



Role of asthma and intolerance to acetylsalicylic acid on the redox profile in nasal polyp tissue

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Abstract

Purpose Nasal polyposis is a chronic inflammatory disease of the mucosa of the nasal cavity and paranasal sinuses. The etiology of nasal polyposis is unclear; however, it may be associated with asthma and intolerance to acetylsalicylic acid, possibly altering the redox profile. The study intends to compare the redox profile in polyps surgically removed from three clinical groups of patients with nasal polyposis who were divided according to the presence of asthma and intolerance to acetylsalicylic acid.

Methods Patients were divided into three groups: nasal polyposis only ($n=30$); nasal polyposis and asthma ($n=19$); and nasal polyposis, asthma and intolerance to acetylsalicylic acid ($n=10$). The following redox evaluations were performed: enzymatic antioxidant activity of superoxide dismutase, glutathione peroxidase, hydrogen peroxide consumption and glutathione S-transferase; non-enzymatic antioxidant levels of vitamin C, vitamin E and glutathione; levels of the oxidative damage biomarkers carbonyl groups (measuring protein damage) and malondialdehyde (measuring lipid peroxidation); and nitrite and nitrate levels.

Results Compared with the polyposis only group, hydrogen peroxide consumption, glutathione S-transferase, vitamin E and malondialdehyde were lower in the asthma group. Total glutathione (0.12 ± 0.01 vs. $33.34 \pm 10.48 \mu\text{mol/mg}$) and nitrite and nitrate (0.06 ± 0.01 vs. $15.95 \pm 1.38 \text{ nmol/mg}$) levels were higher in the nasal polyposis, asthma and intolerance to acetylsalicylic acid group.

Conclusions In patients with nasal polyposis, asthma may alter the redox profile associated with the hydrogen peroxide and lipid damage pathways, whereas asthma and intolerance to acetylsalicylic acid increase nitrite and nitrate and total glutathione levels.

Keywords Nasal polyposis · Antioxidant · Oxidative stress · Redox profile · Oxidative damage · Nitric oxide

Introduction

Nasal polyposis (NP) is a chronic inflammatory disease of the mucosa of the sinuses that is commonly seen in the out-flow tract of one or more of the paranasal sinuses or the mucosa of the nasal cavity [1]. NP is frequently encountered in otolaryngology clinics, with an incidence of 0.5–4% in the general population, and it may be associated with different diseases such as asthma, cystic fibrosis, chronic sinusitis and also with acetylsalicylic acid (AAS) intolerance [2]. The NP association with asthma ranges from 5 to 20%, and NP associated with asthma and AAS intolerance, called the Widal triad, ranges from 5 to 36% [3].

The etiology of NP has remained unclear so far [4], but inflammation is considered to be the most important

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mechanism in NP development [2]. Despite most reported studies focusing on the inflammatory mechanisms, recent studies have pointed out the role of free radicals in NP [5].

Free radicals and other reactive species (RS) are generated by the normal metabolism of the cell. In several cells and tissues, RS are involved in energy production, immune cell responses, cell growth regulation, intercellular signaling, and cell differentiation, among other very important functions. Physiologically, RS are kept in balance with the antioxidant defense system in the body, a condition called redox homeostasis. Nevertheless, when produced in excess, RS may lead to oxidative stress (OS), which has detrimental effects such as lipid peroxidation, protein oxidation, and enzyme inactivation due to damaged active sites, and oxidative damage to carbohydrates and nucleic acids [6]. However, RS may be neutralized in vivo by antioxidant defense mechanisms [7].

The present study is the first to evaluate the redox profile in polyps surgically removed from three clinical groups of patients: patients with NP only (NPO); patients with NP and asthma (NPA); and patients with NP, asthma and AAS intolerance (NPAA).

Methods

Study design and characteristics of the studied population

The study was conducted by screening patients referred for polyp removal by endoscopic surgery. The patients signed a written informed consent after detailed information was provided. Asthma patients were previously diagnosed by the physician, according to the guidelines for asthma management [8]. Only patients with eosinophilic asthma were included. Intolerance to acetylsalicylic acid was identified by the same physician, characterized by previous cutaneous and/or respiratory reaction to acetylsalicylic acid.

