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Rosso, Marinela; Prgomet, Drago; Marjanović, Ksenija; Pušeljić, Silvija; Kraljik, Nikola

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HEAD AND NECK

Pathohistological changes of tracheal epithelium in laryngectomized patients

Marinela Rosso · Drago Prgomet · Ksenija Marjanović · Silvija Pušeljić · Nikola Kraljik

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Abstract Total laryngectomy results in a permanent disconnection of the upper and lower airways. Thus, the upper airways are bypassed and can no longer condition, humidify, and filter the inhaled air, leading to damage of the tracheobronchial epithelium. There is little scientific information available about the effects of tracheostoma breathing and the degree of mucosal damage in laryngectomized patients. The aims of this study were to determine the histopathologic findings and investigate the potential impact of using a heat and moisture exchanger (HME) on the tracheal epithelium in long-term tracheostomy patients. Tracheal mucosal biopsies were taken from a total of 70 patients. Specimens were stained with hematoxylin and eosin and examined by a light microscope. Normal pseudostratified ciliated columnar epithelium was found in only 9 (12.9 %) cases; while, 17 (24.3 %) cases had some degree of basal cell hyperplasia. Squamous metaplasia was the most common finding (50 %). Pre-invasive lesions (mild and moderate squamous dysplasia) were found in only one patient who used an HME, and in eight (11.4 %) non-users. Although the HME cannot completely restore the physiological functions of the upper respiratory track, it delivers a better quality of air to the lower airways and has a positive effect on tracheal mucosa.

Keywords Laryngectomy · Heat and moisture exchanger · Pulmonary rehabilitation · Trachea

M. Rosso (✉)
Department of Otorhinolaryngology and Head and Neck Surgery, University Hospital Center Osijek, J. Huttlera 4, 31 000 Osijek, Croatia
e-mail: rossom@net.hr

D. Prgomet
Department of Otorhinolaryngology and Head and Neck Surgery, University Hospital Center Zagreb, Zagreb, Croatia

K. Marjanović
Institute for Pathology and Forensic Medicine, University Hospital Center Osijek, Osijek, Croatia

S. Pušeljić
Clinic for Pediatrics, University Hospital Center Osijek, Osijek, Croatia

N. Kraljik
Institute of Public Health for the Osijek-Baranja County, Osijek, Croatia

Introduction

Under intact anatomical and functional conditions, the upper airway provides a passage for air to be breathed in and out of the lungs; heats, humidifies, and filters the air, and is involved in coughing, swallowing, and speech [1]. Total laryngectomy results in a permanent disconnection of the upper and lower airways; thus, the upper airway is bypassed, eliminating its multiple functions [2]. Patients then breathe through a tracheostoma, and the tracheal epithelium becomes directly exposed to unprepared ambient air. During nasal breathing, inhaled air with a temperature of 22 °C and 40 % relative humidity is conditioned by the upper airways to reach a temperature up to 32 °C and relative humidity up to 99 % at the level of the trachea. The same air inhaled through a tracheostoma reaches a temperature of 27–28 °C and 50 % relative humidity [3]. This results in drying out of the tracheal and bronchial epithelia, which responds by increasing the production of mucus. This increase, together with the greater viscosity of the mucus, may result in mucus plugs or crusts as the mucus desiccates. The normal mucociliary clearance

mechanism is also disrupted. This loss of heat and moisture from the respiratory mucosa has the potential to damage the respiratory epithelium [4, 5].

