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Cytomorphologic and ultrastructural study of nasal mucosa by means of brushing: a comparison between asymptomatic and rhinitic subjects*

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SUMMARY

The authors compared the cytomorphologic and ultrastructural features of nasal epithelium collected by means of brushing from asymptomatic subjects with these of patients affected by nasal polyposis (NP), allergic (AR) and non-allergic rhinitis (NAR). A brushing of nasal epithelium was taken from each member of both groups, and analysed under light and electron microscopy. The observation showed normal ciliary patterns and preserved intercellular joints in the great majority of asymptomatic subjects, while in all subjects of the pathologic group the junctions appeared variously damaged or absents, with ciliary abnormalities. The damage to the intercellular joints, rather than the alterations of ciliary patterns, seemed to represent in this study the border between the absence of symptoms and the presence of chronic inflammation. Therefore, a reduced tightness of the intercellular joints could contribute to the impairment of the mucociliary clearance, priming the vicious circle that leads to the condition of chronic inflammation.

Key words: brushing, nasal mucosa, intercellular joints, nasal polyposis, rhinitis

INTRODUCTION

Since many years the cytological examination of nasal secretions has been included among the laboratory diagnostic tests, and performed with various methodologies (blowing, washing, scraping), with different results in terms of reliability and information obtained. By means of electron microscopy the study of nasal mucosa can be extended to the epithelial cytostructure, with a detailed depiction of the thinnest ultrastructural components (cytoplasm and organules, cilia, intercellular junctions) [1].

In the literature many methods for sampling tissue fragments of nasal mucosa for an ultrastructural study (scraping, biopsy, imprints) have been reported, their application being extremely dependent on the invasiveness of the chosen technique.

In 1981 Rutland et al. [2] reported the use of a brush for "scraping" a small amount of nasal mucosa to obtain, by means of light microscopy, detailed morphostructural information about the epithelium. The presence of cilia and their aspects together with the degree of reciprocal adhesion of the cells included in the sample was analysed. Gilain et al. [3] also managed to evaluate the different cellular components involved in inflammatory processes of nasal mucosa, while Grygorczyk [4] adopted this sampling technique to avoid the use of proteolytic enzymes to isolate cells. Among these mentioned, the "brushing" technique has proven to be the most tolerated by patients, and has been largely used to collect tissue fragments to be histologically examined [5-10].

In collaboration with the Department of Biomedical Sciences of our University we developed a system of storage of the sampled material, using fixing procedures and preserving in resine techniques that allowed us to keep bioptical fragments in an unchanged state for long periods of time, ready for further evaluations under light and electron microscopy.

The aim of the study was to evaluate the cytological and ultrastructural features of samples of nasal mucosa, collected by means of brushing from a group of asymptomatic subjects, to compare their various cytomorphological parameters with these of patients affected by nasal polyposis (NP), and rhinitis (R) of allergic (AR) and non-allergic (NAR) origin.

MATERIALS AND METHODS

Forty subjects (20 males, 20 females, mean-age 34.3 years) were included in the study, as a reference group. All subjects reported no exposure to irritants or pollutants in their living or working environment, and no history of recent or previous nasal pathologies. No nasal respiratory difficulties were referred, skin PRICK and RAST tests for allergens were negative, and anterior rhinoscopy and nasal endoscopy were normal.

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All subjects were submitted to active anterior rhinomanometry (AAR) and the mucociliary transfer time (MCTt) was calculated, as described by Passali et al. [11]. An inactive tracer (vegetable charcoal powder) and a water-soluble substance (saccharin powder at 3%), were placed on the heads of inferior turbinates, and both the dust transit time at oro-pharyngeal level (normal values: < 10-14 min.), and the time needed to stimulate the sweet taste perception (normal values: < 11-20 min.) were recorded.
The clinical normality of this group was thus assessed either objectively (anterior rhinoscopy, nasal endoscopy), or functionally (AAR), using the following criteria:

- absence of symptoms
- negative skin PRICK and RAST for allergens
- aspect and degree of mucosal trophism
- absence of secretions
- absence of nasal polyps.
- Total nasal resistance at rhinomanometry: < 0,30 Pa/ml/sec. [12].
- mucociliary transfer time

All members of this group satisfied the parameters considered, but one in which the MCTt was increased. This group was thus considered suitable as reference for our study.

