ORIGINAL ARTICLE

A Comparative Histological Study of the Surface Epithelium and High Endothelial Venules in the Subepithelial Compartments of Human Nasopharyngeal and Palatine Tonsils

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ABSTRACT

Objective: To compare the thickness and organization of surface epithelium, and high endothelial venules in subepithelial compartments of human nasopharyngeal and palatine tonsils, with reference to functional differences.

Study Design: Comparative cross-sectional.

Place and Duration of Study: The Anatomy Department of CPSP Regional Centre, Islamabad, from January to December 2005.

Methodology: Thirty samples each of human nasopharyngeal, right palatine and left palatine tonsils were collected by convenience sampling technique. Haematoxylin and eosin stained paraffin sections were examined for surface epithelium. Thickness of stratified squamous and pseudostratified ciliated columnar epithelium was measured, while organization was observed in case of stratified squamous. The high endothelial venules in subepithelial lymphoid compartments were counted.

Results: The surface epithelium of nasopharyngeal tonsils (stratified squamous and pseudostratified columnar inclusive) was 63.21±1.93 µm, and that of palatine (stratified squamous) was 143.99±5.94 µm thick ($p<0.001$). The mean count of high endothelial venules in subepithelial compartments of nasopharyngeal was 1.15±0.06 and that of palatine tonsil was 0.93±0.08 ($p=0.042$). Organization of stratified squamous epithelium was poor in 26 out of 30 nasopharyngeal, and well in all palatine tonsils ($p <0.001$).

Conclusion: The surface epithelium of nasopharyngeal tonsil being thinner and poorly organized than that of palatine tonsil might act as a less effective barrier between the antigenic stimulus and subepithelial lymphoid compartments. This may contribute towards higher level of immune response by these compartments of the former, which is endorsed by higher number of high endothelial venules as compared to the latter.

Key words: *Tonsil. Epithelium. Lymphoid tissue.*

INTRODUCTION

Tonsils are dense aggregations of lymphoid follicles along with diffuse lymphoid tissue, covered over by epithelium.[1] Human palatine tonsils and the nasopharyngeal tonsil (adenoids) are the largest components of Waldeyer's ring.[2] The lymphoepithelial elements of the Waldeyer's ring are strategically located to perform regional immune functions because these structures are exposed to both airborne and alimentary antigens.[3] The mucosal surface of palatine tonsils is coated by non-keratinous stratified squamous epithelium. The nasopharyngeal tonsils are covered by ciliated columnar pseudostratified with goblet cells and non-keratinous stratified squamous epithelium.[4,5]

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Transfer of lymphocytes from the blood to the tonsils is essential for their immunological competence but they are devoid of afferent lymphatics.[6] Naive lymphocytes enter these organs through specific blood vessels known as High Endothelial Venules (HEVs).[7] These HEVs are a pre-requisite for development of the Germinal Centre (GC).[8]

The adenoids are more reactive and lymphocyte proliferation is significantly higher in adenoids than in palatine tonsils in response to antigenic stimulation.[9] Allergic edema in adenoids has also been reported to be significantly different from that of palatine tonsils with a further suggestion that thicker epithelium of the palatine tonsils might be responsible by making the contact between the antigen and subepithelial reactive lymphoid compartments difficult.[10] These are the areas where lymphocytic migration through HEVs and immunological reactions take place.[6] These functional differences should base on concurrent structural differences. A comparative study of surface epithelium and underlying HEVs (an indicator of reactivity of lymphoid tissue beneath epithelial barrier) of the human nasopharyngeal and palatine tonsils is lacking in the literature.

The present study was designed to compare the height, organization of the epithelium, and number of high endothelial venules underneath the epithelium of adenoids and palatine tonsils.

METHODOLOGY

Thirty sets of samples were collected by convenience sampling technique for this comparative cross-sectional study at the time of adenotonsillectomy from the patients in whom the indication of adenotonsillectomy was either chronic adenotonsillitis or non-inflammatory nasal obstruction. All cases with recent episodes of acute tonsillitis or acute adenoiditis, antibiotic therapy in the immediate pre-operative period and neoplastic growths of the tonsils were excluded. Each set of samples comprised of a nasopharyngeal, right palatine and left palatine tonsil from the same patient. The patients ranged between 5-13 years of age, were all non-smokers and had no history of known allergies.

