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Iron homeostasis and methionine-centred redox cycle in nasal polyposis

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Abstract

Nasal polyposis is a multifactorial disease with a strong inflammatory component. Its pathogenesis is often associated with ROS production catalysed by redox-active iron. This study aimed to characterize the roles of iron homeostasis and redox status in the pathogenesis of polyposis. Nasal polyps (NP) from asthmatics and non-asthmatics and turbinates from controls and NP-patients were analysed for ferritin, ferritin-bound iron (FBI) and levels of methionine-centred redox cycle proteins. The ferritin content in both NPs was significantly higher than in adjacent turbinates. No differences in FBI were observed between both NP groups and both turbinates groups, while in NPs it was significantly higher. In NP-turbinates the highest levels of redox proteins were observed. In conclusion, re-distribution of iron occurs upon the development of NP. While FBI is elevated in NPs, the adjacent turbinate remain iron-poor and low-inflammatory, suggesting the formation of virtual boundary between these tissues.

Keywords: *Iron, ferritin, ROS, asthma, nasal polyposis, methionine-centred redox cycle*

Introduction

Chronic rhinosinusitis (CRS) is a common health problem in the western world. Nasal polyposis (NP), in which the nasal cavity is filled with oedematous masses of soft tissue originating from the sino-nasal mucosal lining, is considered to be the most severe form of CRS [1]. The majority of nasal polyps originate in the region of the middle nasal meatus and the ethmoid sinuses and they can spread throughout the nasal cavity. However, nasal polyps almost never originate in, nor do they involve the inferior turbinate or the inferior nasal meatus. The turbinates, located laterally in the nasal cavities, are composed of a thin bone covered by a thick, vascular, glandular and respiratory epithelial tissue layers. There are three turbinates on each side of the nose. The inferior turbinates are the largest turbinates and are responsible for airflow direction, humidification, heating and filtering of the inhaled air.

The pathogenesis of NP has not yet been fully understood and is considered to be multifactorial. In adults, NP is clearly associated with asthma, aspirin hypersensitivity and allergy. In children, it is mostly associated with cystic fibrosis (CF). Some evidence exists regarding the possibility of a genetic pre-disposition [2]. It has been suggested that polyps are formed at mucosa-to-mucosa contact points. A possible explanation could be that mucosal contact stimulates the release of pro-inflammatory cytokines from the epithelium [3].

Reactive oxygen-derived species (ROS), including free radicals (FR) can cause damage to vital biomolecules, often leading to loss of cellular function and death. A variety of ROS is present in biological

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systems and is actively involved in many physiological and pathological processes. The rate of FR-production is balanced under normal conditions by tissue defense mechanisms. However, during periods of oxidative stress, the levels of ROS may overwhelm the body immunity with subsequent damage to proteins, lipids, DNA and RNA, which in turn alter their biological activity and cause cellular damage [4,5]. Irreversible and irreparable carbonylation is one mode of protein modification caused by ROS. Carbonylated proteins tend to be more hydrophobic and resistant to proteolysis, forming high molecular weight cytotoxic aggregates [6]. Such aggregates have been found in asthma and in CF patients, where NP is common, and also in several other conditions [7].

Multiple enzymatic and non-enzymatic antioxidant defense systems actively protect the cell organelles from the injurious effects of ROS [8]. Any change in the delicate balance between oxidant production and local antioxidant defense can result in pathologic conditions. Indeed, ROS have been implicated as causative agents in many pathologic conditions [9]. A recent study has demonstrated that the development of NP may be associated with a general increase in cellular fluxes of ROS and is followed by elevation in oxidative stress markers such as malondialdehyde [10]. Moreover, a significant down-regulation of enzymatic and non-enzymatic antioxidants was detected in the serum and polyps of NP patients [10,11].

