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NITRITE/NITRATE AND MALONDIALDEHYDE LEVELS IN NASAL POLYP

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Abstract – The aim of this study was to examine the pathophysiological role of NO (nitric oxide) and MDA (malondialdehyde) in tissue in patients with nasal polyposis. We measured nitrite/nitrate (Nitrite/Nitrate; NO_2^-/NO_3^-) and MDA in tissue and plasma of NP patients (n=20) and controls (n=20). MDA level expressed as the concentration of substances reacting to thiobarbituric acid and production of NO (concentration of nitrite/nitrate in plasma) by the Griess reaction were determined. The level of NO_2^- and NO_3^- in tissue are higher than that of the normal tissue ($p<0.05$). The level of MDA in tissue are higher than that of the normal tissue ($p<0.05$). The level of NO_2^- and NO_3^- in plasma of two groups are similar ($p>0.05$). The level of MDA in plasma is higher than that in the normal controls ($p<0.05$). The change in NO_2^-/NO_3^- and MDA levels of nasal polyp patients was demonstrated. Further studies are required concerning the significance of changes in lipid peroxidation and nitrite levels in pathogenesis of nasal polyp.

Key words: Inflammation, Oxidative stress, Malondialdehyde, Nitric oxide, Nasal Polyposis.

INTRODUCTION

Nasal polyposis (NP) is common health problem which affect 1-4% of the population (1). It is a chronic inflammatory disease of the nasal mucosa, sometimes, affecting patients with allergy, cystic fibrosis or primary ciliary dyskinesia, but mostly occuring in patients without any underlying disease. In all cases, polyps grow in the nasal and paranasal sinus cavities, causing very disabling symptoms and requiring long term treatment with corticosteroids or even surgery The results are expressed as mean values ± standard deviation. Differences between the groups for MDA and NO metabolites values were analyzed by using non-parametric tests (2). The pathophysiology of the disease remains unclear and histomorphologic features of NP are nonspecific, showing edema, inflammatory cell infiltration with numerous eosinophils in epithelium, gland, connective tissue and vessels (16). Nasal polyposis is a multifactorial disease, with infectious, non-infectious, inflammatory, anatomic and genetic abnormalities. Chronic inflammation remains the central major factor in nasal polyps although the majority of NP are idiopathic (7).

Nitric oxide (NO), a free radical gas previous considered merely as an atmospheric pollutant, is now recognized to be one of the key mediators in many physiological and pathological processes. NO formation was first described in endothelial cells, neurons, fibroblasts, platelets, macrophages, neutrophils and epithelial cells (13). Malondialdehyde (MDA) is an indicator of lipid peroxidation that is a general mechanism of tissue damage by free radicals. There are a few articles about the possible role of free radicals on the formation of nasal polyposis (3,11,14).

In the present study, we aimed to determine nitric oxide (NO) as a mediator of non specific immunoreactions in the nasal tissue of patient

with NP and to show possible relationship between NO levels and etiopathogenesis of NP. In addition to this, the possible role of MDA as a mediator of lipid peroxidation was investigated. These parameters were determined in the sera and tissues of patients with nasal polyp.

MATERIALS AND METHODS

40 subjects (24 female and 16 male) with a mean age of 35.2 ± 10.7 (range 19-54) years were included the present study. The patients group consisted of 20 subjects (12 females and 8 males of a mean age of 36.0 ± 11.2 years, range 19 -54) with nasal polyp who were selected for polypectomy procedure, and the control group consisted of 20 patients (12 females and 8 males of a mean age of 34.0 ± 10.3 years, range 22-54) with septal deviation who were selected for septoplasty.

Informed consent was obtained from all patients, and the study was approved by the Local Ethic Committee. All non-asthmatic, non-smokers were prospectively studied. None of the patients had allergy, systemic disease, acute infection, or history of drug and supplement intake. None of the patients with polyp had received systemic or topical steroids for at least 4 weeks before polyp tissue sampling. Tissue specimens were washed with 0.9% NaCl to remove hematome. They were stored in plastic bottles individually at -70° C until biochemical analyses were carried out. Tissue were weighed, placed in a % 1.15 KCl solution and homogenized for 30 min at 20,000 at + 4° C. Aliquots of homogenates superantants were analysed for MDA and NO_2^-/NO_3^- levels. Venous blood was drawn from all patients using 21-gauge needle and K3 tube with EDTA was used. For preparation of plasma samples, the tubes were centrifuged at 3000X for 10 minute at room temperature. The blood and tissue samples were stored at -70° C until analyses.

The levels of nitrite/nitrate, stable end products of NO_2^-/NO_3^-, were measured by using a flow-injection system based on the Griess reaction (4). Tissue supernatant specimen (400 µl) passed through a column containing copper-coated cadmium, which reduced all NO_2^-/NO_3^-. When the NO_2^- reacted with Griess reagent, it was converted into a diazo indicator and was detectable. Absorbance was measured at 550 nm using a spectrophotemeter. The results were expressed as micromoles per milligram of protein for tissue and micromoles per liter for plasma.

