

# The Influence of Cigarette Smoke on the Epithelium of the Vestibule: an Electron Microscopic Study

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It is known that cigarette smoke induces cytological alterations on the respiratory and olfactory mucosa of the nasal cavity. We evaluated whether cigarette smoking had adverse effects on the epithelium of the vestibule, in the absence of any published ultrastructural studies. We evaluated ten patients suffering from septum deviation, eight of whom were long-term smokers. While each layer of the epithelium obtained from the non-smokers consisted of a homogeneous cell population, each from the long-term smokers consisted of a heterogeneous cell population. The most prominent changes occurred in the shape and size of the cells and nuclei, the number and length of the cytoplasmic projections, the number and distribution pattern of the desmosomes, and the width of the intercellular spaces. We concluded that cigarette smoke produces hyperplastic and dysplastic changes, important factors related with cancer development, on the epithelium of the vestibule.

**Key Words:** Cigarette smoke, vestibule, electron microscopy

## INTRODUCTION

The main route of contamination of the human body with airborne pollutants is through the upper air and food passages. The target organs for air pollution effects on the upper aerodigestive tract include mucosa, olfactory epithelium, auditory receptor cells, glottic epithelium, and adjacent neural and muscular tissues. Strong evidence for

the carcinogenic effect of occupational inhalants in the nasal cavity and paranasal sinuses is seen with exposure to tobacco smoke, hardwood dust, furniture making, and leather tanning.<sup>1</sup> Little attention has been paid to the nasal epithelium in terms of its response to inhaled toxicants, including cigarette, although the nose is one of the major ports of entry for inhaled materials and the nasal epithelium is sensitive to common environmental pollutants.<sup>2,4</sup>

Tobacco-specific nitrosamines are a group of carcinogens that are present in tobacco and tobacco smoke. They are formed from nicotine and related tobacco alkaloids. These nicotine-derived nitrosamines can induce tumors both locally and systemically. So they may be involved in the etiology of tobacco related cancers of the nasal cavity, esophagus, and pancreas.<sup>5</sup> Bioassays with many animals, including rats, mice, hamsters and minks, have shown that one of the tobacco-specific nitrosamines; N-Nitrosornicotine is carcinogenic to the nasal cavity, trachea and esophagus.<sup>6,7</sup> N-nitrosodiethanolamine in tobacco and tobacco smoke also induces carcinomas of the nasal cavity, papillomas of the trachea and tumors of the larynx in some animals.<sup>8</sup>

The surface epithelium of the anterior nasal cavity is generally divided into three morphological types: squamous, respiratory and olfactory.<sup>3</sup> Previous studies have demonstrated that cigarette smoke induces some cytological alterations on the respiratory and olfactory mucosa of the anterior nasal cavity.<sup>4,6,9,10</sup> Although there are no consistent published associations with any environmental toxins to determine patients at risk for squamous cell carcinoma of the nasal vestibule, cigarette

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usage was reported to be prevalent in most cases.<sup>11-13</sup> Moreover, Tesfaigzi, et al.<sup>14</sup> reported that in rats exposed to cigarette smoke 6h/day for 5 days, the number of small mucous cells increased in the respiratory epithelium of the nasal septum in the early stages of squamous differentiation, but that these cells were gradually replaced by squamous metaplastic cells.

To the best of our knowledge there is no published ultrastructural study about the effects of cigarette smoking on the epithelium of the vestibule in humans. So, the aim of the present study was to investigate the effects of cigarette smoking on the epithelium of the vestibule in humans.

## MATERIALS AND METHODS

Biopsy materials from the vestibule were obtained, with written permission, from 10 patients suffering from septum deviation during surgery. The study was approved by the Ethics Committee of Ataturk University. All patients were male and the mean age was  $37.2 \pm 2.2$  years (range 34 to 40). None of them had been exposed to any irritants, such as formaldehyde, ammonia and hardwood dust, except cigarette smoke. They were not alcohol drinkers. Eight of the patients were long-term smokers (about 20 cigarettes per day, for a period of 10 years). Two patients were non-smokers and were age matched with the smokers. Immediately after excision, the biopsy material was fixed in 3% glutaraldehyde and post-fixed in 0.1% osmium tetroxide. Following dehydration in acetone, the blocks were embedded in Araldyde 212. Semi-thin sections were cut on a Nova LKB Bromma ultramicrotome, mounted on glass slides, stained with toluidine blue and examined in a light microscope. Ultrathin sections were placed on grids and stained with uranyl acetate and lead citrate. The ultrastructural examination was performed with a JEOL-100SX electron microscope.