Patients with systemic diseases (diabetes mellitus, arthritis), cataracts, brain vascular disease, Behçet's disease, neurodegenerative diseases, pregnant women, people aged < 18 years and those who refused to join the study were excluded.

Preoperative routine and sample evaluation

Before undergoing surgery to reduce the size of polyps, all patients used 20 mg prednisolone with 500 mg azithromycin on a daily basis for 7 days. They also underwent preoperative nasal endoscopy and paranasal sinus computed tomography (CT) to detect the presence of NP.

Polyps were removed by endoscopic surgery by a specialist surgeon and sent to the research laboratory on dry ice and

immediately frozen at -80°C until further processing. In the processing, polyps were manually macerated, and the samples were mixed in 5 mL of 30 mmol/L of phosphate buffer containing 120 mmol/L KCl and 100 $\mu\text{mol/L}$ PMSF, pH 7.4. After mixing, polyp samples were sonicated three times for 10 s each and centrifuged for 10 min at 1700xg. The supernatant was aliquoted and frozen at -80°C until used for assays.

Polyp size was measured by CT and grouped according to Rouvier's anatomical classification. According to Rouvier's classification, polyps can have the following sizes: mild or small polyp size (stage 1); medium or moderate polyp size (stage 2); and obstructive or large polyp size (stage 3) [9]. Polyps were weighed after surgery.

Enzymatic antioxidant evaluation

Superoxide dismutase (SOD) activity was measured using a RanSOD kit (Randox, UK).

Glutathione peroxidase (GPx) activity was measured using a Ransel Kit (Randox, UK).

Hydrogen peroxide (H_2O_2) consumption was evaluated by measuring absorbance at a wavelength of 240 nm [10].

The glutathione S-transferase (GST) antioxidant assay measures the formation of S-(2,4-dinitrophenyl)-glutathione by GST enzymatic activity by 1-chloro-2,4-dinitrobenzene (CDNB) and glutathione (GSH) conjugation [11].

Non-enzymatic antioxidant evaluation

Vitamin C levels and Vitamin E levels were measured by high-performance liquid chromatography (HPLC) [10].

Total GSH (tGSH) levels were analyzed by measuring the formation of p-nitrophenol from 5,5'-dithiobis-2-nitrobenzoic acid [10].

Oxidative damage evaluation

As an index of protein damage, carbonyl group levels were measured using absorbance at a wavelength of 370 nm [10].

As an index of lipid peroxidation, malondialdehyde (MDA) levels were measured by HPLC [12].

Nitric oxide (NO) evaluation

Indirect NO levels by the nitrite and nitrate (NO_2 and NO_3) assay were measured by spectrophotometry using Griess reagent [10].

Data normalization

All results were normalized using the total protein content by the Bradford method, using BSA as the standard.

All assays in this study were independently performed in triplicate.

Statistical analysis

Variables with a normal distribution are presented as the mean $\pm$ SE and were compared using one-way ANOVA with a post hoc Dunnett test. Variables with non-normal distribution are presented as the median (25% – 75%) and were compared using the Kruskal–Wallis test.

All analyses were performed at a 0.05 level of significance. Statistical analyses were accomplished with the support of the Statistical Nucleus (NAE).

Data transformation was performed for GPx and NO₂ and NO₃. For GPx, the mean was raised to 0.2, and for NO₂ and NO₃, the square root of the mean was calculated to transform these means to fit a normal distribution.

Results

Sample collection was conducted between May 2014 and July 2015. Fifty-nine patients diagnosed with NP were included in the study. Patients were divided into three clinical groups according to the presence of asthma and intolerance to acetylsalicylic acid. The NPO group ($n = 30$) was composed of patients with NP and no other associated

diseases, the NPA group ($n = 19$) was composed of patients with NP and asthma, and the NPAA group ($n = 10$) was composed of patients with nasal polyposis with asthma and AAS intolerance.

Anthropometric and laboratory data from patients are shown in Table 1. No differences were observed in the prevalence of the three clinical groups of NP according to sex, age or weight of the polyp when comparing the three groups (Table 1).

