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MAIN ARTICLE

Effect of antioxidants on the clinical outcome of patients with nasal polyposis

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Abstract

Aim: To investigate the therapeutic effects of antioxidants on the clinical and biochemical outcome of patients with nasal polyposis.

Methods: Thirty-four patients with nasal polyposis were divided into two groups receiving either intranasal steroid or intranasal steroid plus per-oral vitamins A, C and E and selenium. Paranasal sinus computed tomography, endoscopy, and polyp tissue and serum sampling were conducted pre- and post-therapy. Serum levels of malondialdehyde, superoxide dismutase, nitrite and myeloperoxidase and tissue levels of malondialdehyde and superoxide dismutase were measured. Group results were compared using the Mann–Whitney U test and Wilcoxon signed-rank test.

Results: Both groups had significantly lower tissue parameters, computed tomography scores and serum malondialdehyde levels, comparing pre- versus post-treatment results. Post-treatment, the steroid plus antioxidant group had significantly lower tissue malondialdehyde levels and a greater fall in tissue and serum malondialdehyde, compared with the steroid group.

Conclusion: Serum and tissue levels of malondialdehyde (an oxidative marker) were significantly decreased by adding antioxidants to standard therapy. This is the first report of the positive effects of adding antioxidants to steroid therapy for nasal polyposis.

Key words: Nasal Polyps; Oxidative Stress; Antioxidants; Pathology; Therapeutics

Introduction

Nasal polyposis is defined as an inflammatory condition of the nasal and paranasal sinus cavities. It frequently originates from the paranasal sinus mucosa, especially the anterior ethmoid cells.

The aetiology of nasal polyposis is still unknown, but multiple factors can play a role. The most important is inflammation.[1] Most reported studies have investigated the inflammatory mechanisms of nasal polyposis. However, recent work has assessed the role of oxygen free radicals in patients with nasal polyposis.[1–12]

Oxygen free radicals can be defined as molecular species containing one or more unpaired electrons.[13] Oxygen free radicals are neutralised *in vivo* by the body's antioxidative defence mechanisms. Once the balance between oxygen free radical production and antioxidative defence activity is disrupted, oxidative stress can occur, which may result in cell injury or death, subsequent tissue damage, and, finally, chronic disease.[14–16]

Studies investigating the role of oxygen free radicals and antioxidants in nasal polyposis have revealed strong evidence for the involvement of oxidative stress in the pathogenesis of nasal polyposis.[1–12] However, the present study is the first to investigate the therapeutic effects of antioxidants on the clinical and biochemical outcome of patients with nasal polyposis.

Materials and methods

Study population

This study included 44 patients from the ENT department of Dıskapi Yıldırım Beyazıt Training and Research Hospital, with the approval of the institution's ethics committee. All patients provided written, informed consent regarding participation in the diagnostic, interventional and therapeutic aspects of the study.

Diagnosis of nasal polyposis was based on anterior rhinoscopy, endoscopic examination and coronal paranasal sinus computed tomography (CT).

We excluded from the study any patients with systemic disease (e.g. diabetes mellitus, arthritis, cataract,

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cerebrovascular disease or Behçet disease) or a history of topical or systemic corticosteroid use in the previous four weeks or acute upper respiratory tract infection in the previous two weeks.

Baseline assessment

The Lund–Mackay staging system was used to assess paranasal sinus CT scans. The four-stage grading system of Rasp *et al.*[17] was used to stage nasal polyposis in both nasal cavities, based on endoscopic appearances.

Therapeutic protocol

Before treatment, cup forceps were used to obtain nasal polyp specimens from 44 patients with nasal polyposis.

Patients were then randomly divided into two groups, using the sealed envelope technique.

One group received topical intranasal steroid treatment with 100 μg/day mometasone furoate for six months.

The other group received 100 μg/day intranasal mometasone furoate plus per-oral administration of an antioxidant preparation containing β-carotene (pro-vitamin A) 20 mg (= 26.000 IU), vitamin C (ascorbic acid) 200 mg, vitamin E 200 mg (= 200 IU) and selenium 50 μg, for six months.