Methionine (Met) oxidation constitutes a front-line defense against oxidative stress. It is a unique amino acid bearing the $-S-CH_3$ group that can be reversibly oxidized by ROS, turning into the methionine sulphoxide residue $-SO-CH_3$; Met(O). The methionine sulphoxide reductase (Msr) family of enzymes, MsrA and MsrB, serve as catalysts in the reduction of Met(O) back to the reduced Met. Cells lacking MsrA [12] or MsrB [13] are more prone to ROS-induced damage, whereas over-production of MsrA has a protective effect against oxidative injury [14]. In the Methionine-centred redox cycle (MCRC), Msr activity is coupled to thioredoxin-1 (Trx), thioredoxin reductase-1 (TrxR) and NADPH. Trx and TrxR are also involved in a variety of other cellular protective processes against oxidative stress and inflammatory conditions [15,16].

Iron is an essential element in all tissues and cells. On the other hand, excess of labile iron is deleterious and causes cellular injury. This two phase behaviour is also shared by ROS. While low levels of ROS serve as beneficial signalling species, the nature and the amount of specific FR may cause damage at higher levels. Labile and redox-active iron, serving as a catalyst in the production of hydroxyl radicals via the Fenton reaction, is one of the key participants in ROS-induced injury, indicating the development of an inflammatory condition [17]. In a well-protected cell, 95–98% of iron is stored within ferritin, the major iron storing and detoxifying protein, which keeps labile and redox-active iron at sub-micromolar levels [18]. An increase in the amounts of cellular ferritin indicates an initial event where cellular levels of redox-active iron had increased and subsequently detoxified [19]. Various pathologic conditions, including asthma, have been proposed to be associated with an increase in cellular levels of iron and ferritin [20], presumably augmenting ROS production.

The purpose of the current study was to investigate the role of iron and its major storage protein, as well as MCRC proteins, in nasal polyps of asthmatic vs non-asthmatic patients.

Materials and methods

The study was conducted according to the guidelines established by the local Internal Review Board (Helsinki Committee).

Experimental design

Thirty-three patients undergoing nasal or sinus surgery at the Department of Otolaryngology/Head & Neck Surgery were prospectively recruited between July 2006 and March 2008. All patients suffered from perennial allergy, previously diagnosed at the Allergy Clinic (by patient history, skin prick and radioallergosorbent (RAST) testing). Non-allergic patients or patients suffering from seasonal allergy were excluded from the study. No patients suffered from any other systemic disease, none were hypersensitive to aspirin (according to patient history and to the data from the Allergy Clinic), none were smokers and none had been treated by antihistamines or by systemic steroids during the 6 weeks prior to surgery. Each patient underwent a thorough medical interview, a physical examination, which included anterior rhinoscopy and nasal endoscopy and a computed tomography scan.

Twenty-five patients suffered from CRS with NP according to the criteria established by the American Academy of Allergy, Asthma and Immunology and the Task Force on Rhinosinusitis; and the European Position Paper on Rhinosinusitis and Nasal Polyps [21,22]. These NP patients were operated on due to failure of maximal medical treatment (including local and systemic steroids). All NP patients suffered from total or near-total obstruction of both nostrils by polyps originating from the middle and superior nasal meati with involvement of all sinuses, thus receiving maximal endoscopic and Lund-Mackay scores [23,24]. In all NP patients the inferior turbinate, the inferior nasal meatus, the nasal floor and the nasal septum were not affected by the disease. Ten NP patients suffered from concomitant bronchial asthma (previously diagnosed by patient history, physical

examination and pulmonary function tests at the Pulmonology Clinic).

Eleven patients were operated on due to non-rhinologic diseases (endoscopic repair of a cerebrospinal fluid leak and endoscopic skull base surgery). Similar to previous studies, their turbinates served as controls [25]. Based on patient history, none of them has suffered from asthma. Since the nose and sinuses were normal, all controls received the minimal endoscopic and Lund-Mackay scores.

Tissue samples removed during surgery were then analysed. All assays were performed in a blinded fashion.

Samples preparation

Inferior turbinates from the control group ($n = 11$), inferior turbinates from non-asthmatic NP patients ($n = 8$) and nasal polyps from non-asthmatic ($n = 15$) and from asthmatic patients ($n = 10$) were removed and immediately stored at −80°C. Each sample was homogenized using a Cole Parmer Teflon homogenizer in a lysis buffer. Protein concentrations in the lysate were determined using a Bicinchoninic Acid Kit (Pierce, Rockford, IL, USA) in accordance with the manufacturer's instructions.

