Original Article

The Relative Distribution of High Endothelial Venules in the Subepithelial Lymphoid Compartments of Human Palatine Tonsil

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Objective: To study the distribution of high endothelial venules of lymphoid compartments of human palatine tonsils beneath the epithelium of the surface, mouth of the crypt and the crypt.

Materials and Methods: This descriptive study was carried at the department of Anatomy, CPSP regional center, Islamabad, from January to July 2005.Thirty samples of palatine tonsils were collected by convenience sampling technique Haematoxylin and eosin stained paraffin sections were examined. The high endothelial venules in lymphoid compartments beneath the epithelial lining of surface and mouth of the crypt, and reticulated crypt epithelium were studied. Their number was counted and compared statistically.

Results: The high endothelial venules found in the subepithelial compartments as well as within the reticulated crypt epithelium were characterized by prominent nuclei of the endothelial cells with non epithelial cells adherent on the luminal side. The mean count of high endothelial venules was 0.98 ± 0.12, 1.08 ± 0.12 and 2.07 ± 0.21 in the high power fields (under the oil immersion lens using 100X objective) beneath the surface, mouth of the crypt and reticulated crypt epithelium respectively. The difference between the former two was statistically insignificant (p=0.543). The number of high endothelial venules beneath the reticulated crypt epithelium was significantly more than the other two locations (p= 0.000 in each case).

Conclusion: The high endothelial venules are distributed throughout the subepithelal compartments of human palatine tonsils. The number increases gradually yet insignificantly from the surface to the mouth of the crypt, whiles the lymphoid tissue beneath and within the reticulated crypt epithelium has significantly higher distribution of high endothelial venules than other subepithelial locations.

Key words: *Palatine tonsil, epithelium, lymphoid tissue, venules*

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Introduction

The surface stratified squamous non keratinizing epithelium of the palatine tonsils, covers the underlying lymphoid tissue and is continuous with the crypt epithelium. However, the crypt epithelium looses organization and gets reticulated in patches. [1] The functional compartments of the palatine tonsils where the immune reactions occur include the lymphoid follicles, mantle zones of the follicles, extrafollicular areas and the lymphoid tissue of the reticulated crypt epithelium. [2,3] Lymphocyte trafficking plays a critical role in disseminating specifically primed lymphocytes all over the body 1 including the functional compartments of the palatine tonsils. As the palatine tonsils are devoid of afferent lymphatics [4], this trafficking depends upon presence of specialized blood vessels known as high endothelial venules (HEVs), through which lymphocytes migrate by interendothelial and transendothelial routes. [5] Once stimulated by an antigen, the primary lymph

nodules develop germinal centers (GC). The development of HEVs is a prerequisite for the development of GC. [6] T-cell homing within germinal centres (GCs) is required for humoral B-cell responses [7]. As HEVs are essential for this recruitment 5, they are present in direct proportion to the immunological activity of the reactive lymphoid compartments. Although HEVs are mostly situated in the subepithelial interfollicular areas, they are also found within the lower portion of the reticulated epithelium. [1] The antigenic stimulation results in reactive activity by the subepithelial lymphoid compartments. [8] As the surface epithelium gradually transforms into the reticulated epithelium (once the crypt is reached), it seems likely that the lymphoid tissue beneath the former would be relatively less reactive as compared to the one lying beneath and within the later. This reactivity can be indirectly measured by the distribution pattern of the HEVs. The current study was aimed to observe the relative distribution of, and gradual increase in the number of HEVs of lymphoid compartments of human palatine tonsils beneath the epithelium of the surface, mouth of the crypt and the crypt.

Materials and Methods

Thirty samples of palatine tonsils were collected from ENT department of Rawalpindi General Hospital by convenience sampling technique for this descriptive study at the time of tonsillectomy performed by dissection technique on the patients in whom the procedure was done either due to chronic tonsillitis or non inflammatory nasal obstruction. All cases with recent episodes of acute tonsillitis, antibiotic therapy in the immediate preoperative period and neoplastic growths of the tonsils were excluded from the study. The same method has been adopted and documented earlier [1]. The patients ranged in age between 5-13 years. Eighteen were males while twelve were females.

Tonsillitis is a very common condition 9; it is near to impossible to get tonsils which have never been infected. It is inappropriate to consider the tonsils collected from autopsy of patients dying of other diseases/accidents to be absolutely normal. Moreover, accurate history of the current status of tonsils is usually not available in these cases. This led to very strict collection criteria mentioned above, which assured that the samples studied were free from any inflammation and antibiotic therapy for at least one month, making them as close to normal as possible.