## RESULTS

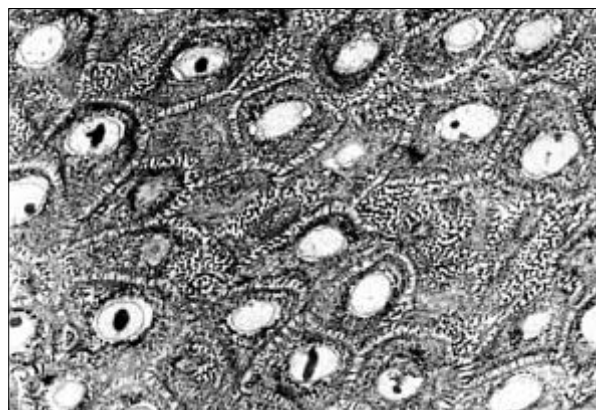
### Non-smokers

The luminal surface of the nasal cavity at this

anatomic level was lined by keratinizing stratified squamous epithelium. The common electron microscopic properties of the cells of the same stratum were similar (Fig. 1). In other words, the polygonal intermediate cells, including large, pale nuclei and tonofilaments in their cytoplasm, were attached to each other by numerous, long and well-formed desmosomes distributed over the entire surface. The ultrastructural features of the various organelles, the width of the intercellular space and the distribution pattern of the desmosomes were similar within the same layer. So, it was observed that each layer of the epithelium of the vestibule obtained from the non-smoker, healthy men consisted of a homogeneous cell population (Fig. 2).

### Smokers

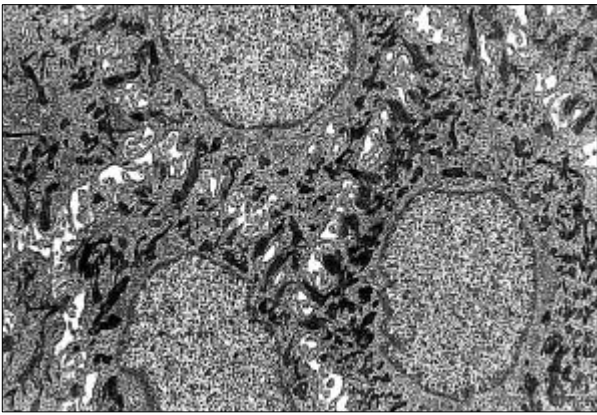
Cellular changes were evident in the cells of all of the stratum, dominantly in the intermediate layers. We observed that each stratum of the epithelium obtained from the long-term smokers consisted of a very heterogeneous cell population. Dysplastic changes were observed (Fig. 3). The most prominent changes occurred in the shape and size of the cells and nuclei, the number and length of the cytoplasmic projections, the number and distribution pattern of the desmosomes, and the wideness of the intercellular spaces. The nuclei of the cells of the same stratum were different from each other in size, shape, and density.



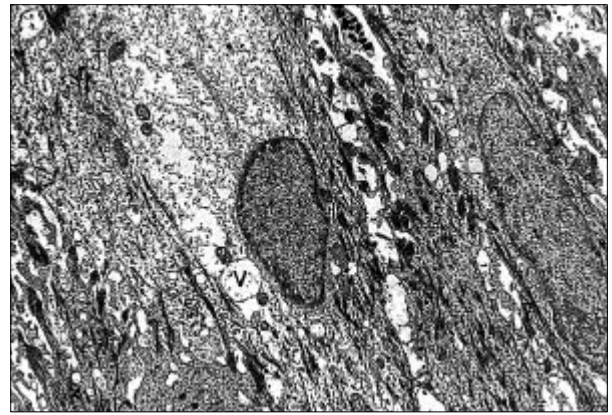
**Fig. 1.** The epithelium of the vestibule of non-smokers. The common electron microscopic properties of the cells of the same stratum are similar. (Methylene blue-oil immersion).