At this time, the only effective non-pharmaceutical method for pulmonary rehabilitation following total laryngectomy is regular use of a heat and moisture exchanger (HME). The HMEs are designed to help laryngectomees regain some of the lost functions of the upper respiratory tract, including warming and humidifying the inspired air and removing particles [6]. The HMEs are synthetic open-cell foam discs, impregnated with hygroscopic salt, to increase the humidifying power, and an antibacterial substance. Filters are placed over the tracheostoma by peristomal adhesion or using a laryngeal tube, in a special holder, and should be replaced at least once a day. During expiration, air passes through the filter, where it deposits some of its moisture and heat. During inspiration, the air passes back through the filter, picking up the previously deposited moisture and heat [7]. Tracheostoma breathing leads to approximately 500 ml of water loss daily, but it is possible to retain 250–300 ml of this loss using an HME in the respiratory system [8]. Furthermore, an HME can filter out particles to clean inspiratory air. Breathing through the filter also increases airway resistance, thereby improving ventilation [9]. Daily consecutive use of an HME contributes to a healthier respiratory tract, restores airway resistance, and helps to maintain optimal lung ventilation. The use of HMEs for laryngectomized patients also has a positive effect on psychosocial functioning and quality of life [10]. The objectives of the present clinical study were to determine the histopathologic findings and investigate the potential impact of HMEs on the tracheal epithelium in long-term tracheostomy patients.

Materials and methods

This cross-sectional study was undertaken at the Department of Otorhinolaryngology and Head and Neck Surgery and Institute for Pathology and Forensic Medicine, University Hospital Center Osijek, Osijek, Croatia. The study included 70 laryngectomized patients, 9 (12.86 %) women and 61 (87.14 %) men, divided into the HME ($n = 35$) or control ($n = 35$) group. The HME subjects were regular users of HMEs for at least 1 year; whereas, control subject had never used an HME. All subjects underwent total laryngectomy and neck dissection due to laryngeal squamous cell carcinoma.

Inclusion criteria were: laryngectomized more than 1 year prior to the study, a stable pulmonary and medical situation, no history of chronic lung diseases before the laryngectomy, treated with postoperative radiotherapy, and a former heavy smoker. All subjects were heavy smokers before the laryngectomy, and no one continued to smoke following the surgery. No subject had a high exposure to air pollution at their work or residence. Exclusion criteria were: acute or chronic pulmonary diseases, loco-regional recurrence, irregular users of the HME, a second primary cancer, non-smokers prior to treatment, and the absence of postoperative radiotherapy.

The patients' ages ranged from 51 to 89 years, with an average of 63 years. The highest incidence rate was recorded in the 60- to 69-year-old age group ($n = 33$). The time since surgery varied from 1 to 28 years, with an average of 5 years. No statistically significant difference was found between the patient's age ($z = -0.10$; $p = 0.920$) and the time since total laryngectomy ($z = -0.118$, $p = 0.906$) in the two groups. There was no statistically significant difference in the number of years of smoking before the laryngectomy ($t = 0.001$, $p = 1.00$), or in the number of cigarettes smoked per day ($t = -0.517$, $p = 0.607$) between the two groups. Patient characteristics are shown in Table 1. At the time of diagnosis, almost all subjects (except one) had advanced (stage III or IV) disease. The mean time for using the HME in the study group was 4.3 ± 2.2 years (min 1, max 7). Provox HMEs (Atos Medical AB, Horby, Sweden) were used in this study.

At baseline, all participants were asked to complete a structured questionnaire covering aspects related to HME use and previous smoking habits. The medical records were reviewed to collect demographic, health, tumor, and surgical data.

Tracheal mucosal biopsies were obtained from a total of 70 patients. The patient's trachea was sprayed with an anesthetic solution, and a preliminary fiberoptic bronchoscopy was performed with the patient sitting upright. Tracheal mucosa biopsies were obtained under direct vision with biopsy forceps, about 4 cm beneath the tracheal stoma. Biopsy specimens were fixed in 10 % buffered

