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Palatine tonsils in smoker and non-smoker patients: a pilot clinicopathological and ultrastructural study

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BACKGROUND: Tobacco smoke is a well-known source of toxic, mutagenic and carcinogenic agents. The aim of the present preliminary study was to investigate the effects of cigarette smoking on lymphoid and non-lymphoid tonsillar tissue.

METHODS: The study group consisted of 12 smoker and 10 non-smoker patients complaining recurrent tonsillitis. Clinical data, histological findings and scanning electron microscopic analyses were considered. To the best of our knowledge, such an approach has not been previously adopted in a similar experimental model.

RESULTS: Smoker patients showed a longer history of recurrent tonsillitis, difficulties in clinical management and evident morphostructural changes than non-smokers.

CONCLUSIONS: These preliminary results suggest a possible interference of cigarette smoking with the therapy response as well as a possible role of tobacco smoke in impairment of inflammatory response. Results are critically analysed and discussed. Literature data on this subject are reviewed.

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Keywords: cigarette smoke; scanning microscopy; tonsils

Introduction

Human tonsils are component of the nasopharyngeal-associated lymphoid tissue and are known to be functionally related to primary and secondary immune response to inhaled or ingested antigens as well as to immune responses initiated in the systemic compartment (1–4).

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Morphostructural changes, extracellular matrix involvement, biological effects of cytokines and cell adhesion molecules have been extensively studied (5–10).

Nevertheless, no histological or ultrastructural studies to date have directly investigated cigarette smoke effects on palatine tonsils. On the basis of the available literature data pertaining human tonsils functional and morphological analysis, the current pilot study attempted to analyse and estimate the adverse effects of cigarette smoking on both epithelial and non-epithelial tonsillar compartments.

Tobacco nitrosamines are a group of inhaled toxicants involved in the aetiology and induction of tumours as well as in functional and morphostructural changes resulting in abnormal tissue response to injury (11, 12).

In this study, two groups of smoker and non-smoker patients complaining recurrent tonsillitis were considered. Clinical data, histopathological and ultrastructural findings were collected and recorded.

Results showed significant differences between the groups regarding clinical and structural findings no previously reported by other investigators. Such features and the possible role of cigarette smoke on tonsillar lymphoid tissue are discussed.

Materials and methods

Clinical data and patient eligibility

Twelve smoker and 10 non-smoker male patients (age ranged from 16 to 24 years, with a mean of 19.5 years) were selected and admitted for the study after having given informed consent signed from patients and from patients parents. The former group revealed a smoking history ranged from 2 to 9 years.

Eligibility requirements for both groups included a diagnosis of recurrent tonsillitis with none of the following other conditions: clinical history of allergy (alimentary, cutaneous or respiratory tract allergies), chronic respiratory pathologies (nasal septum deviation, hypertrophic rhinopathy, asthma, chronic sinusitis), surgical or clinical rhinopharyngeal and oral treatments

(adenoidectomy, nasal polyps surgery, periodontal or peritonsillar abscesses drainage, dental or periodontal surgery).

There were not alcohol drinkers. Each case was characterized by age, presenting signs and symptoms, and mean duration of clinical symptoms antedating clinical diagnosis and tonsillectomy. The patients had a full physical examination, complete blood counts and clinical laboratory tests including leucocyte count and oral bacterial flora analyses. The number and duration of recurrent tonsillitis episodes as well as response to antibiotic treatment and history of medical conditions were recorded. Such clinical informations gathered on each patient were further outlined by considering available medical records, parents report and, when possible, by contacting each respective attending doctor. Smoker patients were further divided into three subgroups according to Fagerstrom Test for Nicotine Dependence scale (13, 14) testing smoke dependence as follows: very low dependence (score ranged from 0 to 3 = 1 patient); low-to-moderate dependence (score ranged from 4 to 8 = 7 patients); high dependence (score ranged from 9 to 11 = 4 patients). More commonly, signs and symptoms registered in both groups were related to recurrent infections, sleep disturbance with snoring and daytime somnolence. After a period of clinical examination and data recording, patients underwent tonsillectomy. Tonsils were removed by dissection and haemostasis was controlled with suction cautery, pressure and/or suture ligature. Patients follow-up revealed no post-operative complications. Clinical data are reported in Table 1.