According to Rouvier's classification of the polyp size, no significant differences were found in the redox profile of the three groups (data not shown).

Triglyceride levels were significantly lower in the NPA group (121.6 ± 4.36 mg/dL) compared with the NPO group (149.1 ± 3.92 mg/dL) and compared with reference values (triglycerides > 150 mg/dL). Eosinophils were higher in the NPA group ($529.24 \pm 27.93/\mu\text{L}$) and in the NPAA group ($539.38 \pm 40.72/\mu\text{L}$) compared with the NPO group ($337.48 \pm 25.13/\mu\text{L}$) and compared with reference values (eosinophils $50\text{--}500/\mu\text{L}$) (Table 1). Evaluation of oxidative damage, non-enzymatic antioxidant and NO₂ and NO₃ levels can be observed in Fig. 1.

MDA levels were significantly lower in the NPA group compared with the NPO group, but were similar between the NPAA and NPO groups (Fig. 1a). No differences were found among the analyzed groups for the carbonyl levels (Fig. 1b).

Table 1 Anthropometric and laboratory data of nasal polyposis (NP) patients

Dependent variable	NPO	NPA	NPAA
Gender (male)	20 (67%)	12 (63%)	4 (40%)
Age (years)	46.7 $\pm$ 2.72	50.05 $\pm$ 2.93	42.5 $\pm$ 3.91
Polyp weight (g)	2.43 $\pm$ 0.18	2.18 $\pm$ 0.21	2.57 $\pm$ 0.18
Glucose (mg/dL)	95.81 $\pm$ 1.62	92.65 $\pm$ 1.81	92.25 $\pm$ 2.64
Creatine (mg/dL)	0.88 $\pm$ 0.03	0.96 $\pm$ 0.04	0.87 $\pm$ 0.06
Uric acid (mg/dL)	5.43 $\pm$ 0.3	5.24 $\pm$ 0.34	5.24 $\pm$ 0.49
Cholesterol (mg/dL)	171.24 $\pm$ 5	160.59 $\pm$ 5.56	168.88 $\pm$ 8.11
Triglycerides (mg/dL)	149.1 $\pm$ 3.92	121.6 $\pm$ 4.36*	141.38 $\pm$ 6.36
HDL (mg/dL)	67.14 $\pm$ 2.18	70 $\pm$ 2.42	68.13 $\pm$ 3.53
LDL (mg/dL)	93.89 $\pm$ 3.92	89.06 $\pm$ 4.36	86.25 $\pm$ 6.35
Erythrocytes (mil/ μL)	4979 $\pm$ 0.09	4.91 $\pm$ 0.1	4.79 $\pm$ 0.15
Hemoglobin (g/dL)	15.51 $\pm$ 0.32	15.15 $\pm$ 0.36	14.69 $\pm$ 0.53
Leukocytes (/ μL)	7970.19 $\pm$ 393.73	7610 $\pm$ 437.61	7807.5 $\pm$ 637.91
Basophils (/ μL)	70.76 $\pm$ 5.73	51.71 $\pm$ 6.4	60.13 $\pm$ 9.3
Eosinophils (/ μL)	337.48 $\pm$ 25.13	529.24 $\pm$ 27.93*	539.38 $\pm$ 40.72*
Neutrophils (/ μL)	4018.71 $\pm$ 243.33	4003.47 $\pm$ 270.45	4046.5 $\pm$ 394.25
Lymphocytes (/ μL)	2365.43 $\pm$ 174.91	2613.03 $\pm$ 194.41	2784.88 $\pm$ 283.4
Platelets (/ μL)	234810 $\pm$ 13419.11	285706 $\pm$ 14914.51	287125 $\pm$ 21741.45

Data are shown as the mean $\pm$ SE unless otherwise noted

NPO patients with NP only, NPA patients with NP and asthma, NPAA patients with NP, asthma and intolerance to acetylsalicylic acid