Follow up

Patients were followed up monthly. Ten patients did not attend follow-up sessions and were thus excluded. Thirty-four patients completed the study.

A second paranasal sinus CT scan and endoscopic examination were performed six months after the beginning of treatment.

Laboratory analysis

Tissue and blood samples were collected just before the beginning of therapy, and follow-up samples were collected on completion of six months' treatment, at the last follow-up session.

Tissue samples were immediately rinsed with 0.9 per cent sodium chloride to remove any blood, and kept at −30°C until needed.

Blood samples were centrifuged at 3000 rpm for 10 minutes to obtain serum. Serum samples were kept at −30°C until needed.

Serum malondialdehyde. Serum levels of malondialdehyde, the end-product of lipid peroxidation, were used to assess superoxide radical production. Serum malondialdehyde concentration was determined using the method of Yoshioka *et al.*[18] This method reacts malondialdehyde with a thiobarbituric acid reagent under acidic conditions to generate a pink product, determined spectrophotometrically at 532 nm; tetramethoxypropane is used as an external standard. Serum malondialdehyde concentration was expressed as nmol/ml.

Tissue malondialdehyde. The tissue malondialdehyde concentration was determined spectrophotometrically using the method of Mihara and Uchiyama.[19] Tissue malondialdehyde concentration was expressed as nmol/g tissue.

Serum and tissue superoxide dismutase activity. The activities of both serum and tissue superoxide dismutase were measured as an index of the antioxidative defence system. Total superoxide dismutase activity (i.e. Cu–Zn and Mn) was determined according to the method of Sun *et al.*[20] This method is based on the inhibition of nitroblue tetrazolium reduction by the xanthine–xanthine oxidase system, acting as a superoxide generator. Activity was assessed in the ethanol phase, after 1.0 ml of a 5:3 ethanol-chloroform mixture (volume for volume) was added to the same volume of serum sample and centrifuged. One unit of superoxide dismutase activity was defined as the enzyme amount causing 50 per cent inhibition of the nitroblue tetrazolium reduction rate. Results were expressed as units per ml serum, or as units per mg protein for tissue.

Serum myeloperoxidase activity. Serum myeloperoxidase activity was assessed by measuring H_2O_2-dependent oxidation of O-dianisidine. In its oxidised form, O-dianisidine is brown, and can be measured spectrophotometrically at 410 nm. Results were expressed as U/ml. One unit of myeloperoxidase activity was defined as the amount of enzyme causing an absorbance change in 1 minute at 410 nm and 37°C.[21]

Serum nitrite. As nitric oxide rapidly degrades to nitrate and nitrite in aqueous solution, the serum nitrate and nitrite levels were estimated, to provide an index of nitric oxide production. The serum nitrite concentration was measured using the Griess reaction, by adding 1 per cent naphthylethylenediamine to 2 per cent sulphanilamide in 5 per cent concentrated phosphate buffer. After 10 minutes' incubation, the absorbance was determined spectrophotometrically at 540 nm. Results were expressed as μmol/ml.[22]

Statistical analysis

The Statistical Package for the Social Sciences version 11.0 for Windows software program (SPSS Inc, Chicago, Illinois, USA) was used to perform all statistical calculations. Results are expressed as mean ± standard deviation. The chi-square test was used to compare parameters between the two groups. The Mann–Whitney U test for unpaired data was used to compare unpaired results between the two groups. The Wilcoxon signed-rank test was used to compare the same parameters in each patient before and after treatment.

Differences were considered statistically significant at a p value of less than 0.05.

Results

The steroid plus antioxidant group comprised 16 patients with nasal polyposis: 10 men and six women, with a mean age of 45.3 ± 13.5 years (range, 22 to 67 years). The steroid group comprised 18

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patients with nasal polyposis: eight men and 10 women, with a mean age of 45.3 ± 9.5 years (range, 24 to 63 years). There were no statistically significant differences between the two groups regarding age or gender.

Pre-treatment polyp and CT scores for both groups are shown in Table I. There were no statistically significant differences between these two parameters, comparing the two groups before treatment.

Table I shows both groups' pre-treatment results for serum levels of malondialdehyde, nitrite, myeloperoxidase activity and superoxide dismutase activity, and for tissue levels of malondialdehyde and superoxide dismutase activity. There were no significant differences between the two groups for any of these parameters, prior to treatment.