Ferritin concentration

Ferritin concentration was quantified using an indirect ELISA assay in accordance with a procedure developed previously in our laboratory [26]. All antibodies were provided by Dr A. Konijn (Department of Human Nutrition and Metabolism, Hebrew University, Jerusalem, Israel).

Ferritin saturation with iron

Equal volumes of sample and anti-ferritin antibody diluted with lysis buffer were mixed and incubated in a cold room for a period of 72 h. The samples were then centrifuged at 20 000 xg for 20 min, the supernatant was disposed of and the pellet was disrupted with 32% HNO_3 (J. T. Baker, Deventer, The Netherlands). The total amount of iron was measured using a Zeeman Atomic Absoption Spectrophotometer. The level of ferritin saturation by iron was calculated based on the previous measurement of ferritin concentration in the homogenate.

Msr enzymatic activity

Total Msr activity was measured as described by Moskovitz et al. [27]. The method is based on the reduction of Dabsyl methionine sulphoxide into Dabsyl methionine, which is determined by HPLC coupled with a spectrophotometric detector at 436 nm [28].

Measurement of MCRC proteins

MsrA, MsrB, Trx and TrxR were quantified using the Western blot techniques with some modifications [29]. The antibodies used in the MsrA and MsrB assays have been previously characterized [30,31]. For Trx and TrxR detection, the samples were treated with primary antibody (generously provided by Dr S. G. Rhee, Division of Life and Pharmaceutical Sciences, Ewha Womans University, Seoul, Korea) and secondary antibody (Zymed, CA). The membrane was exposed to a light-sensitive film. The bands were evaluated with a densitometer. The data is presented as ratios to β-actin.

Protein carbonyl content (PCC) assay

PCC value often serves as an indicator of the metabolic rate and as a measure for the production of ROS in tissues; 1 ml homogenates of samples (2–5 mg/ml protein) were mixed with 10 mM 2,4-DNPH (Sigma, MO) in 2 M HCl, two sample tubes and the same amount of 2 M HCl (Gadot, Haifa, Israel) for two control tubes. The mixtures were incubated in a dark place for 1 h, vortexing every 15 min. They were then diluted with 500 µl of 20% trichloroacetic acid (TCA) (Aldrich, WI), incubated on ice for 5 min and centrifuged at 10 000 xg for 10 min at 4°C. The supernatant was discarded; the pellet was suspended in 1 ml 10% TCA and placed on ice for 5 min. After centrifugation under the same conditions, the pellet was suspended in 1 ml of ethanol/ethyl-acetate 1:1 mixture, stored for 10 min and centrifuged. The pellet was suspended in 1 ml of 6 M guanidine-HCl (Bio-Lab, Jerusalem, Israel) in K_3PO_4 0.5 M pH 2.5 buffer and centrifuged under the same conditions. The absorbance of the supernatant at $\lambda = 370$ nm was measured spectrophotometrically and the amount of carbonylated proteins was calculated [32].

Statistical analysis

The data was analysed using Kruskal-Wallis test followed by one-way ANOVA test with the Scheffe post-hoc procedure for pairwise comparisons. Differences between mean values with a p-value of 5% or less were considered statistically significant.

Results

Iron status

Ferritin. Ferritin concentration in control samples (turbinate tissue) was 4.4-fold higher than the concentration in turbinates from NP patients ($p < 0.05$). No significant difference was found between ferritin concentrations in non-asthmatic NP and control turbinates. Asthmatic polyps demonstrated 2.8-times

increased ferritin levels when compared to non-asthmatic polyps ($p < 0.05$) (Figure 1A).

Ferritin saturation with iron. No significant differences were found in the degrees of saturation of ferritin with iron, within either turbinates groups (from controls or NP patients) or within the polyps groups (from non-asthmatic and asthmatic NP patients). Interestingly, the amount of iron per ferritin molecule in the nasal polyps was 1.8-fold higher than the amount in the turbinates ($p < 0.05$) (Figure 1B), which is in accordance with the inflammatory status of the polyps.