* $p \leq 0.05$ vs. NPO

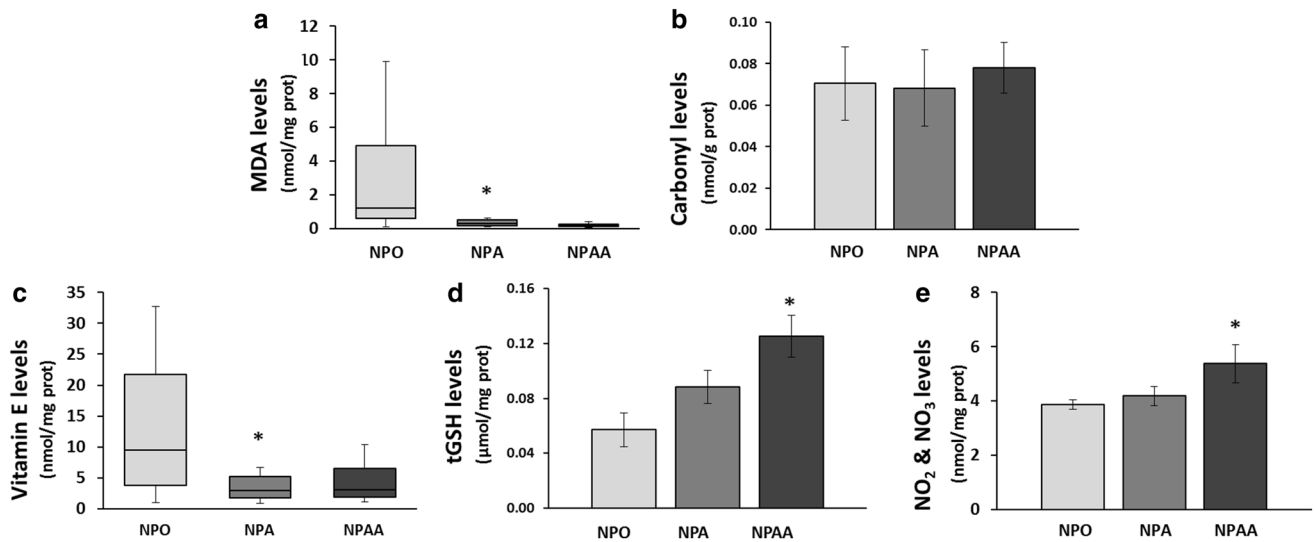


Fig. 1 Oxidative damage and non-enzymatic antioxidants in polyp tissue of patients with nasal polyposis (NP). *NPO* patients with NP only, *NPA* patients with NP and asthma, *NPAA* patients with NP, asthma and intolerance to acetylsalicylic acid. * $p \leq 0.05$ vs. *NPO*

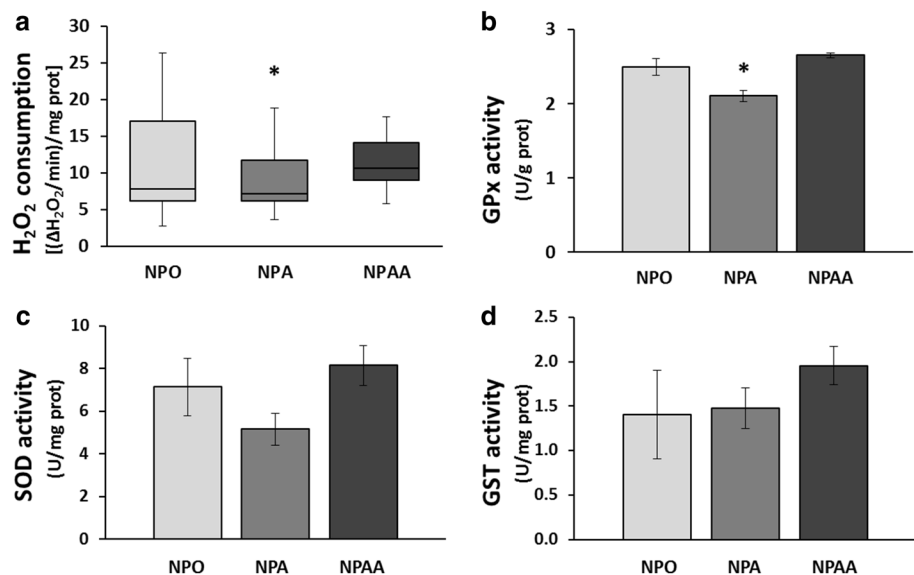
Vitamin E levels were similar to MDA levels and significantly lower in the *NPA* group compared with the *NPO* group, but similar between the *NPAA* and *NPO* groups (Fig. 1c).