We also examined 28 patients (17 males, 11 females, mean-age 42.2 years), with endoscopic evidence of NP confirmed by CT scans, 9 of them with associated allergy confirmed by skin PRICK and RAST tests, and 18 patients (12 males, 6 females, mean-age 35 years), with diagnosis of chronic rhinitis, 9 of non-allergic (NAR) and 9 of allergic (AR) origin, depending on the response to skin PRICK and RAST tests.
Following a pharmacological wash-out of at least 30 days of topic or general treatment (steroids, antihistamines, cromones), all patients of the second group were submitted to AAR, which was altered in all patients with NP and NAR and in 16 out of 18 patients with AR; MCTt was increased in all patients with NP and with NAR, and in 7 out of 9 patients with AR, the 2 remaining allergic patients with normal MCTt and AAR being sensitive to seasonal pollens, not present in the environment at the time of the study. Therefore, AAR and MCTt appeared to be reliable tests to differentiate the normal subjects from the pathologic ones (Table 1).
All subjects who entered the study were asked to sign a form in which the aim of the study and the relative procedures were explained.

From each member of both groups a sample of nasal tissue was taken by brushing (GIMABRUSH), from an accessible zone of nasal mucosa, between the floor of the nasal fossa and the septum. In all cases the procedure was well tolerated, and only in one case did the brush cause slight bleeding, easily stopped by compressing the nose wings for a few minutes. The samples were immediately collected in a test-tube, fixed with glutaraldehyde at 2.5 % and osmium tetraoxide at 1% and dehydrated in conformity with the alcohol growing series. After being passed in propilene oxide, the sample was included in durcupan resin. Half-thin sections (1 micron thick) were prepared, stained with toluidine blue and observed under light microscopy.
To evaluate the percentage of cells found in the samples the sections were then submitted to a morphometric analysis; for each subject 10 cover glasses were harvested, and the cells counted in 10 fields per cover glass.
Eventually, ultra-thin sections of the sample were obtained with an Ultrakut ultramicrotome and analysed under electron microscopy (Electronic Microscope 902, Zeiss), after contrast with uracile acetate and lead citrate.

RESULTS

At the light microscope level, after blue toluidine staining, the following cytological parameters were considered:

- cellular lines
- cellular homogeneity
- reciprocal cellular adhesion
- presence of cilia and their appearance

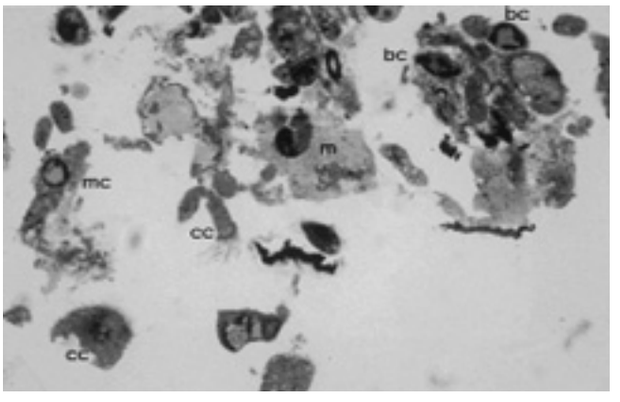

Figure 1. Light microscopy: groups of cells joined in “clusters”, expression of a well represented junction ciliated cells (cc), mucous cells (mc), and blood cells (bc). At the center of the picture a mucous aggregate (m) is evident. (25x).

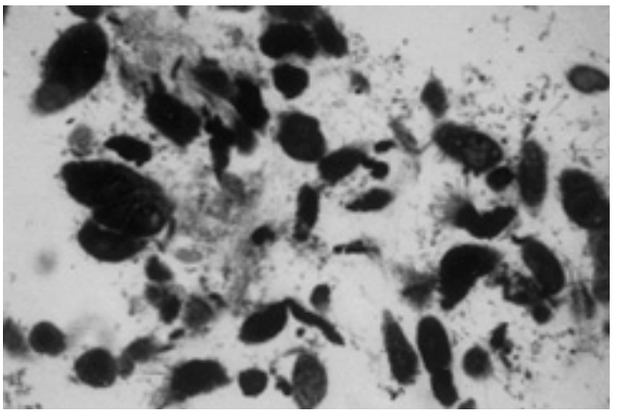

Figure 2. Due to the impairment of intercellular joints, the cellular clusters appear disjointed.