In the steroid plus antioxidant group, there were statistically significant differences for serum and tissue levels of malondialdehyde, and for polyp and CT scores, comparing pre- versus post-treatment results. This group showed no statistically significant differences for any other parameter, comparing pre- versus post-treatment results.

In the group receiving steroid alone, there were statistically significant differences for serum malondialdehyde levels and for polyp and CT scores, comparing pre- versus post-treatment results. There were no other statistically significant differences in this respect (Table I).

Following treatment, there was a statistically significant difference between the two groups' serum and tissue malondialdehyde levels.

Table II shows mean differences for all analysed parameters, comparing pre- versus post-treatment results, for both groups. Mean differences in both serum and tissue malondialdehyde levels were significantly higher in the steroid plus antioxidant group, compared with the steroid group (Figures 1 and 2); i.e. the pre- versus post-treatment decrease in serum and tissue malondialdehyde levels was significantly greater in the steroid plus antioxidant group, compared with the steroid group.

TABLE II
MEAN DIFFERENCE IN ANALYSED PARAMETERS, PRE- *VS* POST-TREATMENT, BOTH GROUPS

Parameter	Difference (mean ± SD)		*p*
	S +AO grp	S grp	
Polyp score R	0.6 ± 0.7	0.5 ± 0.8	0.597
Polyp score L	0.6 ± 0.7	1.0 ± 0.7	0.237
CT score	2.0 ± 2.6	1.9 ± 3.0	0.986
Serum MDA (nmol/ml)	2.7 ± 2.4	0.9 ± 2.2	0.025*
Serum SOD (U/ml)	−0.2 ± 8.1	−4.5 ± 12.7	0.959
Serum MPO (U/ml)	−8.4 ± 29.6	−0.8 ± 25.3	0.551
Serum NO_2 (μmol/ml)	−15.0 ± 64.1	12.3 ± 37.7	0.746
Tissue MDA (nmol/g tissue)	1.3 ± 0.9	0.1 ± 1.2	0.040*
Tissue SOD (U/mg protein)	−9.7 ± 43.8	−8.8 ± 72.6	0.631

*$p < 0.05$, Mann–Whitney U test. SD = standard deviation; S + AO grp = steroid plus antioxidant group; S grp = steroid group; R = right; L = left; CT = computed tomography, MDA = malondialdehyde; SOD = superoxide dismutase activity; MPO = myeloperoxidase activity

Discussion

In recent years, it has become increasingly obvious that oxygen free radicals play an important role in the normal regulatory mechanism of the human body. A certain physiological level of oxygen free radicals is essential for the regulation of cell functions such as intracellular signalling, transcription activation and cell proliferation.[23] However, unbalanced levels of oxygen free radicals may be of primary significance in inflammatory disease.[24] Oxygen free radicals are also involved in the pathogenesis of inflammation, and are considered to cause tissue damage via chemical modification of proteins, lipids, carbohydrates and nucleic acids.[25]

The biological effects of oxygen free radicals are controlled *in vivo* by a wide spectrum of enzymatic and non-enzymatic defence mechanisms (known as the antioxidant system), such as the superoxide dismutase, catalase and glutathione peroxidase reactions. An extreme increase in oxygen free radical production or deterioration in the antioxidant system may result in cytotoxic oxidative stress.[26]

