

# Tonsillar crypts and bacterial invasion of tonsils, a pilot study

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## Abstract

**Conclusion:** We found no clear correlation between tonsillitis and a defect in the tonsillar crypt epithelium. Tonsillitis might be due to immunological differences of subjects rather than a lack of integrity of the crypt epithelium. Further study with larger sample size and normal control is suggested. **Objective:** To investigate histologically if a lack of protection of the deep lymphoid tissue in tonsillar crypts by an intact epithelial covering is an aetiological factor for tonsillitis. **Method:** A prospective histological study of the tonsillar crypt epithelium by immunostaining for cytokeratin in 34 consecutive patients undergoing tonsillectomy either for tonsillitis or tonsillar hypertrophy without infection (17 patients in each group). **Results:** Discontinuity in the epithelium was found in 70.6% of the groups of patients with tonsillitis and in 35.3% of the groups of patients with tonsillar hypertrophy. This is of borderline significance.

## INTRODUCTION

The association of lymphoid tissue with protective epithelium is widespread, eg skin, upper aerodigestive tract, gut, bronchi, urinary tract. The function of the palatine tonsils, an example of an organised mucosa-associated lymphoid tissue, is to sample the environmental antigen and participate with the initiation and maintenance of the local and systemic immunity. The epithelium lining the tonsillar crypts is a stratified squamous epithelium with patches of reticulated epithelium which is much thinner, with a discontinuous basal lamina, but has an unbroken oropharyngeal surface. The epithelium is exceedingly thin in places so that only a tenuous cytoplasmic layer separates the pharyngeal lumen from the underlying lymphocytes. Epithelial cells are held together by small desmosomes anchored into bundles of keratin filaments. Interdigitating dendritic cells are also present. The intimate association of epithelial cells and lymphocytes facilitates the direct transport of antigen from external environment to the tonsillar lymphoid cells. <sup>1</sup> The palatine tonsils themselves are frequently prone to infection as indicated by the frequency of tonsillectomy. Streptococcus is the commonest bacteria causing tonsillitis. Hokonohara et al <sup>2</sup> in an animal experiment demonstrated the susceptibility of tonsils to infection on exposure to Group A streptococci, the pharynx, larynx, trachea, bronchi and lungs being resistant. The

infection apparently occurs through the micropore of the crypt epithelium. A. Jacobi in his presidential address in 1906: "The tonsil as a portal of microbic and toxic invasion" stated: "A surface lesion must always be supposed to exist when a living germ or toxin is to find access.---- Stohr has shown small gaps between the normal epithelia of the surface of the tonsil". He also quoted George B. Wood: "It has been proven by several very thorough and capable investigators that foreign bodies in the crypts can pass through the epithelium into the inter follicular tissue". <sup>3</sup> A.J. Wright observed in 1950: "The epithelium of these crypts is frequently absent in parts, thus bringing the lymphoid tissue into direct contact with the surface." <sup>4</sup> A breach in the continuity of the epithelium could explain the susceptibility of the tonsils to infection and we carried out a pilot study to investigate the integrity of the crypt epithelium by using immunostaining for cytokeratin.

## MATERIAL & METHOD

Ethical approval was obtained from the hospital ethical committee and ethical

standards were followed.

Thirty-four consecutive patients who were admitted for tonsillectomy by author, A.O. were recruited. No one refused to participate in the study.

The indication for tonsillectomy was repeated tonsillitis in one group and large tonsils causing obstructive sleep apnoea without any history of tonsillitis in another group. The tonsils were removed by sharp / blunt dissection with minimal possible trauma to the tonsillar tissue. The two sides were dealt with separately, but in an identical manner. They were marked “side one” and “side two”. Laboratory methods (including the method used for producing the immunohistochemically stained sections). The tonsillar tissue was fixed in 10% buffered formalin. Representative slices from each tonsil were embedded into paraffin wax and 4µ sections stained with haematoxylin and eosin. One section with the best array of crypts from each tonsil was chosen and was pretreated with 0.1% solution of Trypsin in 5% calcium chloride solution for 10 minutes at 37 c for antigen retrieval and thereafter was treated with DAKO monoclonal Mouse Anti-Human Cytokeratin antibody, Clone MNF 116. The later reacts with cytokeratins 5,6,8,17 and probably also 19, it shows an especially broad pattern of reactivity with human epithelial tissue from simple glandular to stratified squamous epithelium (www.dako.co.uk).

