RETICULAR EPITHELIUM AND UNDERLYING HIGH ENDOTHELIAL VENULES OF HUMAN NASOPHARYNGEAL AND PALATINE TONSILS: A COMPARATIVE HISTOLOGICAL STUDY

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ABSTRACT

Background: The tonsils are important structure for immunological functions and are composed of nasopharyngeal and palatine tonsils. They bear a varying degree of antigenic insult. **Objective:** To compare the reticular epithelium and underlying high endothelial venules of human nasopharyngeal and palatine tonsils. **Material and Methods:** This was a comparative cross sectional study, conducted in the Anatomy department of CPSP regional centre, Islamabad, Pakistan from January to December 2005. 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 patches of reticular epithelium. Distance between the patches was measured and high endothelial venules in the subepithelial compartments were calculated. **Results:** The reticular epithelium had same structure in both tonsils. The distance between two patches was 181.18 ± 17.83µm in nasopharyngeal and 726.01 ± 48.89µm in palatine tonsils. Comparison yielded significant statistical difference (P=0.000). The mean counts of high endothelial venules in lymphoid compartments beneath these patches of nasopharyngeal and palatine tonsils were 2.19 ± 0.1 and 1.85 ± 0.12 respectively with nonsignificant statistical difference (P=0.075). **Conclusion:** The results suggested that although the degree of reactivity of reticular epithelium in both types of tonsils might be similar, but the nasopharyngeal tonsil may react to antigenic stimulus more rapidly as compared to palatine tonsil by virtue of more frequent reticulation of epithelium on mucosal surface of former compared to later.

Key words: Tonsil, Epithelium, Lymphoid tissue, Venule.

INTRODUCTION

The tonsils are arranged in the form of a ring known as Waldeyer's ring.[1] The nasopharyngeal tonsil is a mass of lymphoid tissue in the posterosuperior wall of nasopharynx while a pair of palatine tonsils is located on right and left walls of oropharynx.[2] They participate in a variety of immunological functions ranging from innate to cellular and humoral immunity both at local and systemic levels. One of these important immunological functions is the continuous surveillance for foreign antigens at the portal of entry into gastrointestinal and respiratory tracts.[3] The areas responsible for this function of antigen sampling include reticular epithelium which is characterized by disruptions in the basement membrane's continuity, desquamation of the surface cell layers and infiltration of epithelium by non epithelial lymphoid cells which can reach to an extent of loss of clear demarcation between epithelium and lymphoid tissue.[4,5] By virtue of heavy infiltration by lymphocytes, these areas have even been named as lymphoepithelium.[6] Presence of such lymphoepithelium had also been reported in nasopharyngeal tonsils.[7] The reticular epithelium is considered a functional compartment of the tonsils. The proposed functions include provision of a favourable environment for contact between the effector cells of immune responses, direct transport of antigens, continuous synthesis of secretary component and keeping a pool of immunoglobulins.[5] All these functions need a constant recruitment of immunocompetent cells, passage for transendothelial migration, which is provided by high endothelial venules (HEVs).[8] The frequency of these venules is thus considered a proportional indicator of level of reactivity.[9]

The nasopharynx is part of airway and so its structure provides for permanent patency as compared to oropharynx which closes intermittently.[10] Nasopharyngeal tonsil is thus relatively more exposed to antigenic stimulation than palatine tonsil. This varying degree of antigenic insult suggests concurrent differences in structure and relative distribution of reticular epithelia and underlying HEVs in both tonsils. Such comparative histological study is lacking in the literature. With this background, the current study was designed to study and compare the reticular epithelium and HEVs of human nasopharyngeal and palatine tonsils.

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MATERIAL AND METHODS

Thirty samples each of right palatine, left palatine and nasopharyngeal tonsils were collected by convenience sampling technique for this comparative cross sectional study at the time of tonsillectomy with prior permission from Ethical Committee of Institute. Samples were collected from the patients in whom the indication was either chronic tonsillitis or non inflammatory nasal obstruction after informed consent. The samples from patients with recent episodes of acute tonsillitis, antibiotic therapy within a month of operative procedure and neoplastic growths of the tonsils were excluded from the study. Strict observance of these selection criteria ensured that samples collected had a superiority over the ones from autopsies. The selected samples were free of any inflammation or antibiotic therapy for at least one month, rendering them as close to normal as possible. If the samples were otherwise collected from autopsy of persons dying of other reasons, it would have been inappropriate to consider them normal in light of the fact that tonsillitis is a very common condition, and an accurate history of the current status of tonsils is usually not available in these cases.[11,12] After fixing in formaline, 5 µm thick paraffin embedded sections from mucosal surfaces of nasopharyngeal and palatine tonsils (including the crypt in case of later) were stained with haematoxylin and eosin for microscopic examination. The crypts of palatine and mucosal surfaces of nasopharyngeal tonsils were observed in histological sections for patches of reticular epithelium and their morphology was examined and recorded. In order to ascertain the number of such patches per unit length, which indirectly showed their frequency per unit area, the distance between two adjacent patches was measured. It was done either by the ocular micrometer (when the line was relatively straight) or with the help of computer software, image J (in case of a curved line of intervening epithelium).[13] Means and standard deviation were calculated.