Histology and scanning electron microscopy analyses

Tonsils were washed in buffered saline, measured along the three dimensions and immediately processed by conventional methods within half an hour after surgery.

In particular, perpendicular sections to the long axis of each tonsil were fixed in 10% neutral-buffered formalin, dehydrated in graded ethanols, cleared in xylene, and embedded in paraffin. Five-micron-thick sections were subsequently mounted on glass slides, processed as routinely, and stained with haematoxylin and eosin, periodic acid-Schiff (PAS) and Weigert van Gieson. Microscopic observation was performed using an eyepiece grid under the microscope from ×20 to ×100 magnification. The following parameters were recorded for the entire tonsillar area: surface and cryptic epithelial changes, follicles, subepithelial and interfollicular compartments (Table 2).

On the contrary, small fragments (5-mm-thick) immediately collected after removal from the surface of each tonsil were fixed in 2% phosphate-buffered glutaraldehyde, post-fixed in osmium tetroxide, dehydrated with increasing concentrations of ethanol, dried in a critical-point apparatus under CO_2, and examined in a Hitachi emission field scanning electron microscope (SEM) (Hitachi Ltd., Tokyo, Japan) at 25 kV. The morphology of surface and cryptic epithelium, and specialized surface cells was analysed (Table 3).

Light and SEM examinations were performed by two independent observers without knowledge of the clinical history and patient's condition.

Table 1 Patients characteristics, number of annually documented recurrent tonsillitis and Fagerstrom Test for Nicotine Dependence score

Non-smoker		*Smoker*		
A	*AT*	*A*	*AT*	*FS*
16	5	16	6	6
17	5	16	9	7
18	6	17	10	9
18	5	17	8	10
21	6	17	7	6
22	7	19	6	5
22	6	19	5	3
22	5	20	7	4
24	5	20	9	10
24	6	20	8	8
–	–	22	10	9
–	–	23	5	4

A, age; AT, annually tonsillitis; FS, Fagerstrom Test for Nicotine Dependence score.

Table 2 Light microscopic parameters

Tonsillar compartments	*Light microscopy*
Surface and cryptic epithelium	Erosion, ulceration, degeneration, spongiosis Intraepithelial inflammatory cells Hyperplastic or dysplastic changes
Follicles	Size, shape and localization of follicles Secondary follicles with clearly defined germinal centres and mantle zones Enlarged irregular follicles Secondary follicles with inclusions of nests of small lymphocytes within the germinal centres Irregular follicles with features of progressive transformation Diffuse lymphoid reaction with rare follicles
Subepithelial and interfollicular compartment	Collagen matrix organization Fibrosis Oedema, haemorrhage Inflammatory cells Vascularization

Table 3 Scanning electron microscopic (SEM) parameters

Tonsillar compartments	*SEM*
Surface and cryptic epithelium	Morphostructural characteristics and pathological changes M cells (specialized surface cells)
Follicles	
Subepithelial and interfollicular compartment	

Results

Tables 4 and 5 summarize major clinical and morphological results of the present study. The analysis of clinical history as well as the clinical evaluation demonstrated differences between smoker and non-smoker

Table 4 Clinical and morphological results of non-smoker patients

Non-smoker patients	
Clinical data	
Recurrent tonsillitis	5–7 episodes of annually documented tonsillitis with a mean of 5.6 episodes (+)
Therapy resistance	±
Signs and symptoms related to inflammatory episodes	±
Light microscopy	
Surface tonsillar epithelium	
Erosions	±
Ulceration	−
IEICs	±
Spongiosis	±
Dysplasia	−
Cryptic epithelium	
Hyperplasia	+
Degeneration	−
b.m. disruption	−
Follicles	Secondary irregular and enlarged follicles, well defined germinal centres
Subepithelial and interfollicular compartments	Oedema, congestion, inflammatory cells: ±/+
SEM	
Specialized surface cells (M cells)	+/++

−, absent; ±, low/mild; +, moderate; ++, high/severe; IEICs, intraepithelial inflammatory cells; b.m., basement membrane; SEM, scanning electron microscopy.