After fixing in formol-saline, a part of the palatine tonsil including the mucosal surface and the largest and most intact curetting of the nasopharyngeal tonsil were selected for processing. Five µm thick paraffin embedded sections were stained with haematoxylin and eosin for microscopic examination.

In case of palatine tonsils, the surface stratified squamous epithelium either had a straight basement membrane or had thick and thin portions. Three thickest and 3 thinnest patches were localized in either case. In each patch, the height of epithelium was measured, and the number of HEVs in the subepithelial high power field was recorded using 100X objective. Average of those values were calculated for thick and thin patches separately and for one tonsil collectively.

In case of nasopharyngeal tonsils, 3 patches each of stratified squamous and pseudostratified columnar epithelia from the mucosal surface were studied. Both the parameters observed for palatine tonsil were recorded for these epithelial patches. Averages of parameters were calculated for each type of epithelium separately and then collectively as surface epithelium.

Organization of the stratified squamous epithelium was observed in the light of intercellular gaps and intraepithelial presence of non-epithelial cells. The epithelium with less gaps and non-epithelial cells was categorized as well-organized, and that with more of these features as poorly organized.

The data was analyzed statistically using SPSS version 10. After statistical comparison of the means of the quantitative parameters (height and number of HEVs) by independent sample t-test, and that of the qualitative parameter (organization of epithelium) by Chi-square test, the p-value ≤ 0.05 was considered as significant.

RESULTS

All 60 palatine tonsils were covered by well-organized stratified squamous non-keratinizing epithelium separated from the subepithelial lymphoid compartment by a conspicuous layer of connective tissue.

Twenty palatine tonsils had a relatively straight basement membrane (Figure 1), while the rest had a wavy basement membrane all along (Figure 2). Three thick and 3 thin patches of epithelium were studied in either case. In all 60 palatine tonsils (right and left inclusive), statistically significant differences were found between height of the thick patches compared to the thin patches, with the values being more in case of the former (Table I). HEVs were found in the subepithelial compartment (Figure 3) but statistical difference between their average numbers was insignificant beneath thick and thin patches (Table I). The mean values of these variables of stratified squamous epithelium (thick and thin patches inclusive), of all palatine tonsils (right and left inclusive) are shown in Table I. Infiltration of the basal region of epithelium by non-epithelial cells was occasionally seen.

The surface epithelium of all nasopharyngeal tonsils was folded and had invaginations. Out of 30 cases of nasopharyngeal tonsils, pseudostratified ciliated

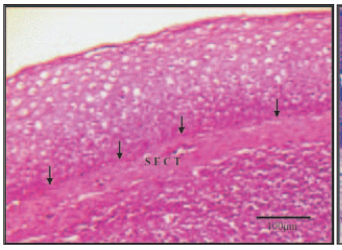

Figure 1: Section of palatine tonsil, showing surface stratified squamous epithelium with a straight basement membrane (arrows) and a conspicuous layer of subepithelial connective tissue (SECT) between epithelium and underlying lymphoid tissue. Haematoxylin and eosin. Photomicrograph. Bar, 100µm.

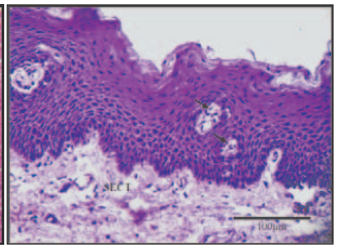

Figure 2: Section of palatine tonsil, showing surface stratified squamous epithelium with alternating thick and thin zones. The subepithelial connective tissue (SECT) is indenting the basal side of epithelium. Cross sections of such an indentation (arrow) can be seen in intraepithelial position. Haematoxylin and eosin. Photomicrograph. Bar, 100µm.

