Histology, Histochemistry and Scanning Electron Microscopy of Tonsil of the Soft Palate of the Young Pigs

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Abstract
The present study was conducted on tonsil of soft palate of 10 young male pigs of 8-10 months age, of local mixed breed. The surface of the tonsil was lined by stratified squamous non-keratinized epithelium. The surface epithelium was modified into reticular epithelium towards the deeper portion of crypts. The crypts or holes of different shapes and size extended up to the propria submucosa. A thick uniform layer of collagen fibres bundles present in subepithelial portion separated superficial and deep portions of connective tissue. The lymphoid follicles of different shapes and size were separated by parafollicular and interfollicular areas which were having more number of fine blood capillaries and high endothelial venules. The glandular acini present in propria submucosa were positive for glycojen, weakly sulphated mucopolysaccharides, hyaluronic acid and salomucins. The glandular acini showed predominance of neutral mucopolysaccharides. The scanning electron microscopy revealed surface of the tonsil of soft palate having longitudinally oriented small folds of mucosa. In between these folds, crypt or holes of varying shapes and dimensions were observed. At higher magnification it showed the flat cells having microplicae of different arrangements.

Key words: High Endothelial Venules, Reticular epithelium, Tonsil of Soft Palate, Young Pigs.

1. Introduction
The mucosa-associated lymphoid tissue of pharyngeal region plays a functional role in protecting the mucosal surfaces of the digestive and respiratory tracts through cell-mediated and humoral immune responses. In the pharyngeal area of pigs, the lymphoid tissue is concentrated in the posterior part of the soft palate, referred as the tonsil of the soft palate. The surface of the tonsil presented numerous invaginations or tonsillar crypts, which penetrated into the lymphoid tissue. Tonsillar crypt epithelium differed from that of stratified squamous epithelium of the oral mucosa. Although the main differences arise from the migration of various kinds of lymphoid cells into the intercellular space of epithelial cells, recent evidence suggested that the structure of the crypt epithelium may be more complex in pigs, including the presence of goblet cells (Ramos et al., 1992). The well-developed lymphoid follicles consisting of germinal centres and parafollicular and inter-follicular areas were populated with subsets of CD4, CD8 positive and B-lymphocytes (Kumar and Timoney, 2006). A lack of systematic

study led to pursue the present study to explore a comprehensive histological architecture of the tonsil of soft palate in the pigs.

2. Material and Methods
2.1 Light Microscopy
The present study was conducted on 10 young male pigs of 8-10 months age, of local mixed breed. The heads were procured from local slaughter house immediately after decapitation. The mid sagittal sections of heads were made to collect tissues. The tissues for histomorphological and histochemical studies were used from 5 heads and fixed in 10 per cent neutral buffered formalin for 48 hours. The fixed tissues were processed for routine paraffin technique of light microscopy. The paraffin sections of 5-6 μ were cut and stained with routine Harris’ hematoxylin and eosin stain, Gomori’s method for reticulum, Weigert’s method for elastic fibres, McManus’ method for glycojen (PAS), Alcian blue method for mucopolysaccharides, and Passa’s method for melanin. The lymphoid follicles, germinal centers, and inter-follicular areas were stained with alkaline phosphatase method of McManus (1960). The lymphoid sinuses and interfollicular areas were stained with Weigert’s method for elastic fibres. The elastic fibres of the muscle bundles were stained with the Verhoeff’s van Gieson method.
Figs 1-8: Photomicrograph of tonsils of soft palate showing: 1. Stratified squamous non-keratinized epithelium, reticular epithelium, crypts and lymphoid tissue. H. & E. x 100. 2. Reticular epithelium, crypt, lymphoid follicles being separated by interfollicular areas. H. & E. x 100. 3. Crypts of varying shapes and size and arrangement of lymphoid tissue. H. & E. x 100. 4. Distribution of collagen fibres in propria submucosa. Note dense arrangement of collagen bundles separating lymphoid and glandular tissues. Crossman’s trichrome x 100. 5. Distribution of reticular fibres in propria submucosa especially around the lymphoid follicles and interfollicular areas. Gomori’s method x 100. 6. Distribution of elastic fibres in the interglandular areas. Weigert’s method x 100. 7. The lymphoid follicles, interfollicular areas and high endothelial venule. H. & E. x 400. 8. Strong PAS positive reaction in mucus type of glandular acini. Note a weak reaction in connective tissue of subepithelial portion. McManus PAS method x 100.