The levels of tGSH were higher in the *NPAA* compared with the *NPO* group, but were similar between the *NPA* and *NPO* groups (Fig. 1d). In a similar manner, NO_2 and NO_3 levels were significantly higher in the *NPAA* group compared with the *NPO* group and were also similar between the *NPA* and *NPO* (Fig. 1e) groups.

Vitamin C levels were undetectable in all groups, even with careful processing of samples and using a very sensitive tool such as HPLC.

Evaluation of enzymatic antioxidant activity can be observed in Fig. 2. H_2O_2 consumption, an assay that is able to evaluate the ability of all H_2O_2 detoxifying enzymes together, was significantly lower in the *NPA* group compared with the *NPO* group (Fig. 2a). In a similar manner, GPx activity, an antioxidant enzyme that is also associated with the detoxification of peroxides, was significantly lower in the *NPA* group compared with the *NPO* group (Fig. 2b). However, there were no significant differences in SOD (Fig. 2c) or GST (Fig. 2d) activities among groups.

Fig. 2 Activity of antioxidant enzymes in the polyp tissue of patients with nasal polyposis (NP). *NPO* patients with NP only, *NPA* patients with NP and asthma, *NPAA* patients with NP, asthma and intolerance to acetylsalicylic acid. * $p \leq 0.05$ vs. *NPO*



Discussion

Many theories have been proposed to explain the etiology of NP. Studies that investigated the role of RS and antioxidants in NP have found strong evidence for the involvement of OS in the pathogenesis of this condition [13].

The present study analyzed the redox profile of these clinical NP groups based on oxidative damage biomarkers and antioxidant defenses to provide a clearer picture of the role of OS in NP and its association with other diseases.

This work showed a significant increase in eosinophils in the NPA group and in the NPAA group compared with the NPO group. Eosinophilia is observed during parasitic infections, allergy, collagen vascular disease, hypersensitivity conditions, or underlying hematologic or malignancies [14]. Given the exclusion criteria for the study we can assume that the increase in the number of eosinophils was due to the presence of asthma as it was observed in both the NPA and NPAA groups. The high concentration of inflammatory cells found in NP potentially results in the overproduction of RS. Inflammatory cells such as eosinophils and macrophages are known to produce elevated amounts of RS. Eosinophils are believed to have an important role in inflammatory diseases, such as allergic asthma, atopic dermatitis, connective tissue diseases, among others [15].

An important mechanism in the development of cell injury due to reactive species is lipid peroxidation of polyunsaturated fatty acids in cell membranes, leading to the formation of by-products such as MDA [16]. Dogru et al. [17] reported higher MDA levels in NP tissue compared to the nasal mucosa as a control. The results from this current study showed low MDA levels in the NPA group compared with the NPO group. Additionally, a downward trend in the NPAA group was observed. The lack of significance due to the number of patients in this group (9 patients) cannot be ruled out.

The tocopherols and tocotrienols inhibit lipid peroxidation [6]. It is well known that vitamin E is absorbed through the diet and can be mobilized to different tissues according to the injury that has occurred. Therefore, it is possible that vitamin E could migrate to the polyp tissue and prevent further damage to the nasal mucosa (lipoperoxidation) [18]. However, the present study cannot rule out that differences in vitamin E levels were due to the diet of patients because diet was not evaluated. Moreover, there are no studies evaluating the levels of vitamin E and vitamin C in clinical groups of NP.

No significant differences were found among the three groups in carbonyl group levels. A study showed that there are increased carbonyl group levels in NP tissue compared to tissue of the nasal mucosa as demonstrated by Ahmad et al. [19].