Some of the nuclei were small and electron-dense (Fig. 4). Many of the cells showed an irregular nuclear membrane (Fig. 5 and 6). Some of the nuclei showed deep indentations (Fig. 6 and 7). The nuclear envelopes were occasionally fragmented and the nuclear content was intermingled with that of the cytoplasm (Fig. 7). Many of the nuclei included a large and prominent nucleolus (Fig. 5). Generally, the peri-nuclear cytoplasm was devoid of tonofilaments and organelles (Fig. 4). Abnormal cells showed a number of large vacuoles in the cytoplasm (Fig. 8). Some fragments of

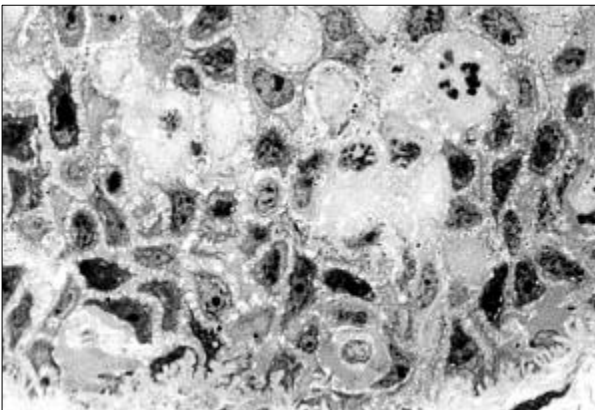
degenerating cells were observed in the inter-cellular space (Fig. 9). Intercellular spaces were wider than normal (Fig. 8 and 10). Cytoplasmic projections crossing these broadened intercellular spaces were thinner and longer than those of the non-smokers (Fig. 10). Desmosomes were not well found (Fig. 8), or showed an abnormal distribution pattern (Fig. 10). Some intracytoplasmic desmosomes were located beneath the plasma membrane (Fig. 10). Tonofilaments were decreased (Fig. 6 and 8). There were lysosomes, including electron-dense granules, in the cytoplasm (Fig. 10).



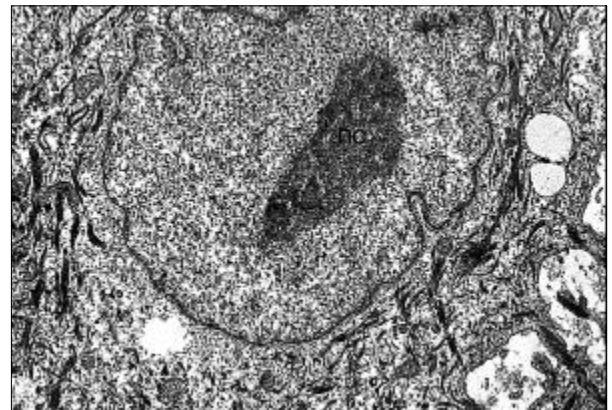
**Fig. 2.** The epithelium of the vestibule of non-smokers. The common electron microscopic properties of the cells of the same stratum are similar. The polygonal intermediate cells, including large, pale nuclei and tonofilaments in their cytoplasm, are attached to each other by numerous, long and well-formed desmosomes distributed over the entire surface. (Uranyl acetate and lead citrate  $\times 5,000$ ).



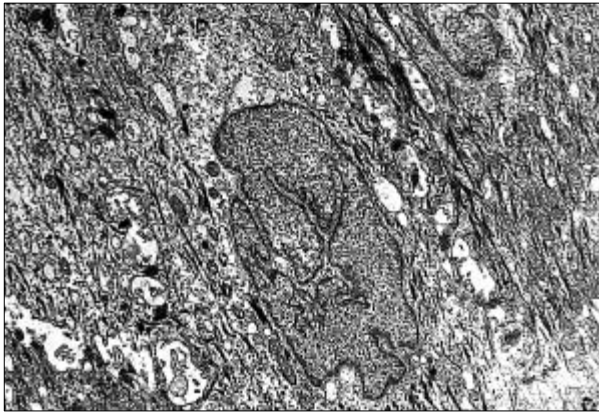
**Fig. 4.** The epithelium of the vestibule of smokers. The nuclei of the cells of the same stratum are different from each other in size, shape, and density. Some of the nuclei are smaller in size and electron-dense. The peri-nuclear cytoplasm is devoid of tonofilaments and organelles. There is a vacuole (v) near the nucleus. (Uranyl acetate and lead citrate  $\times 5,000$ ).