Cellular redox status

Methionine sulphoxide reductase enzymatic activity. The (total) enzymatic activity of methionine sulphoxide reductase (Msr) was measured. A 2.1-fold higher activity was obtained in NP-turbinates when compared to control turbinates. Also, the activity of Msr in the NP polyps was 1.8-fold lower than the NP turbinates ($p < 0.05$). The difference between either of the NP groups, asthmatic or non-asthmatic, and the control group, was statistically insignificant (Figure 2).

Thioredoxin 1, Thioredoxin reductase 1, MsrA and MsrB. The tissue protein level of Trx in NP turbinates was 2.3-fold higher than in the control turbinates. Likewise, the Trx level in NP turbinates was 1.4-fold higher than in non-asthmatic NP and 2.3-fold higher than asthmatic NP ($p < 0.05$, for both) (Figure 3A). The difference found between control turbinates and NP tissues is in accordance with a previous report by Lee et al. [33].

For TrxR, a 2.6-fold increase in TrxR protein level was observed in turbinates from NP patients compared to controls ($p < 0.05$) (Figure 3B). Conversely, the level in NP turbinates was 4.0-times higher than in asthmatic NP ($p < 0.05$) and 1.7-fold higher than in non-asthmatic NP ($p < 0.05$). No difference was found between TrxR levels in controls and in polyps from both groups (non-asthmatic and asthmatic NP), while a 2.4-fold increase was observed in non-asthmatic polyps when compared to asthmatic polyps ($p < 0.05$).

Western Blot analysis and specific antibodies to either MsrA or MsrB were employed for quantification of the amounts of the Msr isozymes. No significant difference was found in MsrA level between turbinates from NP patients and controls. Analogously, no difference was found between NP turbinates and non-asthmatic NPs. However, a marked down-regulation of MsrA was observed in asthmatic NP, when compared to the other groups ($p < 0.05$) (Figure 3C).

Consistent with the trend demonstrated by MsrA levels, no significant alterations were detected in MsrB concentration between both turbinates subgroups, either from NP patients and controls and non-asthmatic nasal polyps. Around a 6-fold decrease was found in asthmatic polyps when compared to non-asthmatics ($p < 0.05$) (Figure 3D).

Protein carbonyl content (PCC). Inferior turbinates, continuously exposed to inhaled toxicants from the environment, are considered to be under light-to-mild inflammatory state [33]. The level of PCC in control turbinates was 0.18 nmol/mg protein. Unexpectedly, the PCC level in NP turbinates was markedly lower