Table 5 Clinical and morphological results of smoker patients

Smoker patients	
Clinical data	
Recurrent tonsillitis	5–10 episodes of annually documented tonsillitis with a mean of 7.5 recurrent and long-lasting episodes (++)
Therapy resistance	+; ++ (high dependence smokers)
Signs and symptoms related to inflammatory episodes	+; ++ (high dependence smokers)
Light microscopy	
Surface tonsillar epithelium	
Erosions	+
Ulceration	+
IEICs	++
Spongiosis	++
Dysplasia	One patient
Cryptic epithelium	
Hyperplasia	−
Degeneration	++
b.m. disruption	+
Follicles	Effacement of follicular architecture (diffuse lymphoid reaction), rare germinal centres
Subepithelial and interfollicular compartments	Oedema, fibrosis, inflammatory cells, congestion, haemorrhage, newly formed capillaries: ++
SEM	
Specialized surface cells (M cells)	−/±

−, absent; ±, low/mild; +, moderate; ++, high/severe; IEICs, intraepithelial inflammatory cells; b.m., basement membrane; SEM, scanning electron microscopy.

patients. Moreover, further differences were also highlighted in smoker subgroups according to Fagerstrom Test for Nicotine Dependence score.

Smokers showed a higher incidence of recurrent and long-lasting infections along with therapy resistance, sleep disturbance, snoring and daytime somnolence. Slight morning cephalgia was sporadically reported in one patient (20 years old, Fagerstrom Test for Nicotine Dependence score 8). Such signs and symptoms and related problems in medical management showed an increased incidence in the subgroup of high dependence smokers. Particularly, two of the patients (17 and 20 years old respectively, both Fagerstrom Test for Nicotine Dependence score 10) experienced at least eight to nine episodes annually of documented moderate-to-severe pharyngeal and tonsillar inflammation with oral temperature above 38°C, enlarged cervical lymph nodes and tonsillar exudate.

On the contrary, a lower incidence of pharyngotonsillitis were reported in non-smoker patients. Low-to-moderate presence of signs and symptoms related to inflammatory episodes characterized this group. Furthermore, therapy resistance and clinical history of sleep disturbance with snoring and daytime somnolence were sporadically noted. One of the 10 non-smoker patients (22 years old, seven episodes annually of tonsillitis) reported a high rate of recurrent moderate pharyngeal and tonsillar inflammation with evident tonsillar exudate, sleep disturbance and morning cephalgia.

The presence of anaerobic bacteria in the oral flora, outnumbering aerobic flora, was demonstrated by laboratory results in smoker as well as in non-smoker patients. No post-operative complications occurred in both groups.

Light and SEM results apparently correlate with clinical findings. Histology of tonsils of smoker and non-smoker patients revealed well-known morphological features of reactive and hyperplastic pattern. Typical secondary follicles with well-defined germinal centres and mantle zones, irregular and enlarged follicles were frequently seen in the same tonsil.

Florid follicular hyperplasia was usually observed in all cases, but a severe impairment of the tonsillar architecture was demonstrated in the smoker group. In these patients reactive diffuse lymphoid hyperplasia and rare small germinal centres in the deep parts of the tissue were predominant (Fig. 1). Dense collagen matrix, fibrosis, oedema and haemorrhage were also noted. Tonsillar crypt epithelium from smoker patients showed focal basement membrane disruption, cellular degeneration and superficial erosions (Fig. 2). A mixture of lymphocytes and neutrophils, desquamated epithelial cells and few erythrocytes frequently filled the crypt lumen.