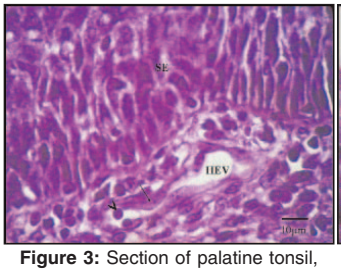

Figure 3: Section of palatine tonsil, showing a high endothelial venule (HEV) beneath surface stratified squamous epithelium (SE). The endothelial cell is seen bulging into the lumen of venule (black arrow). A non-epithelial cell can be seen just adjacent to the endothelial cell (arrow head). Haematoxylin and eosin. Photomicrograph. Bar, 10µm.

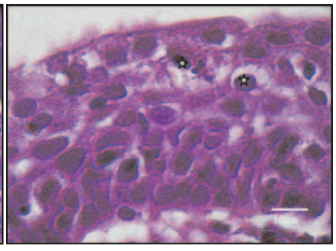

Figure 4: Section of nasopharyngeal tonsil, showing poorly organized surface stratified squamous epithelium. Several non-epithelil cells (stars) can be seen in the epithelium. Haematoxylin and eosin. Photomicrograph. Bar, 10µm.

Table I: Parameters observed on stratified squamous epithelia of palatine and nasopharyngeal tonsils.

Stratified squamous epithelium		Height (μm)	p-value*	Number of HEVs beneath epithelial patches	p-value*
Palatine tonsils n=60	Thick patches	182.40±8.55	0.000**	0.93±0.08	0.931
	Thin patches	106.53±4.00		0.94±0.09	
	Means of thick and thin patches	143.99±5.94		0.93±0.08	
Nasopharyngeal tonsils n=30		62.15±2.90	0.000**	1.24±0.1	0.024**

**=P-value computed by independent sample t-test, **=Significantly different at p<0.05, HEVs=High endothelial venules.*

columnar epithelium was found in 27. These patches had an average height of 64.39±2.55 μm and an average number of 1.05±0.09 HEVs beneath.

The patches of stratified squamous epithelium were found in all 30 cases (Figure 4). The epithelium was categorized into well or poorly organized according to the criteria laid down in methodology. Poorly organized stratified squamous epithelium was observed in 26 out of 30 cases. When compared with palatine tonsil (having well-organized epithelium in all cases) using the Chi-square (Fisher's exact) test, this variable showed significant statistical difference with a p-value of <0.001.

The mean height of patches of stratified squamous epithelium of nasopharyngeal was less, and the mean count of the HEVs beneath these patches was more than their counterparts calculated for palatine tonsils (thick and thin patches put together). The p-values showed statistically significant differences (Table I).

The surface epithelium of palatine when compared with that of nasopharyngeal tonsils (stratified squamous and pseudostratified columnar put together), was found to be thicker, with less number of HEVs beneath. The statistical differences were significant (Table II).

Table II: Parameters observed on the surface epithelia of human nasopharyngeal and palatine tonsils.

Parameter	Surface epithelium of palatine tonsils Mean±SE n=60	Surface epithelium of nasopharyngeal tonsils . Mean±SE n=30	p-value*
Height (μm)	143.99±5.94	63.21±1.93	p=0.000**
No. of HEVs beneath epithelial patches	0.93±0.08	1.15±0.06	p=0.042**

**= P-value computed by independent sample t-test, **= Significantly different at p<0.05 HEVs= High endothelial venules.*

DISCUSSION

A special inclusion criteria was laid down for this study. One set of samples was collected from one patient, and included right palatine, left palatine and nasopharyngeal tonsils. This precaution ensured that all the 3 samples were exposed uniformly to the immunological environment, both in terms of types of pathogens with the time period of their exposure, and the extent of internal immune reactions of the patient, thus increased the validity of comparison.

Adenotonsillitis is a very common condition. The autopsy samples of patients dying of other diseases can not be considered absolutely normal. Strict inclusion and exclusion criteria were laid down for this study to assure a definite history of infection-free and treatment-free one month prior to collection of samples, thus making them as close to normal as possible. The same method of sample collection while studying the normal human tonsils, has previously been adopted and documented.[11]