The MDA assay, which is an important indicator of oxidant stress, was based on spectrophotometry of the pink colored product of thiobarbituric acid-reactive substances. NP ve control tissue was homogenized in phosphate buffer (1:10 w:v, pH 7.4). One milliliter of supernatant was mixed with 0.5 ml trichloracetic acid and centrifuged at 3000g for 10 minutes. Subsequently, 1 ml of the supernatant was mixed with 0.5 ml of 0.7% thiobarbituric acid in water acetic acid solution 1:1 v:v) and boiled for approximately 30 minutes. After cooling on ice for 2 minutes, the absorbance of the samples was read at 532 nm (10). Total thiobarbituric acid-reactive substance (TBARS) were expressed as MDA.

The results were expressed as nanomoles per miligram of protein. Measurement of plasma MDA levels were measured by a modification of the methods of Yagi. The plasma MDA levels were expressed as nanomoles per milliliter The tissue MDA levels were expressed as micromole per liter (17). NO_2^-/NO_3^- and MDA measurements were carried out using a spectrophotometer (Schimadzu UV 1601, Kyoto, Japan). Protein was estimated according to method of Lowry et al (8).

Statistical analysis was performed by Statistical Package for Social Sciences (SPSS) 11.5 software (SPSS Inc., Chicago, IL, United States). The results are expressed as mean values ± standard deviation. Differences between the groups for MDA and NO metabolites values were analyzed by using non-parametric test (Mann Whitney U-test). A p value less than 0.05 was considered statistically significant.

RESULTS

No significant difference was recognized between the patients and the control group in terms of age and gender (p= 0.881). The nitrite concentration in tissue was 17.35 ± 4.02 µmol/mg in control, 22.76 ± 7.22 µmol/mg in patients (p=0.020). The nitrate concentration in tissue was 98.40 ± 12.32 µmol/mg in control, 112.29 ± 20.65 µmol/mg in patients (p=0.045). The level of MDA in tissue was 31.35 ± 6.29 nmol/g in controls and 39.18 ± 10.47 nmol/g in patients (p=0.017). The level of MDA in plasma was 1.15 ± 0.17 nmol/L in control, 1.39 ± 0.35 nmol/L in patients (p= 0.020). The mean plasma nitrite and nitrate concentration are similar (p=0.798, p=0.577, respectively). The results and p values are presented in Table I.

Table 1. Nitrite/ Nitrate and MDA Tissue (Polyp and Nasal Mucosa) and Plasma Levels Results were Expressed as Mean ± SD

	Control Group n=20	Patient Group n=20
Nitrite (µmol/mg pr)	17.35 ± 4.02	22.76 ± 7.22*
Nitrate (µmol/mg pr)	98.40 ± 12.32	112.29 ± 20.65*
MDA (µmol/mg pr)	31.25 ± 6.29	39.18 ± 10.47*
Nitrite (plasma-µmol/L)	11.50 ± 3.23	11.87 ± 2.91
Nitrate (plasma-µmol/L)	93.80 ± 11.45	96.12 ± 15.41
MDA (plasma-nmol /ml)	1.15 ± 0.17	1.39 ± 0.35*

*p<0.05 significance relative to control

DISCUSSION

NO in nasal physiology remains poorly understood, the functions are thought to be host defense, ciliary motility and improved ventilation-perfusion ratio in the lungs by auto-inhalation (9). NO is generated from arginine by a family of enzymes, the NO synthase, and acts as an autocrine and paracrine messenger.

Determination of nitrite in plasma can be used as mediators of NO radical formation. The determination of only plasma nitrite meaningless because it is rapidly oxidized into plasma nitrate (>95% in 1 h) by hemoglobin. It has been shown that the NO concentration found in normal sinuses was sufficient to inhibit the growth of several bacteria (5). This reinforces the idea that under normal condition, NO represents the first line of defense of the paranasal sinuses. There have been some articles dealing with the roles of free radicals in the pathogenesis of NP (6). Dagli et al. have claimed that there is strong evidence related to oxidative stress in the pathogenesis of nasal polyp, and antioxidant can have a preventive role in free-radical-mediated tissue damage in nasal polyp (3). In the study of Karlidag et al. nitrite levels were measured as a marker of tissue NO levels. Although the specificity of nitrite for nasal tissue was not been confirmed, it proved the presence of NO as an end product (6). In our study, it was observed that there is a meaningful increase of NO in nasal polyp tissue when compared with the normal mucosa. Plasma MDA levels have increased as well.

Sato et al evaluated the pathophysiological role of NO in nasal mucosa in patients with perennial nasal allergy. They measured nitrite, nitrate, and 3'-5' guanosine monophosphate in nasal lavage fluid. Their results showed that the production of NO was increased in patients with perennial nasal allergy (12). Brinke at al. evaluated direct relationship between sinonasal mucosa thickness and bronchial inflammation in severe asthma. Their results showed to a significant increased level of exhaled NO, p< 0,01 (15). In our study, we showed that the level of plasma nitrite change increase in polyp tissues. It was also observed that plasma MDA and tissue MDA levels have been parallel in terms of increase. We have observed that plasma NO did not change and that tissue NO increased, which reminded us that NO had not been affected systematically and that the NO increased in nasal polyp tissue could have increased due to changes of local inflammation and blood transfer.

The change in NO_2^-/NO_3^- and MDA levels of nasal polyp patients was demonstrated. Further studies are required concerning the significance of changes in lipid peroxidation and nitrite levels in pathogenesis of nasal polyp.

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