In addition, evident spongiosis, ulceration and erosions of the squamous tonsillar epithelium along with intraepithelial inflammatory cells (i.e. lymphocytes, neutrophils and macrophages) and subepithelial oedema were observed in smoker patients (Fig. 3).

Evidence of slight epithelial dysplasia was present in one patient (22 years old, Fagerstrom Test for Nicotine

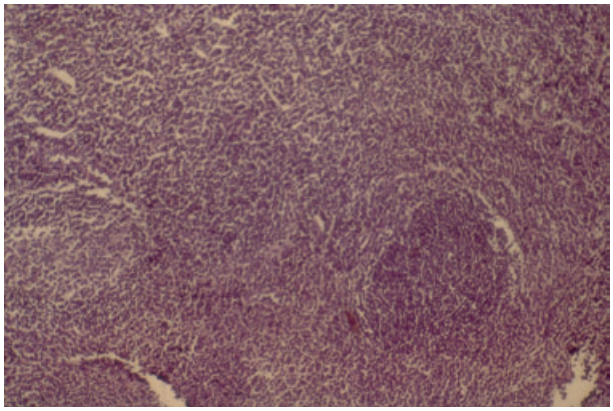

Figure 1 Smoker patient. Diffuse hyperplastic lymphoid reaction with residual germinal centre (light micrograph, haematoxylin/eosin, ×50).

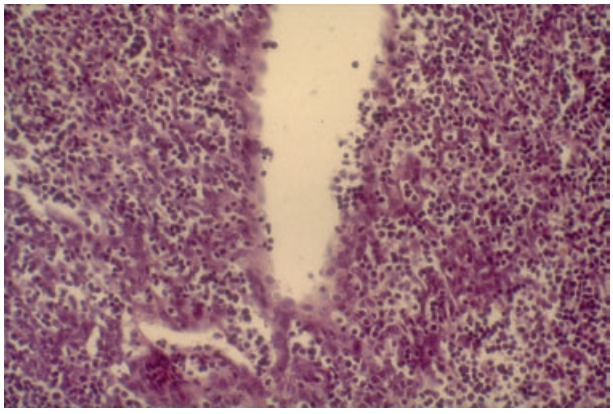

Figure 2 Smoker patient. Altered crypt showing absence of epithelial cells and diffuses inflammatory infiltrates (light micrograph, haematoxylin/eosin, ×100).

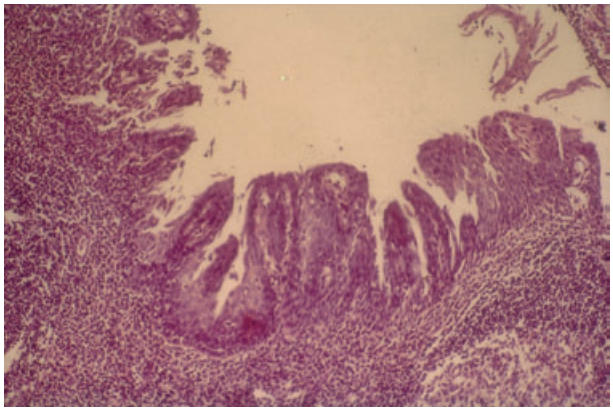

Figure 3 Smoker patient. Evident alterations in surface tonsillar epithelium. Diffuse subepithelial inflammatory reaction is noted [light micrograph, haematoxylin/eosin (H & E), ×50]. Particular of the chronic infiltrate within the stroma (H & E, ×50).