TABLE I
ANALYSED PARAMETERS PRE- AND POST-TREATMENT, BOTH GROUPS

Parameter	Steroid + antioxidant group			Steroid group		
	Pre	Post	*p*	Pre	Post	*p*
Polyp score R	2.1 ± 0.9	1.5 ± 0.9	0.005*	2.6 ± 1.0	2.1 ± 1.2	0.029*
Polyp score L	2.3 ± 1.0	1.6 ± 1.1	0.008*	2.8 ± 0.9	1.8 ± 1.2	0.001*
CT score	15.3 ± 5.5	13.3 ± 6.1	0.007*	17.7 ± 4.9	15.8 ± 5.6	0.023*
Serum MDA (nmol/ml)	10.9 ± 1.9	8.1 ± 1.1	0.002*	11.7 ± 1.9	10.7± 1.5	0.028*
Serum SOD (U/ml)	36.8 ± 7.3	37.0 ± 11.1	0.224	35.5 ± 10.3	40.1 ± 5.2	0.231
Serum MPO (U/ml)	77.4 ± 28.1	85.9 ± 42.4	0.379	72.3 ± 35.3	73.2 ± 28.6	0.879
Serum NO_2 (μmol/ml)	47.6 ± 30.1	62.7 ± 74.8	0.679	51.9 ± 37.4	39.6 ± 19.2	0.236
Tissue MDA (nmol/g tissue)	3.5 ± 2.0	2.1 ± 1.2	0.003*	3.9 ± 1.4	3.8 ± 1.9	1.000
Tissue SOD (U/mg protein)	191.0 ± 65.7	200.7 ± 61.5	0.646	227.8 ± 124.0	236.6 ± 127.2	0.386

*$p < 0.05$, Wilcoxon test. Pre = pre-treatment; post = post-treatment; R = right; L = left; CT = computed tomography, MDA = malondialdehyde; SOD = superoxide dismutase activity; MPO = myeloperoxidase activity

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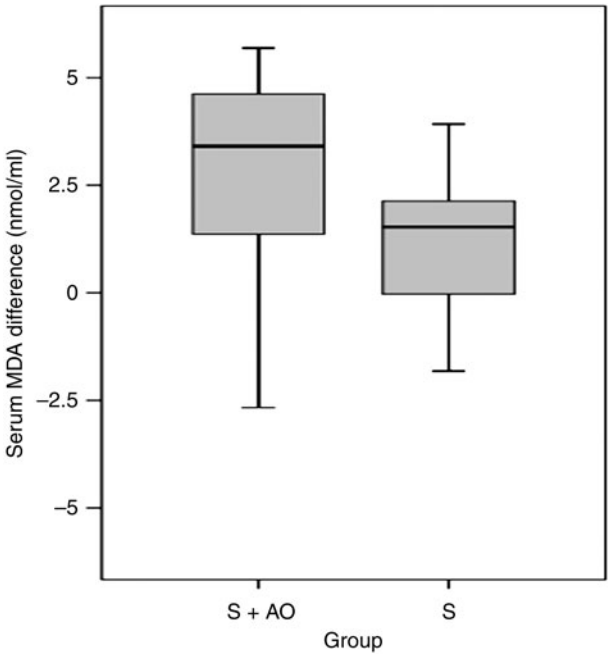

FIG. 1

Difference in mean serum malondialdehyde (MDA) level pre- *vs* post-treatment, for both groups. S + AO = steroid plus antioxidant; S = steroid

Oxygen free radicals are suspected of playing a major role in the pathogenesis of nasal polyposis. In this field, studies have focussed on investigating different oxidative stress markers and antioxidants, by assessing malondialdehyde, nitric oxide, adenosine deaminase, peroxynitrite, glutathione peroxidase, catalase, xanthine oxidase, superoxide dismutase, retinol, β-carotene, α-tocopherol and ascorbic acid, within blood and tissue samples.[1–12] The reported results of such studies have been similar, showing an increase in oxidative stress markers and a deterioration in antioxidant scavenging systems. These studies supply strong evidence that oxidative stress plays a role in the pathogenesis of nasal polyposis.