The field beneath each patch was studied for HEVs. Their number was counted per field and means and standard deviation were calculated. The means of both parameters were then compared by independent sample t test with the help of SPSS version 17. A p-value of 0.05 or less was considered statistically significant.

RESULTS

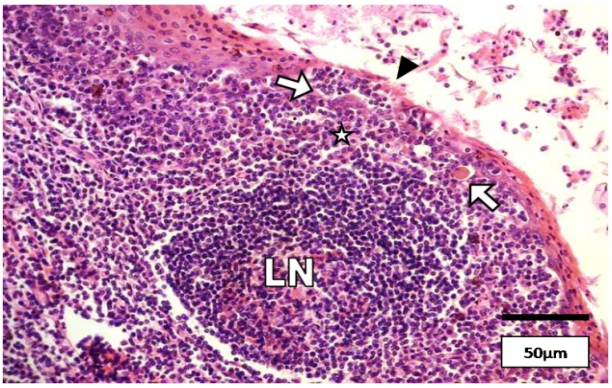
The patches of reticular epithelium on nasopharyngeal tonsils were found to be significantly closer (181.1 ± 17 um) than those of palatine tonsils (726± 48um), on statistical comparison by independent sample t test (p=0.000). Whereas the count of HEVs beneath epithelial patches of both had no significant difference when compared statistically, where number of HEVs in palatine tonsils was 1.85 ± 0.12 and in nasopharyngeal tonsils was 2.19±0.1 (p=0.07) (Table I).

Table I: Mean, Standard Deviation and statistical significance between reticular epithelium of palatine and nasopharyngeal tonsils

Parameter	Palatine tonsil Mean ± SD (n = 60)	Nasopharyngeal tonsil Mean ± SD (n = 30)	P-value
Distance between two patches of reticular epithelium(Um)	726.01 ± 48.89	181.18 ± 17.83	0.000
No. of HEVs beneath patch of reticular epithelium	1.85 ± 0.12	2.19 ± 0.1	0.075

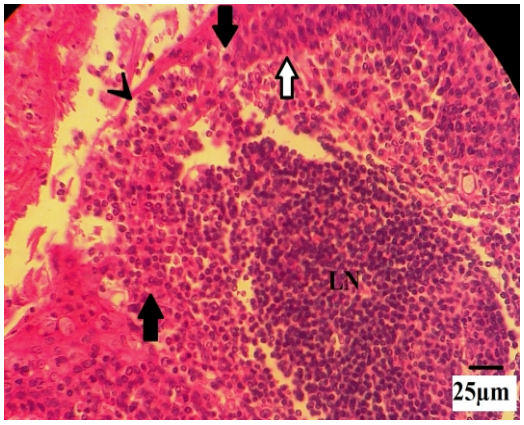
All samples had patches of reticular epithelium. They were usually located on apices of nodules. (Fig: I)

Figure I: Section of palatine tonsil, showing a patch of reticular crypt epithelium (white arrows) overlying a lymph nodule (LN). Some characteristic features of reticular epithelium like loss of demarcation between epithelium and underlying lymphoid tissue (asterisk) and surface disruption (arrowhead) are visible.
(Haematoxylin and eosin. Photomicrograph. Bar approximately 500um



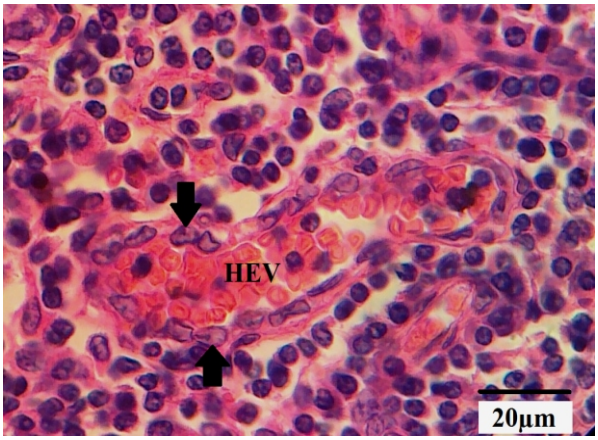
In case of nasopharyngeal tonsil they predominated in the epithelium of folds, while in palatine tonsil, were observed in crypts. The crypts of palatine tonsils showed these patches interspersed with intact stratified squamous epithelium and the boundary between these two epithelia was marked by loss of demarcation between epithelium and subepithelial lymphoid tissue (Fig: II)

Figure II: Section of palatine tonsil, showing a patch of reticular crypt epithelium (black arrows) with heavy infiltration by non epithelial cells overlying a lymph nodule (LN). Demarcation between epithelium and subepithelial lymphoid tissue (white arrow) is apparent in adjacent intact stratified epithelium. Very thinned out epithelial cells (arrowhead) are still present above a portion of reticular epithelium.
(Haematoxylin and eosin. Photomicrograph. Bar approximately 25um).