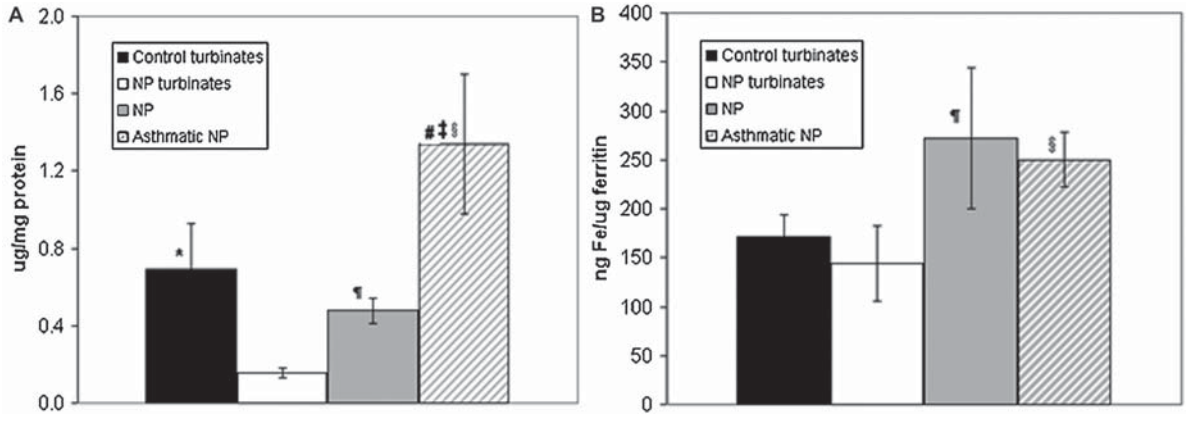

Figure 1. Ferritin concentration (A) and its saturation with iron (B). Ferritin concentration was measured using the ELISA-based 'sandwich' assay, developed in our laboratory. Briefly, the samples from four groups were incubated in 96-well plates pre-treated with goat antibodies against L-ferritin, then rabbit anti-H-ferritin antibodies were added. The second stage was followed by Chlorophenol Red-β-D-Galactopyranoside (Roche, IN) (CPRG)-conjugated goat-anti-rabbit antibodies incubation. The plates were scanned using a microplate reader with a test (570 nm) and reference (630 nm) filters. The results are shown in μg/mg protein. Means ± SE are shown; $^*p < 0.05$ between the turbinates sub-groups; $\#p < 0.05$ between polyps (NP) sub-groups; $¶p < 0.05$ between the turbinates from NP patients and non-asthmatic polyps; $§p < 0.05$ between the turbinates from NP patients and samples from asthma-associated polyps; $‡p < 0.05$ between control turbinates and polyps from asthmatic patients. The differences between the four groups were $p = 0.0001$ (A) and $p = 0.0001$ (B) (ANOVA).

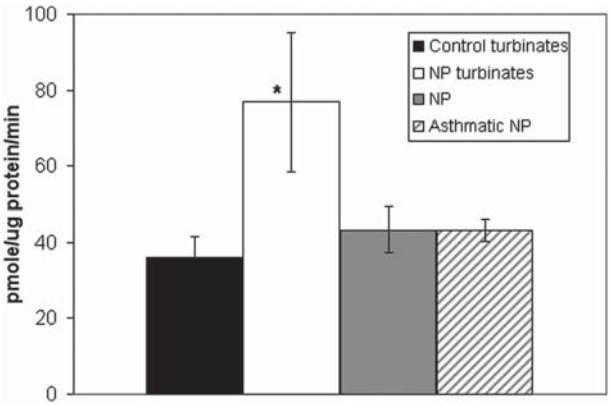

Figure 2. Methionine sulphoxide reductase enzymatic activity. Msr activity was assayed by reducing Dabsyl methionine sulphoxide (substrate) into Dabsyl methionine, which was then determined with HPLC coupled to spectrophotometric detection at $\lambda = 436$ nm. The results are expressed in pmole/µg protein/min. NP, nasal polyps; Control turbinates were obtained from patients operated on due to non-rhinologic diseases; Means ± SE are shown. $^{*}p < 0.05$ between the turbinates sub-groups. The difference between the four groups was $p = 0.001$ (ANOVA).

(0.09 nmol/mg protein) indicating a prominently lower inflammatory status in these tissues, albeit the fact that they are found adjacent to the NP polyps. This low PCC level in NP turbinates hints towards unique characteristics of NP turbinates when compared to other tissues.

A 2.3-fold increase in PCC value was observed in asthmatic nasal polyps compared to control turbinates and a 1.6-fold increase compared to non-asthmatic NP (Figure 4).

Discussion

In this study we investigated biochemical aspects related to the cellular redox status and iron homeostasis in inflammatory airway pathologies, mainly NP, the most severe form of CRS and its interaction with asthma.

There is a well-established association between asthma and CRS with NP [34]. Asthma and CRS co-exist at a higher frequency than would be expected from the prevalence of each disease separately. The prevalence of asthma in the general population in the US is ~5%. Conversely, 20% of patients with CRS suffer from asthma [34]. This rate rises to more than 40% in patients who require functional endoscopic sinus surgery for the treatment of CRS [35]. Among patients who suffer from CRS with NP, at least 50% suffer from asthma as well [21].