After fixing in formol saline, an approximately 0.5 cm thick part of the palatine tonsil, including the mucosal surface and the crypt was processed. Five µm thick consecutive paraffin embedded sections were taken, stained with haematoxylin and eosin for microscopic examination.

The epithelial covering of the mucosal surface, mouth of the crypt and the crypt was observed. Three patches of surface stratified squamous epithelium, two patches of epithelium lining the either side of the mouth of the crypt, and three patches of crypt epithelium with maximum degree of reticulation were selected.

After selection, the slide while being examined using 100X objective under the oil immersion lens, was slightly moved in such a way that the tissue just beneath the epithelial patch came in the focus and the epithelial patch moved just outside the high power field. Any vessel, which had even a single cuboidal endothelial cell, was considered as the HEV. When only a part of the vessel could be seen in the high power field, it was traced outside, if needed, to determine whether it was HEV or not. The HEVs were studied and counted per field, averages were calculated and then compared by independent sample t test with the help of SPSS version 11. A p value of 0.05 or less was considered statistically significant.

Results

The mucosal surface of the palatine tonsil was found to be covered by a well organized stratified squamous non keratinizing epithelium resting on a conspicuous layer of connective tissue. The surface epithelium when traced towards the mouth of the crypt started loosing organization, got thinner and continued with the epithelial lining of the crypt. Thinning of subepithelial connective tissue was also observed. The crypt epithelium showed patches of reticulation with characteristic features of heavy infiltration of the epithelium by non epithelial cells, separation, distortion and transformation of the epithelial cells into star-shaped reticulated cells and loss of demarcation between the epithelium and underlying lymphoid tissue. The extended and branched cytoplasmic processes of the epithelial cells interconnected with one another constituting a complex network. The surface of the reticulated epithelium was disrupted and non epithelial cells were observed within the epithelium and in lumen of the crypt.

Several HEVs were found in the subepithelial compartments. They were characterized by prominent nuclei of the endothelial cells. Non epithelial cells were seen adherent to these endothelial cells on the luminal side (Fig-1). Although located in the lymphoid tissue below the surface epithelium, epithelium of the mouth of the crypt and reticulated crypt epithelium, but were also found within the lower portions of the reticulated epithelium, or even extended throughout the epithelium in areas of greatest reticulation. Some were even observed in the connective tissue separating the surface epithelium and lymphoid tissue (Fig-2).

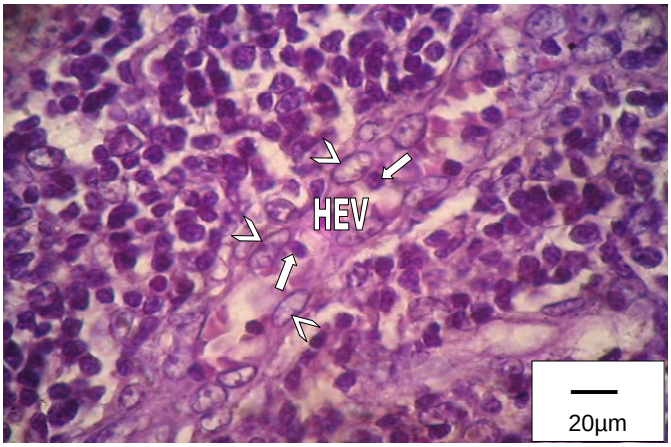

Figure 1: Section of palatine tonsil showing an obliquely running high endothelial venule (HEV) deep to a patch of reticulated crypt epithelium. Nuclei of endothelial cells (arrow heads) are prominent. Several non epithelial cells (arrows) are seen adherent to the endothelial cells on the luminal side. Haematoxylin and eosin. Photomicrograph. Bar, 20µm.