In both types of tonsils, the patches of reticular epithelium showed similar morphological characteristics. They were characterized by heavy infiltration of the epithelium by non epithelial cells which resulted in distortion of, and separation between epithelial cells. The boundary between the epithelium and underlying lymphoid tissue was not distinct. (Fig: I, II) Negligible amount of subepithelial connective tissue was found in some cases. Several HEVs were observed underneath the epithelial patches. (Fig: III)

Fig. III: Section of nasopharyngeal tonsil, showing a High Endothelial Venule (HEV) running beneath a patch of reticulated epithelium. Endothelial cells with bulging nuclei (arrowheads) can be seen bulging into the lumen filled by blood cells.
(Haematoxylin and eosin. Photomicrograph. Bar approximately 20um).



At sites of maximum infiltration by non epithelial cells, the epithelial cells appeared like star-shaped reticulum cells with several cytoplasmic processes constituting a complex network. Reticular epithelium became exceedingly thin in places. The surface of the reticular epithelium was disrupted at sites (Fig: I) and non epithelial cells were observed at these disruptions which probably passed into the lumen through these gaps. Very thinned out epithelial cells with cytoplasmic extensions, were occasionally seen on the surface.(Fig: II) At sites of excessive infiltration, the reticular epithelium rather gave the appearance of lymphoid tissue. Several small blood vessels and capillaries in addition to the HEVs were also found in the region of reticular epithelium.

DISCUSSION

Strict observance of inclusion criteria ensured that one set of samples (including one nasopharyngeal, right palatine and left palatine tonsil each) came from one patient thus shared the same level of antigenic exposure and uniform internal environment. This precaution enhanced the validity of the results.

The palatine tonsils and adenoids form a distinct component of the mucosa-associated lymphoreticular tissues showing features of both systemic and mucosal compartments.[14] Tonsils not

only act as a first line of defence against foreign antigenic insult but also as a route of entry and a site for replication for some pathogens.[15] The epithelium of palatine tonsil may even act as a potential site for oral transmission for HIV.[16] Thus a better understanding of structural characteristics of tonsillar epithelium might help in clarifying the pathogenesis of many diseases.

The crypts of palatine tonsils and the mucosal surfaces of nasopharyngeal tonsils in all cases showed epithelial patches with characteristics of reticular epithelium in agreement with previous studies, directly overlying the lymphatic nodules in accordance with documented literature.[5,17,18] By virtue of this close association, these patches can be compared to those mentioned as "Follicle associated epithelium" and thus might act as functional compartments of tonsils in league with the subepithelial lymphoid tissue.[7,19,20] The reticulation of epithelium may have resulted due to stronger antigenic stimulation whereas the areas which have retained stratified squamous epithelium might have been exposed to this insult at a lesser extent.[5] Close interaction between reticular epithelium and non epithelial lymphoid cells infiltrating through it provides a basis for so called lymphoepithelial symbiosis.[21] It was observed that at sites of heavy infiltration, the reticular epithelium took up the appearance of lymphoid tissue. This coincides with the fact that it also acquires the role of lymphoid compartment.[5]

The palatine and nasopharyngeal tonsils are the sites of allergic sensitization as they are both located at portal of entry to respiratory and digestive tracts yet occupy different parts of pharynx.[22] The nasopharyngeal tonsil lies in that part which is kept constantly patent due to rigid pharyngobasilar fascia, while oropharynx which provides a seat for palatine tonsils is subject to closure intermittently.[10] This difference in location might lead to difference in degree of antigenic stimulation which could explain significantly more patches of reticular epithelium per unit length of nasopharyngeal as compared to palatine tonsil observed in this study. This finding endorses the documentation of different and frequently occurring edema in response to antigenic stimulation in case of nasopharyngeal as compared to palatine tonsil and significantly higher proliferation of lymphocytes in former as compared to later when stimulated by P6 outer membrane protein of non-typeable H influenza leading to the conclusion that nasopharyngeal is a more reactive tonsil.[23,24]

The tonsils are considered as secondary lymphoid organs, and for carrying out their immune functions they depend upon a constant recruitment of lymphocytes. The stimulation of the functional compartments of tonsils and subsequent immune reactions is a dynamic process and development of HEVs is a pre requisite for that.[25,26] Thus the relative abundance of HEVs in functional compartments of tonsils could be correlated with their level of reactivity in direct proportion. Although the patches of reticular epithelium were found to be closely placed in nasopharyngeal than in palatine tonsils, the insignificant difference in number of HEVs beneath these patches suggest no difference in mode or degree of function of individual patch in either case and the previously discussed and documented high reactivity of nasopharyngeal tonsils could be attributed to more frequently located reticular epithelium on them as compared to palatine tonsils.

The functionality of tonsils depends upon several factors including immunocompetent cells, reticular epithelium and cytokines.[4,5,27] However, in light of its structural characteristics and location where it acts as the first line of encounter with antigens, the importance of reticular epithelium in this regard cannot be over looked.

CONCLUSION

The results suggest that although the degree of reactivity of reticular epithelium in both types of tonsils might be similar, but the nasopharyngeal tonsil may react to antigenic stimulus more rapidly as compared to palatine tonsil by virtue of more frequent reticulation of epithelium on mucosal surface of former compared to later.

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