Table 1 Patient characteristics for the HME and Control group separately

Characteristics	HME group ($N = 35$)	Control group ($N = 35$)	P value
Gender (F/M)	8/27	1/34	
Age (years)			0.920
Median	62	61	
Range	51–89	51–79	
Time since surgery (years)			0.906
Median	5	5	
Range	1–14	1–28	
Tobacco consumption (years ± SD)	38.2 ± 12.5	38.1 ± 8.9	1.000
No. of cigarettes/day (years ± SD)	28.1 ± 9.8	27.0 ± 7.1	0.607

formaldehyde. The samples were dehydrated and embedded in paraffin blocks. Serial 4–7 μm thick tissues were cut and stained with hematoxylin and eosin and examined by a light microscope (Olympus Cx40, Olympus, Germany). The histological findings were classified using seven levels: 1—normal epithelium, 2—mild basal cell hyperplasia, 3—moderate basal cell hyperplasia, 4—advanced basal cell hyperplasia, 5—squamous metaplasia, 6—mild dysplasia, and 7—moderate dysplasia. For the classification of basal cell hyperplasia, Auerbach's grading was used; while the World Health Organization (WHO) criteria were used for the classification of dysplasia. The histological findings were grouped in ascending order of pathogenicity as: normal, changes preceding pre-invasive lesions, and pre-invasive lesions. The methods of this study were evaluated and approved by the Ethics Commission of the University Hospital Center Osijek, and the tenets of the Declaration of Helsinki were followed. All participants were informed in detail about the investigation, and all signed informed consent prior to their inclusion.

Statistical analysis

Statistical analysis was performed with SPSS Statistics (Version 17.0.0, SPSS Inc., Chicago, IL, USA). Mean values of continuous variables are expressed as the mean and standard deviation for normally distributed variables and median and range for variables that are not normally distributed. The frequency distribution was determined for each group. To determine the difference in numeric variables between two independent samples, the Student's t test was used for parametric and Mann–Whitney U test for non-parametric analyses. The difference between two independent samples for nominal variables was assessed using the Chi square test. A p value of <0.05 was considered statistically significant.

Results

Bronchoscopically and grossly, there were no macroscopical alterations except dryness and thickening of the tracheal mucosa. Normal pseudostratified ciliated columnar epithelium was found in nine (12.9 %) cases. Among all patients, 61 (87.14 %) had pathological findings. Seventeen (24.3 %) cases had basal cell hyperplasia of some degree. Squamous metaplasia was found in 35 (50 %) and dysplasia in nine (12.9 %) cases. Pathohistological findings for all patients are presented in Table 2.

Histopathological findings were divided into three groups: normal epithelium, changes preceding pre-invasive lesions, and specific pre-invasive lesions (according to World Health Organization classification of pre-invasive lesions in the lung). Normal histology tracheal epithelium was found in almost equal numbers of respondents in both groups, 4 (5.7 %) in the HME group and 5 (7.1 %) in the control group. Changes preceding pre-invasive lesions (mild, moderate, and advanced basal cell hyperplasia and squamous metaplasia) were found in 30 (42.86 %) HME users and 22 (31.4 %) non-users. Pre-invasive lesions (mild and moderate squamous dysplasia) were found in only one (1.4 %) HME user and in eight (11.4 %) non-users. The difference between the HME group and control group was statistically significant for this investigated parameter ($\chi^2 = 6.786$, $p = 0.023$; Table 3). Additionally, chronic inflammatory changes of the tracheal mucosa were present in 63 (90 %) patients.

Table 2 Summary of histological findings

Findings	HME group ($N = 35$)	Control group ($N = 35$)	All patients
Normal epithelium	4 (5.7 %)	5 (7.1 %)	9 (12.9 %)
Mild basal cell hyperplasia	6 (8.6 %)	2 (2.9 %)	8 (11.4 %)
Moderate basal cell hyperplasia	2 (2.9 %)	5 (7.1 %)	7 (10 %)
Advanced basal cell hyperplasia	0	2 (2.9 %)	2 (2.9 %)
Squamous metaplasia	22 (31.4 %)	13 (18.6 %)	35 (50 %)
Mild dysplasia	0	5 (7.1 %)	5 (7.1 %)
Moderate dysplasia	1 (1.4 %)	3 (4.3 %)	4 (5.7 %)
Total	35 (50 %)	35 (50 %)	70 (100 %)