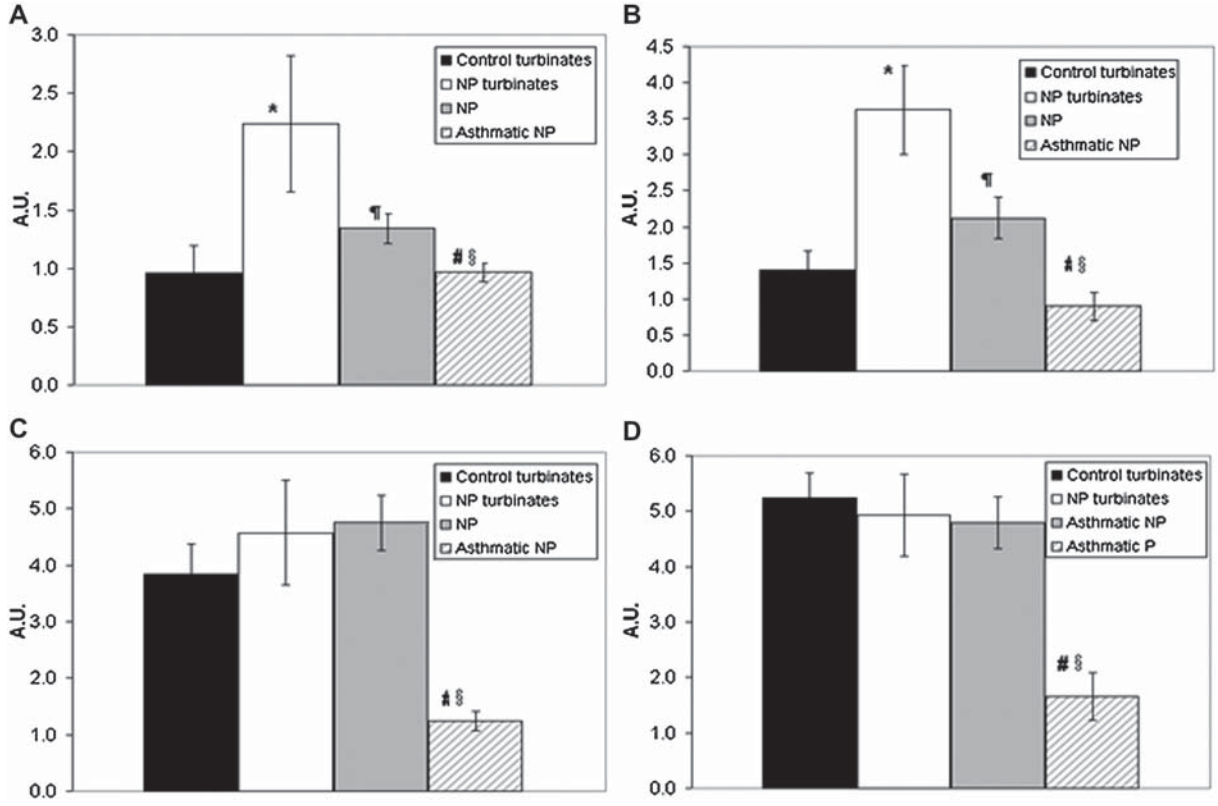

Figure 3. Amounts of Trx (A), TrxR (B), MsrA (C) and MsrB (D). Samples from four groups were run on SDS-PAGE, transferred to nitrocellulose membranes and then exposed to the antibodies against the respective protein. This exposure was followed by incubation with secondary antibodies, conjugated to HRP (horseradish peroxidase). The graphs represent densitometry data normalized per β-actin. The results are shown in AU (arbitrary units). NP, nasal polyps; Control turbinates were obtained from patients operated on due to non-rhinologic diseases. Means ± SE are shown. $^{*}p < 0.05$ between the turbinates sub-groups; $\#p < 0.05$ between polyps (NP) sub-groups; $\P p < 0.05$ between the turbinates from NP patients and non-asthmatic polyps; $\S p < 0.05$ between the turbinates from NP patients and samples from asthma-associated polyps. The differences between the four groups were $p = 0.047$ (A), $p = 0.048$ (B), $p = 0.005$ (C), $p = 0.005$ (D) (ANOVA).

