prior to using medication (4). On the other hand, oral NO increases during de novo deposition of dental plaque (5). Also, it has been proven that salivary NO levels change in patients with periodontitis (6,7) and gingivitis (8). Further investigations showed that salivary concentration of NO in patients with Sjogern's syndrome significantly increased in comparison to healthy subjects (9). Recently, we have also found a decrease in salivary NO concentration in patients with diabetes mellitus (10). One of the leading symptoms of Parkinson's disease, as well as Sjogren's syndrome and diabetes mellitus, is the reduced secretion of saliva. However, there is no data on salivary NO concentration in patients with Parkinson's disease. Therefore, the aim of the present study was to investigate saliva flow rate and salivary concentrations of NO in patients with Parkinson's disease as well as to establish correlation between saliva flow rate and salivary concentration of NO in those patients.

Subjects and Methods

1. SUBJECTS

Study group included 16 patients with Parkinson's disease of both sexes (8 male and 8 female), 60-70 years of age, who were medically treated. The diagnosis of Parkinson's disease was made on the basis of clinical examination at the Neurology Clinic, University of Sarajevo Clinics Center. Control group consisted of 16 subjects of both sexes (8 male and 8 female), 60-70 years of age, who were healthy according to their subjective and objective findings. A medical history was obtained for all subjects and they received a complete dental check-up.

2. MEASUREMENTS OF NON-STIMULATED SALIVA FLOW RATE

Whole non-stimulated saliva samples were collected into tubes during a 15 minute period. Saliva flow rate was determined by dividing the volume of saliva by the collection time.

3. SALIVA SAMPLING

In all subjects, NO concentrations were determined after 2 days of low NO_2^- diet for. Subjects were told not to eat or smoke for 1 hour before the examination. Non-stimulated saliva was collected after the subjects washed their mouths with tap water for one minute in order to reduce bacterial contamination. Freshly secreted saliva was collected within the first 30-60 seconds after mouth washing. The samples were then refrigerated until essay.

4. NITRITE MEASUREMENT

NO concentration in the saliva was determined by measuring nitrite concentration, a stable metabolic product of NO with oxygen, as determined by Griess reaction (11). Briefly, equal volumes of saliva and Griess reagent were mixed at room temperature. After 5 min, the absorbance was measured at 570 nm using Perkin Elmer 550 S spectrophotometer. Nitrite concen¬tration was determined by a standard curve prepared with sodium nitrite (1-200 UM).

5. STATISTICAL ANALYSES

The measurement results were expressed as mean ± SEM. Differences between means were statistically compared by Student's t-test, and differences at $P<0.05$ were considered significant. Correlation coefficients were determined by employing Spearman's test.

Results

Non-stimulated saliva flow rates in patients with Parkinson's disease and healthy subjects are presented in Figure 1. The saliva flow rate was significantly lower in patients with Parkinson's disease (0,2±0,03 mL/min; X±SEM) than in healthy subjects (0,55±0,02; $p<0,0001$). Results of salivary NO_2^- concen¬trations are presented in Figure 2.

Samples obtained from patients with Parkinson's disease had significantly lower salivary NO_2^- concen¬trations (5,02±0,64) than the healthy persons' samples (22,39±1,24; $p<0,0001$). There were no significant sex-related differences in salivary NO_2^- concentrations, either in the control group or in the group of patients with Parkinson's

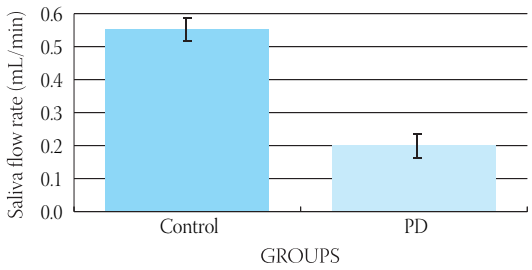

FIGURE 1. Saliva flow rate (ml/min.; mean±SEM) in patients with Parkinson's disease.
CONTROL – control group
(healthy subjects; N=16; 8 male and 8 female)
PD - patients with Parkinson's disease
(N=16; 8 male and 8 female)

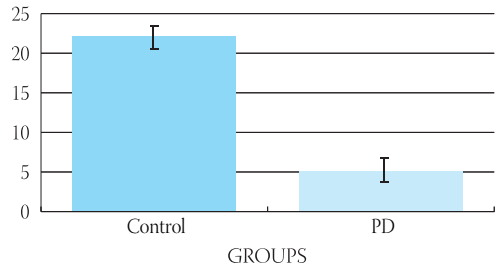

FIGURE 2. Salivary NO concentration (μmol/L; mean±SEM) in patients with Parkinson's disease.
CONTROL – control group
(healthy subjects; N=16; 8 men and 8 women)
PD - patients with Parkinson's disease
(N=16; 8 male and 8 female)

disease (data not shown). No correlation was found between salivary NO_2^- concentrations and saliva flow rate in patients with Parkinson's disease (data not shown).