In the lower respiratory tract, NO levels are low, normally less than 20 p.p.b [20]. Raised NO levels indicate inflammation, which can have many causes, including allergy and infection [21]. In the upper respiratory tract and paranasal sinuses, the situation is even more complex, as high constitutive NO levels (20–25 p.p.m.) are produced due to the bactericidal role of NO [22]. The vasodilator effects of NO are also known, and this is an important factor for the maintenance of normal vascular function [23]. In the nasal epithelium, the inducible form of nitric oxide synthase (iNOS) is present with high activity, especially in patients with NPAA [24]. The increase in NO₂ and NO₃ in the NPAA group can be due to induction of iNOS and high concentrations of inflammatory cells, as seen by the increased eosinophils in this group.

Li et al. [25] showed that in endothelial cells, NO increases the intracellular tGSH concentration by inducing γ -glutamylcysteine synthetase, the rate-limiting enzyme of tGSH synthesis, as well as cellular cystine uptake. tGSH is considered the most important intracellular non-enzymatic antioxidant [6]. In lung tissue samples from asthmatic patients, increased tGSH levels are frequently observed, which may be associated with inflammation in the lungs and can be considered an adaptive response to the OS [26]. Researchers have reported an increased level of tGSH in blood in asthmatic patients, which may demonstrate that tGSH is a systemic inflammatory component of the disease [27]. There are no data in the literature regarding the oxidative profile of NPAA patients. It is noteworthy that we observed increased tGSH and NO levels in the NPAA group, but not in the NPA group.

Asthmatic patients are exposed to oxidative stress due to the generation of RS as a consequence of chronic airway inflammation. High levels of H₂O₂, NO, and isoprostane are present in the exhaled air of asthmatic patients along with high levels of oxidized protein products in peripheral blood, pulmonary epithelial lining fluid and urine. These data provide broad evidence of OS in asthma [28]. Asthma patients also show remarkable increases in carbonyl groups in plasma [19].

OS in asthma may be further aggravated by the fact that these patients have diminished enzymatic antioxidant defenses such SOD, catalase and GPx [19]. Moreover, asthmatic patients also have low GPx activity in the breath condensate of exhaled air [29]. It has been reported that GPx plays a significant role in the peroxyl scavenging mechanism, reducing fatty acid hydroperoxides and H₂O₂ and maintaining the functional integration of cell membranes [19]. In the present study, no significant differences were found in SOD or GST activity. However, we found a significant decrease in H₂O₂ consumption and GPx activity in the NPA group compared with the NPO group. Importantly, the H₂O₂ consumption assay measures the activity of enzymes

responsible for detoxifying H_2O_2 , such as peroxiredoxins, catalase, and GPx together.

Although steroids can reduce inflammation and alter the redox profile, patients included in this study received the same corticosteroids and antibiotic treatment, and the differences found in the redox profile of patients were not influenced by drug treatment. It is believed that corticosteroid medication only influenced the reduction in polyp size to optimize endoscopic surgery [30].

Notwithstanding, patients present high polyp recurrence rate and may require two or more polypectomies. For this reason, medicament management of nasal polyposis is recommended, since long term systemic steroid treatment prevents eosinophil infiltration, as well as prevents polyp size increase [30].

The present study indicates that asthma and intolerance to acetylsalicylic acid alter the redox profile of polyp tissue, and may consequently contribute to changes in inflammatory profile. Recent studies are indicating the use of antioxidant and anti-inflammatory diet for treatment of OS- and inflammation-related diseases such as rheumatoid arthritis [31] and systemic chronic inflammation [32]. We hypothesize that antioxidant and anti-inflammatory diet may prevent polyp size increase while reducing recurrence rate by impairing ROS formation and pro-inflammatory signaling. Future studies will be necessary to elucidate if an antioxidant and anti-inflammatory diet or supplementation may reduce polyposis size and recurrence, as well as the severity of polyposis symptoms such as rhinorrhoea, headache or facial pain, and sneezing.

In conclusion, this study shows that the redox profile of polyp tissue is altered in patients with asthma and intolerance to acetylsalicylic acid. Polyps of patients with asthma (NPA group) have decreased enzymatic defense against peroxides and have lower lipid peroxidation. Moreover, polyps of patients with NPAA have increased levels of NO and tGSH.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were approved by the ethics committee of the institution and was conducted in accordance with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The patients signed a written informed consent after detailed information about the study was provided.

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