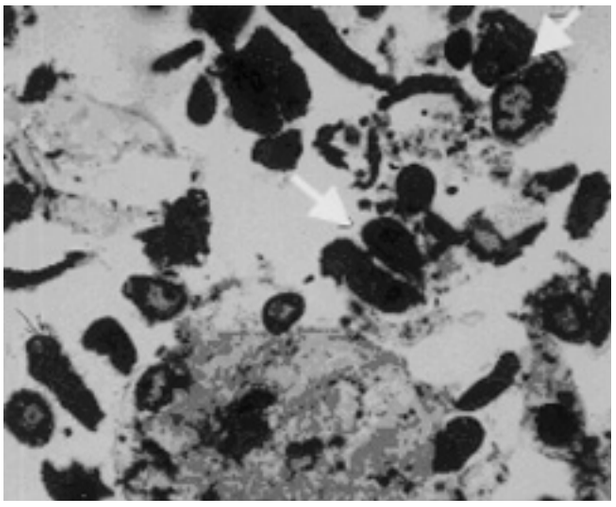

Figure 3. Light microscopy: good cellular adhesion (arrows). A reduction in the quantity of cilia is evident.

The great majority (39 out of 40 = 99 %) of asymptomatic patients (the reference group), according with data published by Nazer et al. [13] showed: a distinct cellular polymorphism, with a good representation of ciliated cells (65%), mucous cells (20%), basal cells (5%), macrophages (5%) and lymphocytes (5%); presence of cilia: ciliated cells appeared globous, with distinct nucleus and evident cilia at their top; cellular adhesion: we observed groups of cells joined in "clusters", expression of a well represented junction apparatus, as confirmed later under electron microscopy; strong basophily: due to the presence of large mucus granules within the mucous cells, a sign of secretory activity (Figure 1).

In the pathologic group, the cellular clusters above described appeared disjointed, with a marked cellular hypercromia; these findings were attributed to, respectively, an impairment of intercellular joints and a mucous hypersecretion, typical of inflammatory activity (Figure 2).

As was not expected, though with a good cellular adhesion, a reduction in the quantity of cilia was found in one subject (Figure 3), at first enrolled (in accordance with the listed criteria) in the asymptomatic group. We must stress that this subject, among those included in our study, was the only one who had spent many years in a polluted area.

The ultra-structural patterns of the same specimens were then analysed under electron microscopy.

The intercellular joints of the control group were well represented in all cases observed (Figure 4). They appeared as desmosomes, formed by a symmetric overlapping of cytoplasmatic membranes belonging to contiguous epithelial cells.

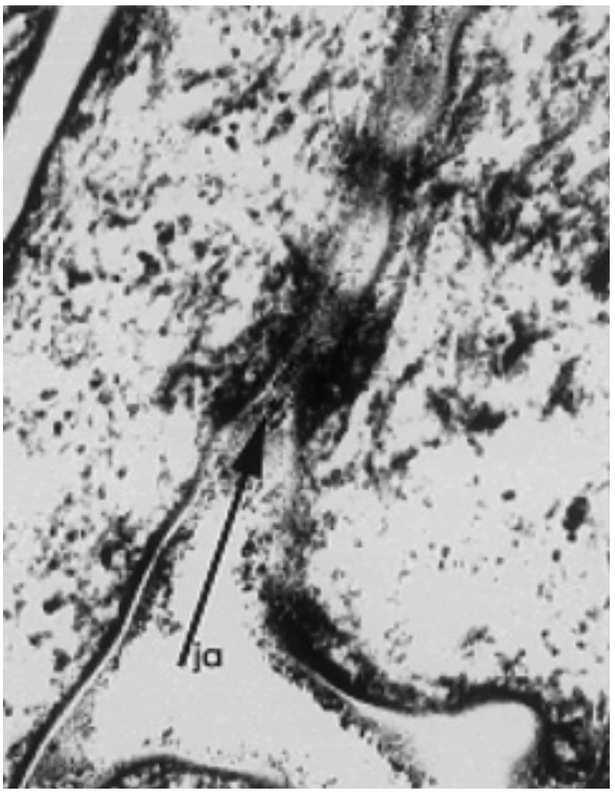

Figure 4. A normal and well represented junctional apparatus (ja) is evident. The electron microscope shows the desmosomial apparatus and the macula occludens. (12.000x).

The electron microscopy confirmed the ciliary impairment in the subject of the control group (Figure 5).

In all subjects belonging to the second group the ultra-structural analysis showed:

- A marked cellular disjunction (Figures 6 and 7) through damage or disappearance of intercellular joints with increased intercellular space. This was particularly evident for mucous cells during the secretory phase, as indicated by the increased number of secretory vesicles. Cilia abnormalities with decreased, compounded and cut cilia (Figure 8).
- Mucous hypersecretion, together with a reduced number of cilia on the cellular surface, may account for the delayed MCTt found in all patients of this group.