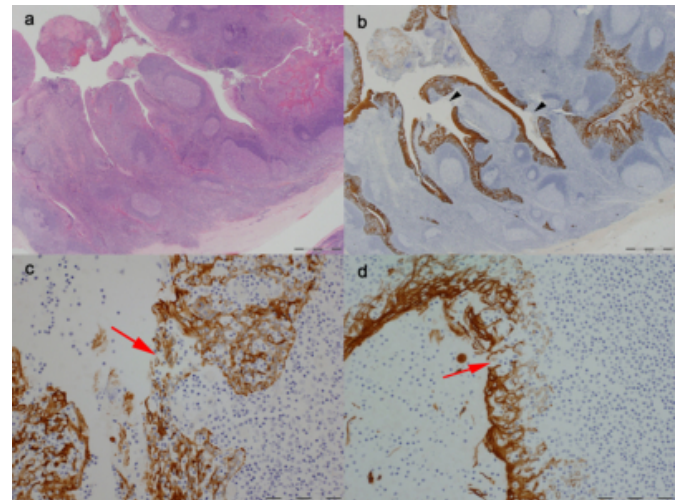
The sections were examined by light microscopy and apparent discontinuities in the crypt epithelium were sought and recorded as being present or absent. All the crypt epithelium in each slide was systematically examined. Foci of artefactual discontinuity were not included (Figure 1b). Apparent epithelial discontinuity was regarded as being present if an imaginary line could be traced from the crypt lumen to the underlying lymphoid tissue without crossing visible epithelium (Figures 1c and d). The microscopic examination was carried out without knowledge of the patient’s identity or medical history.

Any ulceration of the tonsillar surface was noted.

The examination was done by a single observer ( JRM )

**Figure 1**

Figure 1: Photomicrographs of tonsillar tissue. Low power H and E stained section of unremarkable tonsil, original magnification x 12.5 (a). AE1/3 immunostained section of same area showing artefactual discontinuity of squamous epithelium (arrowheads), original magnification x 12.5 (b). High-power view of apparent epithelial discontinuities (arrows), AE1/3 immunostain, original magnification x 200 (c and d).



**RESULTS**

There were 17 patients (M13, F4) in the tonsillitis group and 17 patients (M5, F12) in the non-infective ( hypertrophy ) group. The age of the infective group ranged from 4 to 32, mean 14.9 years of age and the non-infective group ranged from 2 to 55, mean 10.7 years of age. None of the 68 tonsils had ulcers histologically. Of the total 68 tonsils examined, there was an epithelial discontinuity in 21 (30.9%). The distribution of the epithelial discontinuity is shown in Table I and Table II

**Figure 2**

Table 1: Diagnosis vs. discontinuity

	Infection	OSA	Total
No discontinuities	5 (31.3%)	11 (68.8%)	16
One or more discontinuities ( Unilateral or bilateral)	12 (66.7%)	6 (33.3%)	18
<b>Total</b>	<b>17</b>	<b>17</b>	<b>34</b>

P (Fisher’s exact test) = 0.08

**Figure 3**

Table 2: Diagnosis vs. number of discontinuities

	Infection	OSA	Total
No discontinuities	5 (31.3%)	11 (68.8%)	16
One discontinuity	9 (60.0%)	6 (40.0%)	15
Two discontinuities	3 (100.0%)	0 (0.0%)	3
Total	17	17	34

P (Fisher's exact test) = 0.06  
 P (Cuzik test for trend) = 0.02

The finding of Table 1 suggest that there is more infection in those with discontinuities of the crypt epithelium but it is only of borderline significance.

The data in Table 2 is limited as only three cases had bilateral discontinuities and all had infection.

**DISCUSSION**

Background: The recurrent nature of acute tonsillitis has been attributed to the bacteria surviving intracellularly, thus avoiding antibiotic exposure and subsequently escaping the cellular confine to cause reinfection <sup>5</sup>. The presence of a bacterial bio film in the tonsillar crypts might explain the chronicity and recurrent nature of some form of tonsillitis. The bacteria in the bio film would be out of reach of antibiotics <sup>6</sup>. Anders Ebenfelt and colleagues in their careful study came to the conclusion that the inflammatory process takes place outside the surface of the epithelial cells. It takes place in the secretion with hardly any direct contact between the bacteria and the epithelial surface and no penetration of bacteria in the cells. This is contrary to conventional wisdom and also would not explain how tonsillitis is associated with peritonsillar abscess <sup>78</sup>. It has been reported that the bacteriological profile of tonsillar core swab culture and fine needle aspirate culture is very similar in groups of patients with tonsillitis <sup>9</sup>. Several authors have reported that there is a high bacterial load in tonsils in groups of patients with tonsillitis compared to control groups without tonsillar pathology <sup>91011</sup>. The bacterial load is equally high in groups of patients with tonsillar hypertrophy without tonsillitis compared to control subjects without any tonsillar pathology <sup>1011</sup>. Both tonsillitis and tonsillar hypertrophy are associated with marked elevation of immunologically active cell population in the tonsils compared to the control <sup>11</sup>. It would be reasonable to deduce that high bacterial profile in the tonsils in some subjects causes tonsillitis and in others it leads to tonsillar hypertrophy without clinical symptoms of tonsillitis. Immunological studies are often based on tonsillar