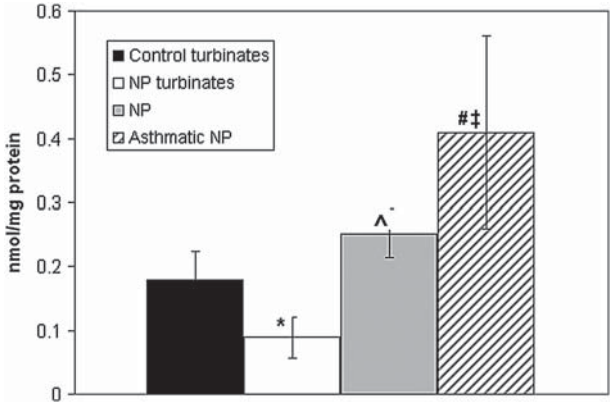

Figure 4. Protein carbonyl content. Protein samples were derivatized by making use of the reaction between 2, 4-dinitriphenylhydrazine (DNPH) and protein carbonyls. Formation of the Schiff base produced hydrazone that was analysed spectrophotometrically at $\lambda = 370$ nm. The carbonyl content (nmol/mg protein) was then calculated. NP, nasal polyps; Control turbinates were obtained from patients operated on due to non-rhinologic diseases. Means $\pm$SE are shown. $^{*}p < 0.05$ between the turbinates sub-groups; # $p < 0.05$ between polyps (NP) sub-groups; $\hat{}p < 0.05$ between control turbinates and non-asthmatic polyps; ‡$p < 0.05$ between control turbinates and polyps from asthmatic patients. The difference between the four groups was $p = 0.0001$ (ANOVA).

According to the 'one airway, one disease' model [36], there are overwhelming similarities between the upper and lower airways. The nose, sinuses and bronchi are all linked anatomically and are lined with the same pseudo-stratified respiratory epithelium, possessing similar innate and acquired immune mechanisms of defense. Interactions between the upper and lower airways occur via several pathways, including neural reflexes and vascular mechanisms. In addition, the same triggers, whether viral, irritant or allergenic, may lead to the onset of both CRS and asthma. Possibly, the same genetic factors play a role in the manifestation of sino-nasal and/or bronchial diseases. The higher co-morbidity of asthma and CRS and the fact that treatment of CRS shows positive effects on asthma control support this model [36].

ROS constitute major contributors to oxidative stress. Often labile iron serves as a potent catalyst for the production of highly reactive free radicals. These are involved in a broad variety of acute and chronic inflammatory processes, including asthma [20] and NP (current study). Generally, accumulation of iron in a tissue may serve as an indication for an ongoing inflammatory process [37]. The level of ferritin, the main cellular iron storage and detoxifying protein, and the amount of ferritin-bound-iron (FBI) enable evaluation of the cellular iron status.

A remarkable increase ($\times$2.8, Figure 1A) in ferritin concentration was found in asthmatic compared to non-asthmatic polyps. However, the FBI values in nasal polyps and NP of asthmatics were similar. These findings indicate that the total ferritin iron is higher in asthmatic NP. Ferritin levels in turbinates (either controls or those adjacent to NP) were markedly lower than in nasal polyps, strongly indicating that NP involves a major inflammatory component, most prominent in asthmatics. This finding is in accordance with the current literature [34].

An unexpected observation in this study is that the levels of ferritin (with the same FBI) and consequently the total amount of cellular ferritin-iron are markedly lower in turbinates which are found near NPs when compared to all other groups. These cellular iron levels are much lower than in the turbinate controls and more prominently lower than in their adjacent NP tissue. These seem to indicate that during the development of the NP, by a yet unknown mechanism, the NP recruits iron from neighbouring cells for the persistent inflammatory process and establishes an iron-concentration gap between the NP and its adjacent turbinate, with a virtual barrier between them. The decreased level of ferritin has to be associated with down-regulation of this protein. According to Oliveira et al. [38] we may assume that the sizeable augmentation in the concentration of Trx observed in NP turbinates can induce binding activity of the Iron Regulatory Protein-1 (IRP-1), thus leading to a lower tissue ferritin concentration. Whether this recruitment represents an initial step in the pathophysiology of NP or whether it follows the initiation of the inflammation needs further investigation.