Dependence score 9). Numerous capillaries (five to eight per site) particularly distributed beneath the epithelial surface, with evidence of erytrorrhages were noted in

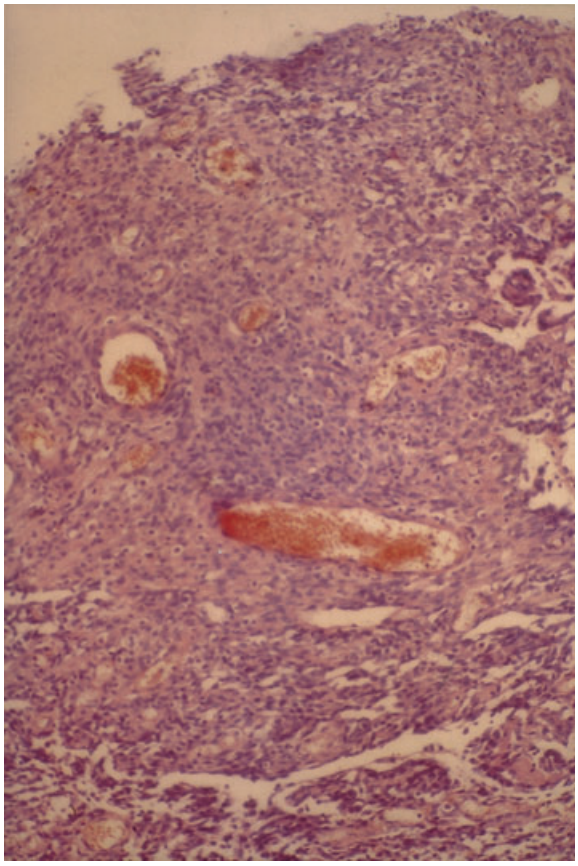

Figure 4 Smoker patient. Loss of surface epithelial lining is observed. Numerous vessels and vascular congestion are evident (light micrograph, haematoxylin/eosin, ×50).

two patients (17 and 20 years old, Fagerstrom Test for Nicotine Dependence score 10; Fig. 4). Generalized vascular congestion was present in all of the samples from smoker patients along with scattered neutrophilic infiltrates and occasional fibrin deposition.

On the contrary, tonsils from non-smoker patients disclosed typical secondary follicles or enlarged irregular follicles, regularly distributed throughout the tonsillar tissue (Fig. 5). Hyperplastic cryptic reticular epithelium with intraepithelial lymphocytes was noted. Cellular degeneration and basement membrane alteration in the crypts were not observed. Slight spongiosis and erosions but no ulceration of the tonsillar surface epithelium were sometimes present. Rare and scattered fibrosis was observed in three patients (18, 22, and 24 years old, six, seven and six episodes annually documented of recurrent tonsillitis respectively). Slight oedema and moderate vascular congestion were also noted.

Scanning electron microscopic studies of tonsils from non-smoker patients showed the surface epithelium consisting of squamous and sometimes keratinized cells in an irregular pattern of microridges with prominent and occasionally cerebroid-like appearance. Specialized surface cells with short microvilli were present (Fig. 6). Lymphoid cells and macrophages close to the crypt lumen were noted. On the contrary, presence of surface