Table 3 Difference between normal epithelium, changes preceding and pre-invasive lesions in two examined groups

Findings	HME group ($N = 35$)	Control group ($N = 35$)
Normal	4 (5.71 %)	5 (7.14 %)
Changes preceding pre-invasive lesions	30 (42.86 %)	22 (31.43 %)
Pre-invasive lesions[a]	1 (1.43 %)	8 (11.43 %)
Total	35 (50.0 %)	35 (50.0 %)

[a] $\chi^2 = 6.786$, $p = 0.023$

Discussion

After total laryngectomy, morphologic alterations of the tracheal epithelium can occur, likely due to permanent stimulation of the tracheal epithelium by inhaled air stream through the tracheostoma, the loss of the natural air-conditioning function of the upper respiratory tract, and

reduced filtering of solid particles and aerosols. Pre-existing chronic inflammation, smoking habits, radiotherapy, poor nutrition, and vitamin deficiencies must be considered in laryngectomized patients. All of these factors can lead to changes in the respiratory epithelium, but it is difficult to determine the nature of them.

Some studies found that smoking can cause metaplasia of the mucosa of the tracheobronchial tree. Knudtson [11] found changes in the tracheal epithelium in 80 % of heavy smokers. Auerbach et al. [12] found changes in the epithelium in 78 % of smokers, and only in 16 % of non-smokers. Bertram et al. [13] confirmed the hypothesis that many changes seen in the bronchial epithelium as a consequence of smoking are reversible, and structural recovery can be expected within 2 years after smoking cessation. Other studies suggested that abnormal histopathologic changes in tracheobronchial mucosa are related to chronic inflammatory lung diseases and bronchiectasis [14, 15]. Vitamin A deficiency may induce changes in mucous cells, leading to squamous cell metaplasia [16, 17]. In addition, respiratory mucosa is often affected when patients are treated with radiotherapy for head and neck cancers [18, 19]. Manifestations of mucosal dehydration and variations in the quality of stoma care also play a part [20].

From a diagnostic and therapeutic point of view, our patients were a relatively homogenous group. Anatomical and physiological changes after a total laryngectomy and tracheotomy can cause morphological changes in the lower respiratory tract; although, studies on these changes are limited. Griffith et al. [21] found squamous metaplasia of the epithelium of the carina in 10 of 12 specimens studied, and patients' changes were compatible with intra-epithelial neoplasia in two cases. In addition, Friedberg et al. [22] found normal tracheal epithelium in only one tracheotomized patient. Roessler et al. [6] postulated that the alterations of tracheal mucosa depend on the extent of the pre-existing damage, and on the level where the specimen is taken. They found squamous metaplasia predominantly at the carina and beneath the stoma.

In our study, 61 (87.1 %) laryngectomized patients had altered tracheal epithelium, but the criteria of severe dysplasia and carcinoma in situ, which are strong predictors of progression to invasive squamous cell carcinoma, were not fulfilled in any patient. Normal respiratory epithelium was found in only nine (12.9 %) cases. Abnormal epithelial proliferations act as precursors for malignant transformation, but are not really considered pre-invasive lesions on their own. Basal cell hyperplasia and squamous metaplasia are the intermediate lesions that give rise to squamous dysplasia. These lesions are associated with tobacco smoking, but are also seen as a result of chronic airway irritation, air pollution, vitamin A deficiency, and chronic inflammation [23].

Basal cell hyperplasia is defined by the presence of three or more layers of basal cells in otherwise normal respiratory epithelium. Multiplication of the layers of the basal cell zone is a common finding; seventeen (24.29 %) cases had some degree of basal cell hyperplasia. In this study, basal cell hyperplasia was characterized using Auerbach's grading [24], as follows: mild (3–4 layers), moderate (4–6 layers), and advanced (more layers). Based on this grading, eight cases (11.4 %) had mild, seven (10 %) had moderate, and two (2.9 %) had advanced basal cell hyperplasia.