Table 1. Percentage of positive results of Active Anterior Rhinomanometry (AAR) and of Mucociliary Transfer Time (MCTt); only in two cases of AR were both tests negative, while in all patients with NP and with NAR both tests were 100% positive.

TEST (Normal values)	NP (28 patients)	AR (9 patients)	NAR (9 patients)
AAR (< 0,30 Pa /ml/sec)	28 > 0,30 Pa /ml/sec =100%	7 > 0,30 Pa /ml/sec = 77%	9 > 0,30 Pa /ml/sec = 100%
MCTt (<10-14 min.)	28 > 15 min.=100%	7 > 15 min =77%	9 > 15 min =100%

DISCUSSION

The junctional apparatus of nasal mucosa acts as a defence barrier for subepithelial structures, such as the endothelium and the endings of the trigeminal nerve. A damaged epithelium, such as that observed in the pathologic group of our series, is no longer capable of offering resistance to irritating agents (atmospheric pollutants, viruses, bacteria, fungi, allergens). This may expose the endothelium and trigeminal C fibres [14], with release of CGRP (Calcitonine Gene-Related Peptide) and P substance [15] at the nervous endings, and neurogen inflammation following [16]. The resulting increase in capillary permeability may start the symptoms of nasal hyperreactivity (sneeze, watery rhinorrea, nasal obstruction).

Elwany [17] found that the most important difference between normal subjects and patients with AR and NAR was a decreased number of intercellular gap junctions. Thus one can hypothesise that the increased permeability of nasal epithelium in chronic rhinitis may be due to a selective damage to the intercellular joints rather than to functional alterations, as reported by Jin et al. [18]. In a recent paper, in fact, Wan et al. [19] postulate that the development of asthma in patients sensitive to Dermatophagoides may be caused by direct damage to the intercellular tight junctions by a proteinase of environmental origin.

In a study on patients with cystic fibrosis, besides the reduction in the number and conformation of cilia, Carson et al. [20] demonstrated the disappearance of intercellular junctions of nasal epithelium. These findings indicate that there are acquired structural lesions that could derive from chronic inflammation and/or from the host response, that further alter the systems of ionic transport and then cause a reduction of ciliary function.

In our series the damage to the desmosomes of the nasal epithelium, rather than the alterations of ciliary patterns, indicated the border between normality and chronic inflammation. The integrity of the junctions may thus be the critical point for the chronicisation of some pathological conditions, such as those examined in our study. It has, in fact, been hypothesized [21] that ciliary dyskinesia, so far considered as the primum movens in nasal chronic diseases, could not be the only or the principle cause of chronic rhinosinusitis. On the other hand, a damaged epithelial component, such as a reduced tightness of the intercellular joints, could contribute to the impairment of the mucociliary clearance, starting the vicious circle that leads to chronic inflammation.

CONCLUSIONS

The examination of the samples under electron microscopy was particularly aimed to evaluate the intercellular junctions during chronic rhinosinusitis. The junctions appeared preserved in the great majority of asymptomatic subjects. By contrast, they seemed to be variously damaged or absent in all subjects of the pathologic group. This was frequently associated with a decrease in number, or even disappearance, of the cilia. In this group the damage to the junctional apparatus was constantly linked to symptoms of rhinitis or to the presence of polyps. It is significant, in our opinion, that one subject, though showing clear abnormalities (reduction of cilia, delayed MCTt), had been included in the reference group because of the absence of symptoms and signs of chronic rhinitis. This might be due to the integrity of his junctional apparatus, as emerged from ultrastructural observations.

In conclusion, this technique of sampling and studying the nasal mucosa permitted us to build a morphostructural guideline, to which different nasal pathologies could be referred. This quick, clear, safe and harmless sampling procedure may be of use to verify the presence or absence of those ultrastructural “markers” that, in our series, seemed to characterise some of the most frequent nasal pathologies.

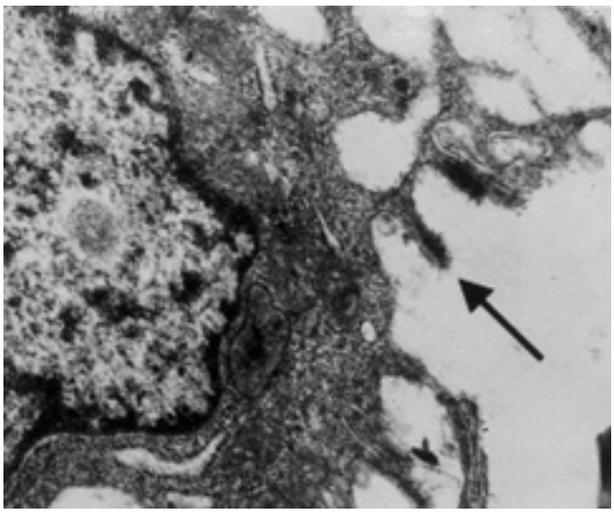

Figure 5. Electron microscopy: a damaged ciliary pattern (arrow). (12.000x).