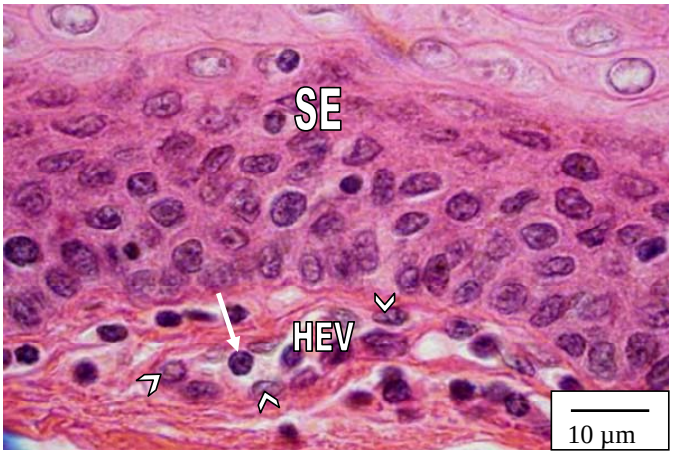

Figure 2: Section of palatine tonsil, showing a high endothelial venule (HEV) in the connective tissue beneath the surface stratified squamous epithelium (SE). Prominent and bulging nuclei of the endothelial cells (arrow heads) are apparent. Non epithelial cells (white arrow) within the lumen can also be seen. Haematoxylin and eosin. Photomicrograph. Bar, 10µm.

The Average count of HEVs beneath epithelial patches (surface epithelium, epithelium of the mouth of the crypt and the crypt reticulated epithelium are shown in Table-1. A statistical comparison by independent sample t test revealed that the mean number of HEVs beneath the crypt reticulated epithelium was significantly more than the same beneath the epithelia of surface and mouth of the crypt (p = 0.000 in each case), while a comparison of the same parameter from mouth of the crypt epithelium and surface epithelium yielded insignificant result (p = 0.543), in spite of higher value observed for former.

Table 1: High Endothelial Venules Beneath the Epithelia of Different Locations of Palatine Tonsils

Epithelium of	HEVs in subepithelial compartments Mean ± SE (n = 30)
Surface	0.98 ± 0.12
Mouth of the crypt	1.08 ± 0.12
Crypt reticulated	2.07 ± 0.21

HEVs = High endothelial venules

Discussion

Lymphocytes are intrinsically mobile and circulate continuously between the blood and secondary lymphoid tissues. When lymphocytes first enter these organs, then adhere to, and finally migrate across specific blood vessels known as high endothelial venules (HEVs). [10] The lymphoid compartments of palatine tonsils lie beneath the epithelium 3 and need lymphocyte homing through HEVs for their functions 2. In this study, the reactivity of these compartments was indirectly measured by the number of HEVs.

The surface stratified squamous epithelium was observed to be well organized. It gradually lost organization at the mouth of the crypt and continued with the crypt epithelium where patches of reticulated epithelium were found. The functional compartments of the palatine tonsils where the immune reactions occur include the lymphoid follicles, extrafollicular areas and the reticulated crypt epithelium 3 but not the surface epithelium. Presence of HEVs in the lymphoid tissue beneath the surface epithelium and even in the subepithelial connective tissue indicates that some level of immune reactivity occurs even deep to the thick barrier of surface epithelium.

The gradual loss of organization, and thinning of the epithelium and subepithelial connective tissue from surface to mouth of the crypt observed in this study, seems to be a step towards the thinner epithelium and absence of subepithelial connective tissue in the crypts of palatine tonsils of humans 1, and horses. [11] Although a trend of increase in the average number of HEVs beneath the mouth of the crypt as compared to surface was observed, but the insignificant statistical difference between the two suggests that the lymphoid tissue beneath mouth of the crypt is still as reactive as that beneath the surface, inspite of thinning of epithelium.

The main structural features of the reticulated epithelium observed for this study are in accordance with the ones documented earlier. [1, 11, 12] At sites of excessive infiltration, the modification of the reticulated epithelium was of such a degree, that it presented the appearance of lymphoid tissue rather than epithelium, comparable to lymphoepithelial symbiosis.[13] These sites can be considered as functional compartments, as previously suggested.[14] This is further endorsed by a high mean count of HEVs below and within the reticulated crypt epithelium.

The mean number of HEVs beneath the patches of reticulated epithelium was found to be significantly more than the surface and mouth of the crypt. This shows that the lymphoid tissue beneath and within reticulated epithelium is more reactive than other subepithelial compartments of the palatine tonsils. This observation can be compared to more number of reactive cells, interrupted basement membrane and vascular elements within the reticulated epithelium than non reticulated epithelium of palatine tonsils.[15] The reactivity of the reticulated epithelium can be emphasized further by the fact, that it was named as lymphoepithelial organ found in palatine tonsils. [16]

Conclusion

The high endothelial venules are distributed throughout the subepithelal compartments of human palatine tonsils. The number increases gradually though insignificantly from the surface to the mouth of the crypt, whiles the lymphoid tissue beneath and within the reticulated crypt epithelium has significantly higher distribution of high endothelial venules than other subepithelial locations.

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