Redox status and iron homeostasis in issues are strongly coupled to each other and could affect the cellular potential to counteract or support free radical formation and the consequent injury. Here, we examined the redox status by monitoring the protein levels and activity of components of the methionine centred redox cycle proteins (MCRC), including methionine sulphoxide reductase (Msr—MsrA and MsrB), thioredoxin (Trx) and its reductase (TrxR). The roles of these enzymes in the ageing process and in various pathologies has been extensively studied and published [39]. Recent publications reported a variety of changes in ROS-related proteins parameters in NP, which did not culminate into a clear mechanism. These include a decrease in glutathione peroxidase in the NPs, unchanged levels of superoxide dismutase (SOD) and elevation of xanthine oxidase in NPs [10,40]. It is noteworthy that these results are not contradictory to ours.

The concentrations of Trx and TrxR, as well as the total Msr enzymatic activity, were significantly elevated in NP turbinates compared to either control turbinates or to NPs (Figures 2 and 3). This finding may indicate an increased demand for protection against oxidative stress, specifically in NP-turbinates, and is in agreement with the hypothesis that a barrier had been established around the nasal polyps. Since hydrogen peroxide can migrate freely between cells, these elevated levels may represent an increased oxidative stress originating from the nasal polyps and spilling over to the adjacent turbinates.

The levels of the MCRC proteins in asthmatic nasal polyps were markedly lower than in other tissues (Figures 2 and 3). This finding is in accord with recent reports showing that asthma exacerbation leads to elevation in Trx concentration [41] and that administration of exogenous Trx is beneficial for asthmatic patients [42,43]. Rapid degradation of Trx, under the continuous severe inflammatory state present in asthmatic NP, may account for these markedly lower levels of MCRC proteins.

Figure 2 depicts the combined activities of both isoforms of Msr—MsrA and MsrB. As shown, the activity is the same in non-asthmatic and in asthmatic NP tissues, while the levels of both proteins, as determined by Western blot analysis, are lower in asthmatic NP (Figures 3C and D). These discrepancies can be explained by the fact that asthmatic nasal polyps constitute severely inflamed tissues, characterized by a high rate of leucocytes infiltration, especially of neutrophils, accounting for about half of the total cells of the polyp [44–46]. Neutrophils have been shown to possess the Msr proteins, but with a specific activity considerably higher than in other cells from the same organism [47,48]. Also, the Msr proteins in these tissues may undergo some post-translational modifications, due to the high inflammatory state, and, therefore, high demand for repair of ROS-induced damage.

The PCC level of NP-turbinates was markedly lower than in control turbinates. This observation is in agreement with the lower level of iron found in this tissue and is evidently a consequence of lower fluxes of ROS, including free radicals, in NP-turbinates. As expected, the PCC value in NPs, an inflamed tissue with elevated fluxes of ROS, is high and even higher in the more severely inflamed asthmatic-NPs. These results are compatible with the differences in Trx and TrxR levels and in Msr total enzymatic activity.

In a previous preliminary study on CRS patients (unpublished data) we found that many of our NP patients suffer concomitantly from perennial allergy. Thus, including only patients suffering from perennial allergy in the current study has prevented considering allergy as a confounding factor. Due to ethical and medical considerations we could not collect turbinates from asthmatic-NP patients. Obviously, it would be interesting to characterize such a tissue and to investigate whether or not it also supports the suggestion of a barrier around asthmatic NPs. Another limitation of our study is the relatively small study groups. However, we were able to perform statistical analysis in all comparisons.

Conclusions

Re-distribution of iron occurs upon the development of nasal polyps. While iron content is elevated in NPs the adjacent tissue (turbinate) remain iron-poor and with low inflammation, suggesting the formation of virtual boundary between these tissues. These processes are in accord with the severe inflammation present in asthmatic-NPs.

Declaration of interest

This study was supported by research grants from Dr Avraham Moshe and Pepka Bergman Memorial Fund, from the Israel Science Foundation (316/05) and from Joels Landovsky Fund at the Hebrew University of Jerusalem. M. Chevion is the incumbent of the Dr William Chair of Heart Studies at the Hebrew University of Jerusalem.

No competing financial interests exist.

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