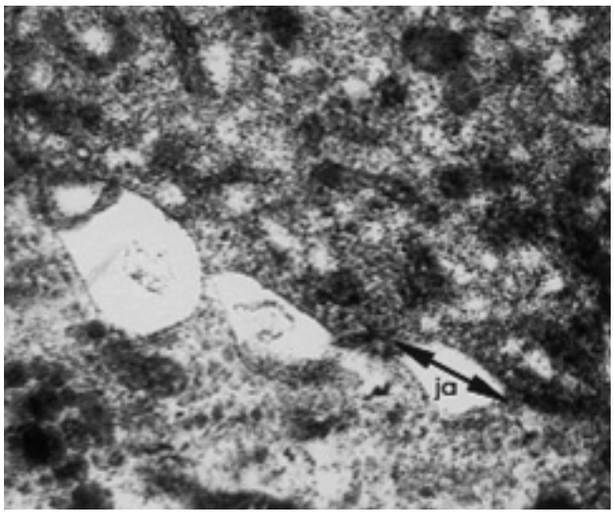

Figure 6. Electron microscopy: increased intercellular gaps among junctional apparatus (ja) (12.000 x).

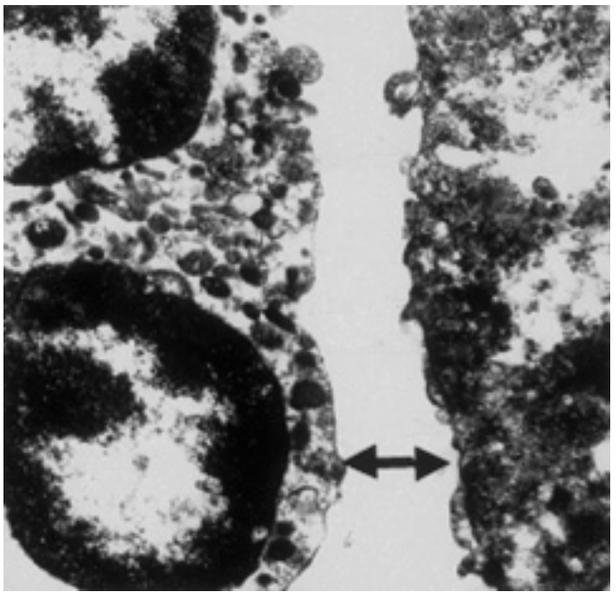

Figure 7. Electron microscopy : the alteration of cellular junctions with increased intercellular space are evident (double arrow) (20.000x).

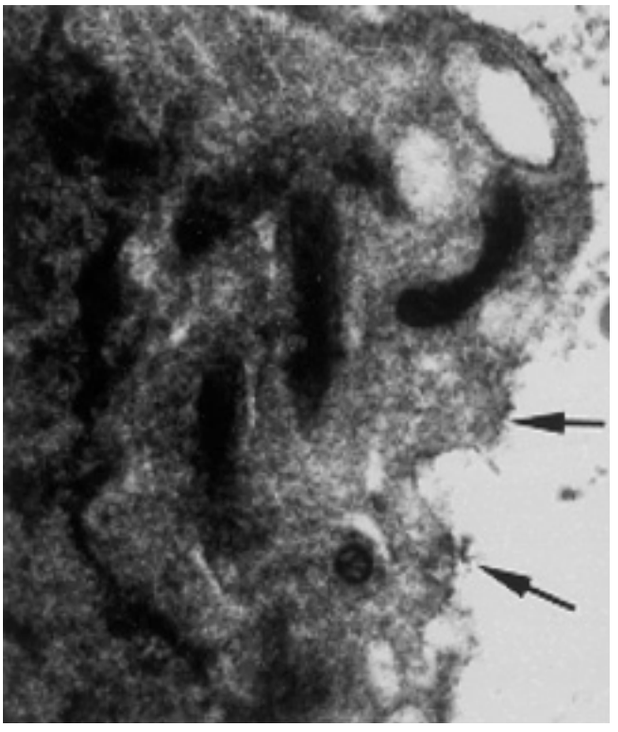

Figure 8. Electron microscopy: anomalies and disappearance of cilia (arrows) (12.000x).

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