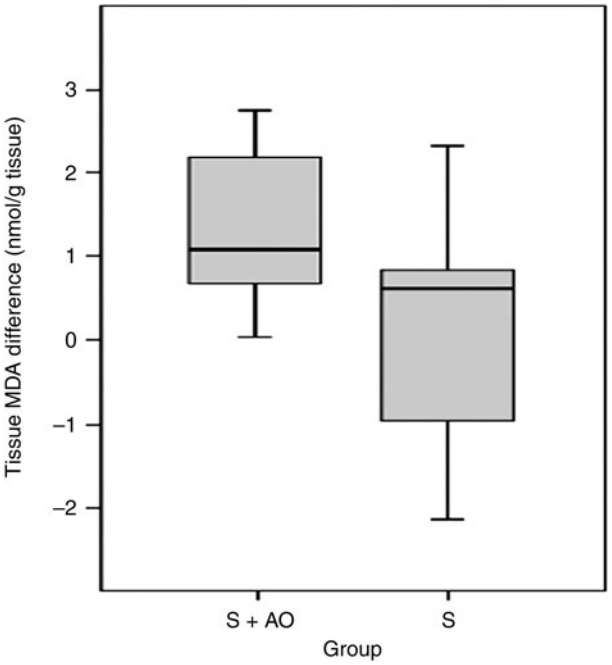

FIG. 2

Difference in mean tissue malondialdehyde (MDA) level pre- *vs* post-treatment, for both groups. S + AO = steroid plus antioxidant; S = steroid

Research on the pathogenesis of nasal polyposis has focussed mainly on mucosal oedema, which has been ascribed to an impaired ion transport mechanism within the nasal epithelium.[3] Dysfunction of ion transport pumps can result in intracellular oedema and deregulation of cell functions. Cochrane showed that oxidants impaired the cellular membrane of the ion transport pumps, resulting in an increase in intracellular sodium and a decrease in intracellular potassium.[27] Epithelial damage is important during initial polyp formation. Norlander *et al.* demonstrated that epithelial disruption may be essential for initial polyp formation in the sinus mucosa.[28] Dağlı *et al.* demonstrated that tissue damage related to free radicals occurs in cases of nasal polyposis; they speculated that oxygen free radicals lead to mucosal oedema in the early stage and to epithelial damage in the advanced stage.[3]

These studies have identified a significant association between oxidative stress and nasal polyposis. Some authors have hypothesised that antioxidants may have a preventive or therapeutic role in oxygen free radical mediated tissue damage. The present study aimed to investigate the therapeutic effects of antioxidant therapy on the clinical and biochemical outcomes of patients with nasal polyposis. Nasal or oral steroids are the main therapeutic agents used in the treatment of nasal polyposis.[29] Adding antioxidants to these treatment modalities may have a therapeutic effect. In the present study, both steroid and steroid plus antioxidant regimens resulted in diminished serum malondialdehyde levels and improved clinical polyp and CT scores. The post-treatment decrease in both serum and tissue malondialdehyde levels was significantly greater in the steroid plus antioxidant group than the steroid group, highlighting the systematic and local therapeutic effects of antioxidants.

Vitamin E is a major lipid-soluble antioxidant present in all cellular membranes, and protects against lipid peroxidation.[30] Vitamins A, E and C are antioxidants which can act directly against a variety of oxygen free radicals, including peroxy radicals, superoxide radicals and singlet oxygen.[30] Selenium, an essential component of glutathione peroxidase, is important in the decomposition of hydrogen peroxide and lipid peroxides.[30] In the current study, we found that adding antioxidant vitamins and selenium to steroid therapy may decrease lipid peroxidation in cellular membranes, and result in better malondialdehyde levels in both serum and tissue. We speculate that antioxidants may have a therapeutic role in limiting the

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epithelial damage phase of polyp formation, within the sinus mucosa.

- **Previous nasal polyp studies have indicated a relationship between oxidative stress and polyp pathogenesis**
- **This study investigated the effect of antioxidant therapy (plus standard therapy) on nasal polyps patients' clinical and biochemical outcomes**
- **Antioxidants produced reduced serum and tissue malondialdehyde levels, but no proven therapeutic benefit**
- **Antioxidants may have a future role in the prevention and treatment of nasal polyps**

These study results suggest a potential therapeutic biochemical effect of adding antioxidants to standard steroid therapy for nasal polyposis, although we could not identify a clear clinical difference based on polyp or CT scores. The small size of our patient sample represents an important limitation. Further studies could employ placebo groups, and investigate different antioxidants in different doses.

Conclusion

This was the first study to investigate the therapeutic effect of adding antioxidants to standard steroid therapy for nasal polyposis. Results indicated that antioxidants resulted in a better biochemical outcome as regards serum and tissue malondialdehyde levels. In future, antioxidants may play a major role in the prevention and treatment of nasal polyposis. However, more comprehensive clinical studies are needed.

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