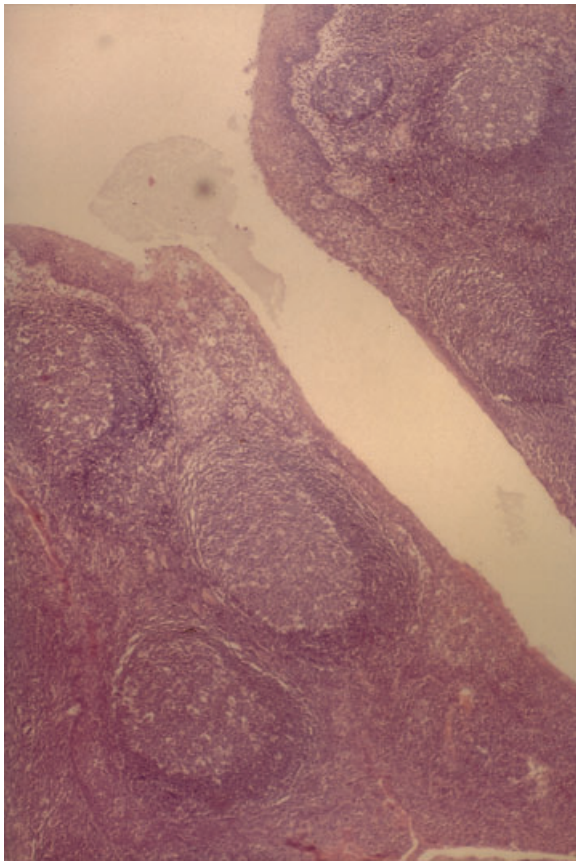

Figure 5 Non-smoker patient. Typical pattern of hyperplastic tonsillitis. Numerous irregular and enlarged follicles with clearly defined germinal centres are showed. No epithelial alteration is present (light micrograph, haematoxylin/eosin, ×50).

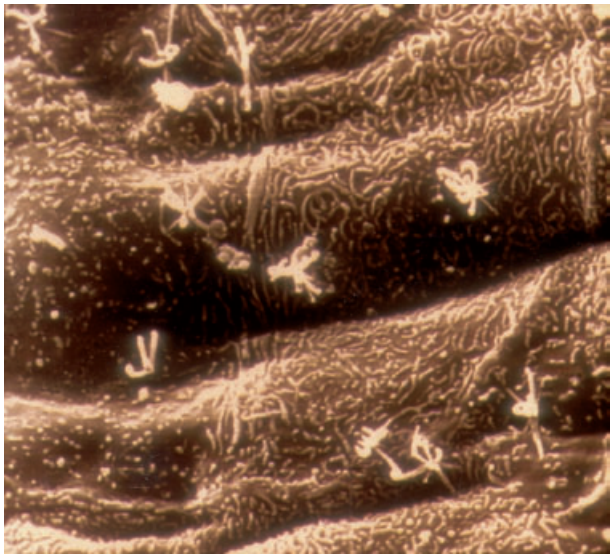

Figure 6 Non-smoker patient. Surface tonsillar epithelium showing numerous specialized cells with clearly surface microvilli (scanning electron micrograph, ×1000).

cellular debris along with erythrocytes and inflammatory cells characterized tonsillar surface in smoker patients (Fig. 7). Moreover, difficulties in evaluating

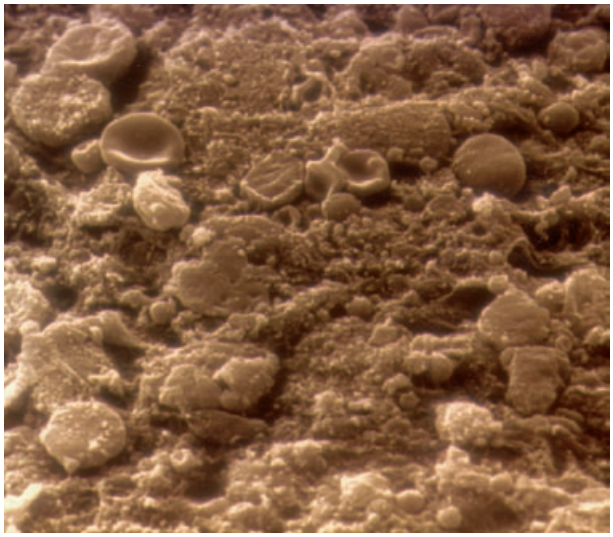

Figure 7 Smoker patient. Surface erythrocytes and mononuclear cells along with absence of specialized surface cells and altered epithelium are observed (scanning electron micrograph, ×1000).

tonsillar crypts and absence of specialized cells were findings of such a group.