Squamous metaplasia indicates a change from the ciliated mucous-secreting epithelium to a stratified squamous epithelium [25]. This was present in 35 (50 %) cases. The lamina propria beneath the metaplastic epithelium showed evidence of chronic inflammation: lymphocyte infiltration, fibrosis, and increased vascularity. Keratinization of the squamous epithelium was not noted.

Severe squamous dysplasia and carcinoma in situ, which occur in the tracheobronchial epithelium, are recognized precursors of invasive squamous cell carcinoma of the lung [23]. In this study, squamous dysplasia was found in nine (12.7 %) cases; of which, five were slight and four moderate. Mild dysplasia (grade I) shows proliferation or hyperplasia of cells of the basal and parabasal layers that does not extend beyond the lower third of the epithelium. Cytological atypia is generally slight with only mild pleomorphism of cells or nuclei. Mitoses are not prominent, and they are usually basally located and normal when present. Architectural changes are minimal. Moderate dysplasia (grade II) shows a proliferation of atypical cells extending into the middle one-third of the epithelium. The cytological changes are more severe than in mild dysplasia and increased and abnormal mitoses may be present. However, stratification and maturation are relatively normal. In severe dysplasia, the disarray extends into the upper third of the epithelium but does not reach the surface [26].

Squamous cell carcinoma evolves through basal cell hyperplasia, squamous metaplasia, and dysplasia to carcinoma in situ and invasive carcinoma [27]. The cause and the interval of time required for the development of these changes is unknown. In this study, the interval between the laryngectomy and biopsy varied from 1 to 28 years. Most of these lesions are not visible, and tracheal biopsies are not necessarily representative of the epithelium of the whole tracheobronchial tree; therefore, the appearance of lesions in bronchial specimens is fortuitous. Pre-invasive lesions are relatively frequent in lung resection specimens from cigarette smokers with lung cancer [28]. The risk and rate of progression of pre-invasive lesions to invasive squamous cell carcinoma as well as the mechanisms of progression and regression are not clear [29].

There are no published reports evaluating the influence of an HME on the tracheal epithelium. In this study, normal

histology tracheal epithelium was found in almost equal numbers of respondents in both examined groups. Changes preceding pre-invasive lesions were found in 30 (42.86 %) HME users and 22 (31.4 %) non-users. The differences between the two examined groups for these two parameters were not statistically significant ($p > 0.05$), but there was a statistically significant difference ($p = 0.023$) for pre-invasive lesions. Precancerous changes were found in eight non-users (control group), but in only one subject in the HME group. It is possible that the use of the HME can prevent the tracheal epithelium changes from evolving to preceding pre-invasive lesions and pre-invasive lesions.

Some limitations of this study are the relatively small number of respondents and the lack of similar studies to enable comparisons; thus, we need to be careful in making final conclusions. Based on the significant difference found between groups for the pre-invasive lesions, our results suggest the HME may prevent dysplastic changes, but wider, multi-center studies are needed to further examine the HME and its effects on changes in the tracheal epithelium in long-term laryngectomized patients.

Patients who have had cancer are much more prone to develop a second primary tumor in a different site. Patients with head and neck carcinomas are at high risk for developing second primary lung cancer [30, 31]. Moreover, in laryngeal carcinoma patients, there is an increased incidence of metaplasia and dysplasia of the tracheobronchial mucosa, which might be a predisposing factor in carcinoma of the lung.

Conclusion

Breathing through a tracheostoma after a total laryngectomy causes changes in the tracheal epithelium in the vast majority of patients. In addition, a total laryngectomy may be a predisposing factor for carcinoma of the lung, and lung diseases are the second leading cause of mortality in laryngectomized patients. Pulmonary rehabilitation using an HME may prevent precancerous changes; thus, an HME should be proposed to all laryngectomized patients. Future research should be aimed at further improving lung health in laryngectomees.

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

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