tissue <sup>121314151617</sup> and it would be tempting to conclude that tonsils have a special immunological function inducing researchers to choose tonsils, but the easy accessibility of tonsillar tissue might be the other explanation or at least a partial one. The tonsils may however have some special immunological function, eg polymeric IgA1 deposited in glomerular mesandium in IgA nephropathy is at least in part of tonsillar origin <sup>18</sup>. There is an inverse relationship between tonsillitis and asthma <sup>19</sup>. Scrutiny of subpopulation of IgG secreting cells from tonsils and bone marrow showed CD38 low IgG secreting cells were present exclusively in tonsils <sup>20</sup>. The concentrations of disease- related prion protein in variant Creutzfeldt-Jakob disease are not uniform throughout the lymphoreticular system but are consistently higher in tonsils <sup>21</sup>. New findings: As mentioned earlier there has been a historical concept that there are actual gaps in the epithelial covering in the tonsillar crypts allowing entry of pathogens to the tonsillar parenchyma <sup>4</sup> and we have tried to examine the validity of this concept in our study. We have demonstrated by cytokeratin immunostaining that an apparent breach in the crypt epithelium occurred in 30.9% of the tonsils examined. The epithelial breach is commoner in the tonsillitis group of patients (70.6%) than the tonsillar hypertrophy group of patients (35.3%) but the difference is only of moderate significance. It is thus not possible to conclude that the susceptibility of tonsils to infection is due to a breach in the epithelial lining. Comparison with other studies: Sako, Wake and Watanabe in their immunohistochemical study using anti-keratin antibodies and transmission electron microscopy of the epithelium of the tonsillar crypt observed that the infiltration of lymphocytes into the crypt epithelium induces changes in keratin expression of the epithelial cells. The change of keratin molecules makes the stratified flattened epithelium transform into the network of star shaped reticular cells. The electron microscopy showed, in the deep portion of the crypt, strikingly long and slender cytoplasmic processes of the epithelial reticular cells adherent to each other constructing a lace-like appearance. The widened intercellular spaces were filled with amorphous electron-lucent materials and lymphocytes <sup>22</sup>. Tang, Hori, Osamura and Tsutsumi in their extensive immunohistochemical analysis characterised the lymphoepithelial complexes of the tonsillar crypt. The reticular epithelial network was displayed by cytokeratin immunostaining. They observed some common morphological characteristics between the tonsillar reticular mucosa and thymic medulla. Although the tonsillar reticular epithelium has certain specific and important roles in the

differentiation of the intra epithelial lymphoid cells they particularly noted the lack of secretory function of the epithelial cells compared to other examples of mucosa-associated lymphoid tissue eg Peyer's patches. Ultrastructurally wide intercellular space (channel) was often present between the epithelial cells, and lymphoid cells were distributed therein<sup>23</sup>. They did not specifically look for areas of "missing epithelium". The evidence from our observation and findings of the recent observers mentioned above might indicate that susceptibility to tonsillitis is determined by an immunological difference of the subjects rather than a mechanical failure in the tonsillar structure. Weakness: The weakness in our study is a lack of normal control without any tonsillar pathology. It is not easy to recruit normal control for obvious reasons. The relatively small sample size of this pilot study (although large enough for analysis) is another weakness. Confocal study might have demonstrated the epithelial breach more clearly.

## CONCLUSION

Tonsils are very prone to infection despite their presumed protective function. A breach in the tonsillar crypt epithelium has been reported before. We examined the crypt epithelium by cytokeratin immunostaining in a tonsillitis group and a tonsillar hypertrophy group of patients. There was a breach in the crypt epithelium in a proportion of cases in both groups but the difference was only of a borderline significance. The evidence from our observation and work of the recent observers indicate that susceptibility to tonsillitis might be determined by an immunological difference of the subjects rather than a mechanical failure in the tonsillar structure. A further study with larger number of patients including normal control (ethics permitting) including confocal studies would be worthwhile to confirm the findings of our pilot study.

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