The stratified squamous epithelium is the type of epithelium present on surfaces that are subject to abrasion but protected from drying, e.g., oropharynx.[12] As stratification of epithelium usually correlates with transepithelial impermeability,[13] it may also be a barrier between the antigen exposure through oral cavity and the reactive lymphoid tissue of the tonsils, as has been suggested previously.[10] It is further supported by the suggestion that chronic inflammatory conditions of the tonsils may occur due to a local dysfunction of the epithelial structures at either rhino or oropharyngeal level.[14] Lymphocytes are intrinsically mobile and circulate continuously between the blood and secondary lymphoid tissues. Naive lymphocytes first enter, then adhere to, and finally migrate across specific blood vessels known as High Endothelial Venules (HEVs), which have structural characteristics for transendothelial migration of lymphocytes.[7,15] As entry of lymphocytes is pivotal for reactivity of the tonsils after antigenic exposure, the number of HEVs beneath epithelial patches has been considered as indicators of reactivity of lymphoid tissue beneath that particular epithelium in the current study.

The epithelium lining the palatine tonsils is documented to be of two types: reticulated and non-reticulated. The surface stratified squamous epithelium is of non-reticulated variety.[16] All the 60 palatine tonsils studied were found to be covered by well-organized stratified squamous non-keratinizing epithelium on the mucosal surface. The morphological features and multilayered arrangement of epithelial cells strata and a conspicuous layer of subepithelial connective tissue resembled the same observed previously.[11]

The straight basement membrane of 20 palatine tonsils observed in this study might indicate uniform exposure and uniform reactivity deep to tonsillar surface epithelium in these cases. On the other hand, the surface epithelium of 40 palatine tonsils showed papillae of subepithelial connective tissue, resulting in alternating thick and thin areas of epithelium. Considering the surface epithelium as a barrier, areas of

low and high reactivity of the lymphoid tissue deep to mucosal surface of palatine tonsils may be suggested by significant statistical differences between heights of thick against thin patches. However, statistically insignificant difference in the number of HEVs beneath the same patches suggests otherwise and variable thickness of surface epithelium might have no effect on the level of reactivity of underlying lymphoid tissue after all.

The stratified squamous epithelium of nasopharyngeal and palatine tonsils differed in several respects. It was significantly poorly organized and thinner in case of the former. The greater thickness of epithelium which had previously been implicated for delayed allergic response in palatine as compared to nasopharyngeal tonsils,[10] is now statistically confirmed by these results. The significantly higher count of HEVs beneath the stratified squamous epithelium of nasopharyngeal as compared to palatine tonsils shows more activity in subepithelial lymphoid compartments of the former, further endorsing the fact that this epithelium of latter is a better barrier than the former.

Several factors had been documented to be responsible for reactivity of tonsils in response to antigenic stimulation, other than surface epithelium barrier. These include the immunocompetent cells of the subepithelial lymphoid compartments, reticulated crypt epithelium and cell surface receptors.[3,17-19] Various cytokines may also play a role in this regard.[14] However, this fact cannot be overlooked that the surface epithelium is the first line of antigenic contact. By virtue of the results of overall comparison of surface epithelium of both types of tonsils, it is suggested that the thinner surface epithelium of the nasopharyngeal tonsils might act as a lesser barrier for the reactivity of the lymphoid tissue beneath, as compared to that of the palatine tonsils. This is supported by significantly higher number of HEVs beneath surface epithelium of nasopharyngeal as compared to palatine tonsils. This thinner surface barrier might act as one of the factors attributing to frequently seen oedema and significantly higher number of proliferating lymphocytes in response to antigenic stimulation in nasopharyngeal as compared to palatine tonsils reported earlier.[9,10] The role of reticulated crypt epithelium and relative distribution of immunocompetent cells in functional compartments cannot be overlooked, and a comparison of these factors among both tonsils might further explain the structural differences underlying the physiological and pathological variations. As the nasal passages are used continuously as compared to the oral passage, they are more likely to be attacked by foreign invaders. Thus, a suggested higher level of reactivity of the nasopharyngeal as compared to the palatine tonsils, based on the structural differences between the two could be explained.

CONCLUSION

The surface epithelium of nasopharyngeal tonsil being thinner and poorly organized than that of palatine tonsil might act as a less effective barrier between the antigenic stimulus and subepithelial lymphoid compartments. This may contribute towards higher level of immune response by these compartments of the former, which is endorsed by significantly higher number of high endothelial venules as compared to the latter.

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