Discussion

Palatine tonsils represent a readily available source of lymphoid tissue useful in characterizing immunological functions. Numerous studies demonstrated the ability of specialized epithelial cells (M cells) as well as of subsets of dendritic cells to regulate the initiation, immunogenic direction and tolerogenic responses to different antigens (1, 2, 10). Moreover, palatine tonsil epithelium and cryptic reticular epithelium are closely associated in order to provide a favourable environment for the effector cells of the immune response (15–19). On the contrary, the lymphoid compartment along with extracellular matrix glycoproteins, stromal and epithelial elements, mitogen- and antigen-induced cytokine responses all create specialized microenvironments to support cell–matrix and cell–cell interactions in immune defence mechanisms (20, 21).

The aim of this study was to investigate the effects of cigarette smoking on lymphoid and non-lymphoid tonsillar compartments with special reference to the histological structural alterations and ultrastructural epithelial changes in comparison with clinical findings. Smoker and non-smoker patients were considered. To the best of our knowledge there are no studies investigating such a condition.

Smoker patients were characterized by a history of severe recurrent pharyngotonsillitis along with difficulties in therapy management and associated signs and symptoms of snoring, sleep disturbance, daytime somnolence and morning cephalgia. In this group of patients light and SEM analyses showed alterations in both lymphoid and non-lymphoid compartments.

On the contrary, histology and SEM features of non-smoker patients revealed the presence of M cells and

reactive regular or irregular follicles consistent with the diagnosis of chronic tonsillitis. Minimal epithelial and cryptic changes were sometimes noted in non-smoker patients with a longer clinical history of recurrent tonsillitis. Nevertheless, such changes did not reach the morphostructural alterations observed in smoker patients.

Cigarette smoking is related to a wide variety of still poorly known chemical, metabolic and toxic mechanisms leading to oxidative injury in both cell membrane and cytoskeleton (11, 12). Moreover, damages of the cellular metabolisms and of the vascular compartment induce a cascade of events that may contribute to impaired immunological responses (22–24).

Structural and microenvironmental changes observed in tonsils of smoker patients may reflect smoking-induced difficulties in adequate immune response with subsequent increased incidence of pharyngotonsillar infections and related disturbances. In particular, fibrosis and oedema could be related to impairments of cell-to-cell and cell–matrix interactions. Epithelial ulceration, cryptic cells degeneration, absence of M cells and altered basement membrane may contribute to delayed and impaired activation, initiation and regulation of immune responses in both lymphoid and non-lymphoid compartments. Moreover, neutrophilic infiltrates, vascular congestion and increased subepithelial vascularization may further be associated with an abnormal inflammatory response leading to alterations in collagen matrix and basement membrane organization as well as in cell migration.

As reported by other investigators as well as confirmed in this study, a longer smoking history along with the daily number of cigarette smoked clearly correlate with both clinical findings of recurrent infections and histological and ultrastructural damages (25, 26). It may also be speculated that high rate of recurrent pharyngotonsillitis could increase at least the effects of cigarette smoking. On the contrary, cigarette smoking may enhance the effects of recurrent infections as highlighted by difficulties in clinical and therapeutic management.

Numerous studies provide evidence of impairment of cell-mediated immunity induced by tobacco smoke. Altered neutrophilic, fibroblastic and lymphocytic activity, decrease of pulmonary dendritic cells, increased number of neutrophils and monocytes along with large amount of proteases and altered secretion of regulatory cytokines have been reported (27–30). Such findings are consistent with an imbalance in tobacco smoke-induced immune response leading to chronic inflammatory diseases as well as contributing to tumour induction and development. Our preliminary results seem to confirm published experience testing similar correlations. Neutrophilic infiltrates, interstitial fibrosis, diffuse lymphoid reaction, epithelial changes and vascular damage in tonsils from smoker patients all provide support for an impaired inflammatory response.

Unfortunately, few patients have been included in this study to permit a clear understanding of the pathological mechanisms evidenced. This is due to the eligibility criteria used in order to critically analyse both clinical and morphological findings.

Further studies are still in progress and a larger number of patients is considered to clearly elucidate the role of cigarette smoking on lymphoid and non-lymphoid tonsillar compartments.

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