Discussion

Our study clearly shows that the mean saliva flow rate in patients with Parkinson's disease is significantly higher than the one found in healthy subjects. Various studies conducted earlier, presented incongruency of data on the excretion of saliva in Parkinson's disease. Certain studies reported hypersalivation while others reported a decrease in saliva secretion (12). According to the most authors on the subject matter, a very common symptom of Parkinson's disease (almost 75% of the recorded cases), is the condition of hypersalivation. This has instigated numerous research projects aimed at discovering an efficient means of dealing with this symptom. Moreover, further research confirmed that the symptom of hypersalivation in Parkinson's disease is not the result of overproduction in the salivatory glands, but cause is merely in the difficulty of swallowing (13). Beside that, further studies have shown that the quantity of non-stimulated saliva excretion is significantly decreased in patients with Parkinson's disease who did not use any medication as well as in patients who were on medications for longer periods of time (14). Moreover, the studies have confirmed statistically non-significant differences in the quantity of secreted saliva between medicated and non medicated patients. The results of our quantitative study on the secretion of non-stimulated saliva in patients with Parkinson's disease are in accordance with the results of the above study. In our study, all patients whose saliva secretion was examined were on antiparkinson's drugs for longer periods of time. The results indicate that medication is not the only agent responsible for decreased saliva secretion. Taking into account that when Parkinson's disease is concerned there is a deficiency of dopamine, we conjecture that this deficiency is precisely the reason for the increased quantity of saliva secretion. It was proven that reduced saliva secretion is a symptom of Sjogern's syndrome. The reduction, in this disease, is a consequence of the structural destruction of the secretory, acinar parenchyma caused by lymphocytic infiltration of the salivatory gland. On the other hand, numerous studies have shown that in the case of the latter disease, an increase of NO concentration in the saliva exists. It is believed that this increase is the result of an increased activity of iNOS which is induced by cytokines from the lymphocytes. The production of NO induced by iNOS is permanent and brings about the production of large concentrations of NO, which may cause tissue destruction. It is precisely this that leads scientists to believe that the reduced saliva secretion in Sjogern's syndrome results from the destruction of acinar parenchyma. Our results in investigating NO concentrations in patients with Parkinson's disease have shown that NO concentrations are significantly reduced. We were unable to compare our results with other studies for in the literature at hand, we were unable to find any indication of previous works on this issue. In contrast to Sjogern's syndrome, the reduced saliva secretion in Parkinson's disease is not the result of a disrupted salivatory gland, and in all probability does not lead to the induction of iNOS. The concentration of NO in Parkinson's disease is partially instigated by ingested nitrates, and partially from acinar cells. NO from acinar cells is brought about partially by the activity of the constitutive isoforms of NOS, and partially from endogenous production. Endogenous production of NO in acinar cells is stimulated by beta-adrenal stimulation. Taking into consideration that the concentration of dopamine is reduced in Parkinson's disease, it is thought that

this reduction may be responsible for the decreased NO production in acinar cells. Moreover, our results show no significant statistical correlation between the NO concentrations in the saliva of patients with Parkinson's disease and that of the measured unstimulated saliva rate flow. Due to the unavailability of literary data, we were unable to compare our findings. The oral cavity is inundated with pathogenic bacteria. Saliva plays an active role in defending the organism from these pathogens, destroying them and reducing their number, due to its unique chemical composition. Accordingly, reduced quantities of saliva may bring about worrisome changes in the oral mucous membrane and instigate the development of caries. Since bacterial activity in the oral cavity, in reacting with nitrates, forms NO, the reduced saliva secretion may be the result of an increased concentration of NO in the saliva. However, the results of research are inconsistent so far. Decreased concentrations of NO in the saliva are found in the use of wide spectra antibiotics, in smokers, and in patients exhibiting good oral hygiene. All of this may be related to the reduced amounts of bacteria in the oral cavity. The results of several studies on the oral health and dental status in patients with Parkinson's disease presented better dental statuses compared to the control group (15). On the other hand, in the cases of inflammatory processes in the oral cavity, both increased (6) and reduced (7) concentrations of NO, were confirmed, despite the presence of large amounts of bacteria. It is thought that certain factors from the saliva itself either stop the synthesis or cause decomposition of the formed NO. This is supported by studies which have shown that saliva in patients with parodontitis or gingivitis stops or even blocks NO synthesis in polymorph nuclear leukocytes, which is not the case with healthy patients (16). Currently, it is not clear whether the reduced concentration of NO in inflammatory diseases simply reflects increased inflammatory activity and tissue destruction, or results from pathogenesis. Which implores the question: are NO concentrations in the saliva, except in the case of parodontal diseases, of diagnostic concern?

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