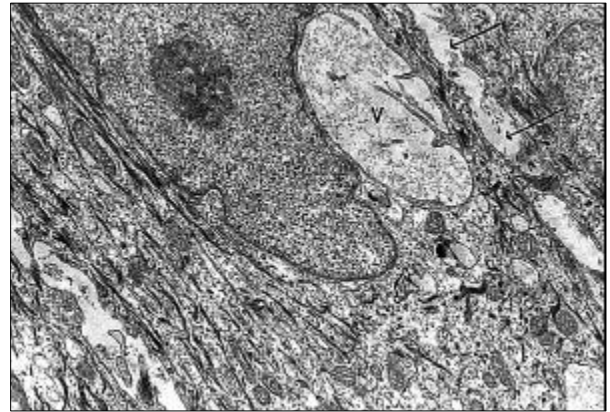
**Fig. 3.** The epithelium of the vestibule of smokers. Dysplastic changes are observed. (Methylene blue-oil immersion).



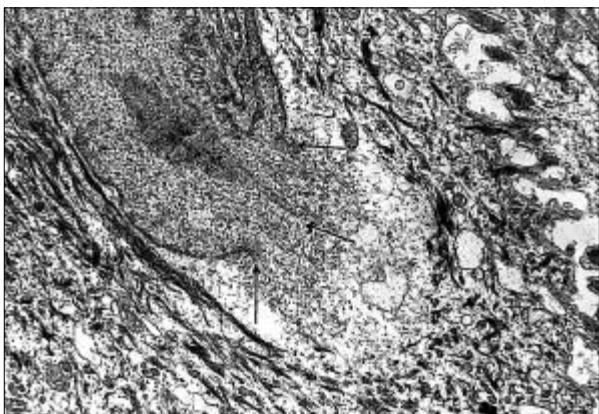
**Fig. 5.** The epithelium of the vestibule of smokers. The nucleus is irregular in shape and includes a large and dense nucleolus (nc). (Uranyl acetate and lead citrate  $\times 8,000$ ).



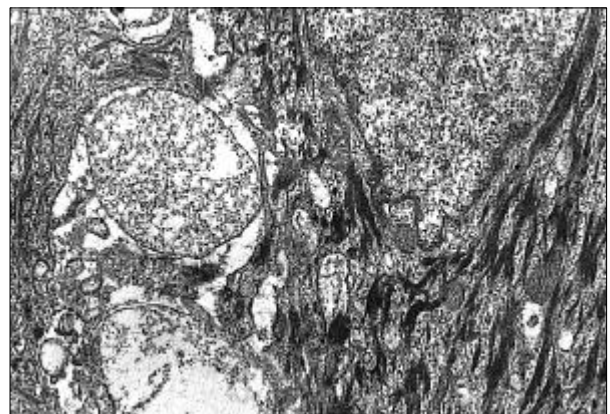
**Fig. 6.** The epithelium of the vestibule of smokers. The nucleus is irregular in shape and shows deep indentations. Tonofilaments are decreased in number and are usually localized in the peripheral cytoplasm. (Uranyl acetate and lead citrate  $\times 5,000$ ).



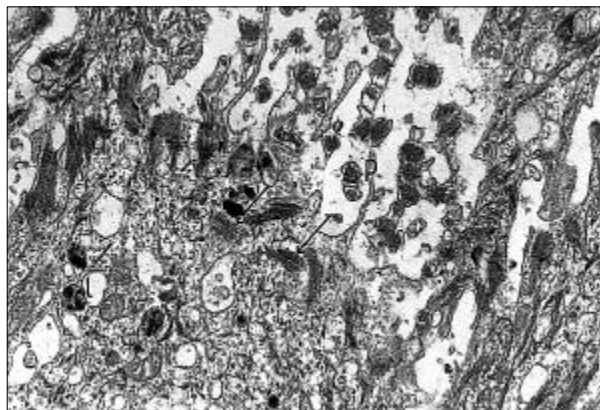
**Fig. 8.** The epithelium of the vestibule of smokers. There is a large cytoplasmic vacuole (v). Desmosomes are not well found (arrow). Tonofilaments are decreased in number (Uranyl acetate and lead citrate  $\times 8,000$ ).



**Fig. 7.** The epithelium of the vestibule of smokers. The nuclear envelope is fragmented and the nuclear content is intermingled with that of the cytoplasm (arrow). (Uranyl acetate and lead citrate  $\times 10,000$ ).



**Fig. 9.** The epithelium of the vestibule of smokers. Some fragments of degenerating cells are present in the intercellular space. (Uranyl acetate and lead citrate  $\times 10,000$ ).



**Fig. 10.** The epithelium of the vestibule of smokers. Intercellular spaces are wider than normal. Cytoplasmic projections (p) crossing this broadened intercellular space are thinner and longer than those of the controls. Desmosomes show abnormal distribution pattern. Some intracytoplasmic desmosomes are located beneath the plasma membrane (arrows). (Uranyl acetate and lead citrate  $\times 10,000$ ).

A few huge cells containing pyknotic or lobulated nuclei were observed, along with many vacuoles.

## DISCUSSION

Cell injury may be defined as a failure of the cell, on challenge, to maintain itself within a homeostatic tolerance limit. This may be an acute, rapidly developing abnormality, such as distension of intracytoplasmic membrane-limited spaces, sometimes accompanied by condensation of the intervening cytoplasmic matrix. A more slowly evolving chronic response may involve the accumulation of secondary lysosomes as evidence of continuing injury. If the cause of injury persists, these acute changes may evolve to a state of adaptation, or may lead to cell death. Cell death itself is only structurally recognizable after the event, by a degree of disruption of cellular morphology so gross as to be clearly irreversible. This will usually include disintegration of the cell membrane, disorganization of the cytoplasmic organelles, and shrinkage or fragmentation of the nucleus. The entire cell may become shrunken and condensed, its organelles barely recognizable. Dying cells often become disconnected from their neighbours and detached or extruded from their position, particularly, in epithelial sheets. The dead cell shows cytoplasmic vacuolization, a watery cytoplasmic matrix and nuclear dissolution.<sup>15</sup> Cigarette smoke induces oxidative changes in both the cell membrane and the cell cytoskeleton.<sup>2</sup> The most common form of response to environmental stress is cell swelling. This may be induced by a wide variety of chemical, physical, metabolic, or toxic agents, of which anoxia is perhaps the most common, clinically relevant example. Interference with the balance of cellular energy metabolism leads to acute malfunction of the energy-dependent pumping systems which maintain the fluid and electrolyte balance of the various compartments of the cell, resulting in abnormal osmotic forces acting across the membrane barriers. These cause swelling of some organelles and the cell itself.<sup>15</sup> We observed many cells, including vacuoles, within their cytoplasm. We suggest that the peri-nuclear region without organelles and filaments shows intracytoplasmic

edema, and cell swelling. Intercellular spaces were wider than those of the controls, perhaps due to the intercellular edema. Cytoplasmic projections crossing these broadened intercellular spaces were thinner and longer than those of the controls. Since the cell membrane is the first point of contact with any toxic substance, it is not surprising that changes in cell surface morphology occur at an early stage in the course of cell injury, particularly when the membrane itself, or its metabolism, is specifically targeted. Impairment of the energy-dependent, sodium pump mechanism of the cell membrane has already been referred to as part of the pathway to cell swelling.<sup>15</sup> We observed some balloon cells, including many vacuoles, within the cytoplasm. Cingi, et al.<sup>4</sup> observed vacuolar degeneration in the cells of nasal mucosa of smoke exposed animals.

Volumetric changes in the nucleus include changes in nuclear number, volume, dimension and contour. Irregularity of contour, increased segmentation, abnormal evagination, or invagination, and deep clefting of the nuclei are common findings in cellular pathology, especially in tumors. In the present study, the nuclei of the cells of the same stratum were different from each other in shape, size and density. Some of the nuclei were shrunken and electron-dense, some showed deep indentations. Many were irregular in shape. The nuclear envelope was occasionally fragmented and the nuclear content was intermingled with that of the cytoplasm. These changes are often interpreted as a reflection of increased metabolic activity, perhaps associated with increased mechanical and dynamic turbulence within the cell, as the irregularity of contour provides an enhanced surface area for nucleocytoplasmic exchange. Alternatively, nuclear irregularity may reflect rapid or unbalanced change in nuclear volume following an acute injury. Some cytotoxic agents, such as methotrexate, may cause nuclear envelope invaginations and protrusions. In lethal injury, rupture of the nuclear envelope is a clear indication of irreversible damage.<sup>15</sup> Cingi, et al.<sup>4</sup> observed pyknosis of nuclei and individual cell necrosis in the cells of nasal mucosa of smoke exposed animals. Aurbach, et al.<sup>16</sup> demonstrated changes such as basal-cell hyperplasia, and occurrence of cells with atypical nuclei

on the bronchial epithelium of cigarette smokers. Nucleoli vary in number and size in different cell types, being more numerous and more prominent in highly active cells.<sup>15</sup> We observed many of the nuclei, including a lot of large and dense nucleolus.

Various specialized, intercellular junctions are common targets for toxic agents, which exert their effects by disruption of such contacts. Separation and disruption of junction specialization in the respiratory epithelium has been claimed as a contributory factor in smoking-related diseases.<sup>15</sup> In the present study we observed that desmosomes were not well found or showed abnormal distribution pattern. Some intracytoplasmic desmosomes were located beneath the plasma membrane. The not infrequent finding of intracytoplasmic desmosomes and other internalized junctions in neoplasia may reflect the instability of cell contact relationship in tumors. Tonofilaments, attached to the desmosomes, provide mechanical cohesion and resistance to trauma.<sup>15</sup> The decrease in the number of desmosomes and tonofilaments may also reflect the instability of cell contact.

Phagocytosis, or heterophagocytosis, is a process of engulfment of extraneous material by cells. This may be an entirely physiological process, but the same mechanism operates in pathological circumstances, in response to many types of cell injury.<sup>15</sup> We observed many lysosomes, including electron-dense granules, within the cytoplasm.

Exposure of the airway surface epithelium to inhaled irritants is frequently accompanied by an initial wave of cell proliferation. Cell proliferation is dependent on the concentration, dose and duration of exposure to the irritant and on its physicochemical nature.<sup>3</sup> Epidermal hyperplasia is a very common manifestation of acute or chronic injury.<sup>15</sup> Radsel and Kambic<sup>17</sup> investigated the influence of cigarette smoke on the pharyngeal mucosa in a clinical and experimental study. They reported a close dependence between the degree of hyperplasia and the number of cigarettes smoked. They observed that the changes related to hyperplasia and dysplasia were more evident with longer smoking history, and that a significant interdependence was observed between the daily number of inhaled cigarettes and the changes on the pharyngeal mucosa. Von Meyerinck, et al.<sup>10</sup>

revealed that the histopathological changes within 90 days in rats due to sidestream smoke from cigarettes were hyperplasia and metaplasia of the epithelium covering the dorsal nasal turbinate bones. Muller and Krohn<sup>18</sup> showed a very clear relationship between precancerous lesions of the larynx and smoking habit. They observed hyperplastic and parakeratotic or keratinizing epithelial changes on the vocal cords. Many studies have reported smoke-induced epithelial hypertrophy, hyperplasia and squamous metaplasia in the conducting airways of rats and mice.<sup>19</sup> The inhaled smoke causes squamous metaplasia and keratinizing hyperplasia in the nasal cavity, trachea and bronchus.<sup>9,19,20</sup> In his study on squamous cell carcinomas of the sino-nasal cavity, Ogawa<sup>21</sup> found squamous metaplasia and squamous dysplasia to be important factors related with cancer development. Chronic inflammation, papillary or stratified proliferation and reserved cell hyperplasia were considered as factors, including squamous metaplasia. He observed some serial changes in the distribution pattern of cytokeratin from metaplasia and dysplasia to microinvasive carcinoma. We also observed dysplastic and hyperplastic changes within the cells of the vestibule. Takahashi, et al.<sup>22</sup> observed squamous cell carcinomas with keratosis, basal cell hyperplasia, squamous metaplasia, and dysplastic changes in the nasal cavity of mice related to minute amounts of cigarette smoke. They suggested that squamous cell carcinoma may pass through the course of basal cell hyperplasia, squamous metaplasia and dysplastic change of the epithelia. The retrospective study of Zheng, et al.<sup>23</sup> found cigarette smoking to be related to an increased risk of cancer of the nasal cavity and sinuses. They confirmed that cigarette smoking is a factor for nasal cancer, and provided further evidence that dietary factors may play a role in the etiology of this malignancy. Cigarette smoking produces squamous cell carcinomas.<sup>1</sup>

The increased use of tobacco has prompted investigators to assess its influence on all tissues and to attempt to diminish these harmful effects.<sup>2</sup> We concluded that cigarette smoking causes dysplastic changes in squamous mucosa of the vestibule and that it is probably an important factor not only for cancers of olfactory and respiratory

nasal mucosa but also of the squamous mucosa of the vestibule.

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