2.2 Scanning Electron Microscopy

Fresh tissues from 5 pig heads were collected for scanning electron microscopy. The tissues were fixed in 2 per cent glutaraldehyde solution for 6 h after thorough washing with chilled 0.1 M phosphate buffer (pH 7.4). The tissues were again washed twice with 0.1 M phosphate buffer and rest of the procedure was carried out at EM-Laboratory, AIRF, JNU, New Delhi. The processed tissues were viewed in scanning electron microscope (Zeiss EVO-40).

3. Results and Discussion

The tonsil of soft palate of pig was lined by stratified squamous non-keratinized epithelium with uniform free surface and internal papillary pegs towards basal surface (Fig. 1) as reported earlier (Belz and Heath, 1996; Casteleyn et al., 2011; Liu et al., 2012) and horse (Kumar and Timoney 2006). However, nasal surface of the soft palate in sheep was lined by pseudostratified columnar ciliated epithelium and oral surface by stratified squamous keratinized epithelium (Cocquyt et al., 2005; Kumar and Singh, 2014). The epithelium was comprised of strata basale, spinosum -

Figs 12-15: Scanning electron micrograph of TSP showing 12. Surface of TSP with crypts or holes of varying shapes and size. x 56 (Bar = 300 µ). 13. Surface of TSP. Note presence of outgrowth in the lumen of crypt. x 128 (Bar = 100 µ). 14. At higher magnification showing longitudinally arrangement of folds. x 217 (Bar = 100 µ). 15. At higher magnification showing presence of closed type of microplicae. x 12280 (Bar = 2 µ).
and superficial. The stratum basale was constituted by a single row of cuboidal to low columnar cells having round to oval vertically oriented nuclei and presented chromatin material distributed irregularly throughout the nucleoplasm. The stratum spinosum was comprised of 4-6 rows of cells having nuclei of varying shapes and size oriented in different directions. The nuclei were less basophilic due to homogeneously distributed fine chromatin material. The large nuclei became more elongated toward the superficial layers. Their eosinophilic cytoplasm had a prickly appearance. Depressions on the outer free surface led into holes or small crypts with small openings. Well-developed crypts were observed as reported earlier in pigs and horses (Belz and Heath, 1996; Kumar and Timoney, 2006).

The surface epithelium was modified into reticular epithelium toward the crypts (Figs. 1, 2). The crypt epithelium was covered by a mixed population of stratified epithelial cells and was modified by the infiltration of leucocytes to form a specialised lympho-epithelium referred as reticular epithelium as reported in pig (Belz and Heath, 1996) and horse (Kumar and Timoney, 2006). The crypts in pig tonsils provided a larger surface area for contact between the crypt epithelium and luminal contents. The basement membrane was interrupted in the areas where infiltration of lymphoid cells was more as reported in the horse (Kumar and Timoney, 2006).

Loose irregular connective tissue, lymphoid and glandular tissues comprised the propria submucosa (Figs. 3, 4, 5, 6) as reported in the horse (Kumar and Timoney, 2006) and sheep (Kumar and Singh, 2014). The superficial and deep portions of the propria were separated by a dense arrangement of collagen fibres.

The lymphoid follicles of different shapes and size mainly possessed germinatal centres but only a few had darkly stained corona (Figs 1, 2, 3) as reported in the horses (Kumar and Timoney, 2006). Lymphoid tissue was separated from adjacent glandular tissue by a dense arrangement of collagen and elastic fibres and a few reticular fibres (Figs 4, 5, 6) and fine blood capillaries and bundles of striated muscles. The lymphoid follicles comprised of small, medium, and large sized lymphocytes, plasma cells and macrophages whereas, the parafollicular and interfollicular areas were having more number of fine blood capillaries and high endothelial venules (Fig 7) as reported in different species (Belz, 1998; Kumar and Timoney, 2006 and Kumar and Singh, 2014).

The stratified squamous non-keratinized and reticular epithelium did not exhibit PAS reaction however, clusters of mucus glandular acini exhibited PAS positive reaction for glycopen, neutral, and acidic mucopolysaccharides (Figs 8, 9) as reported in the horses (Kumar and Timoney, 2006), dog (Arrighi et al., 2011). The acini were also positive for weakly sulfated acidic mucopolysaccharides, hyaluronic acid and sialomucins as demonstrated by Alcian blue reaction. The glandular acini showed predominance of neutral mucopolysaccharides. However, these mucopolysaccharides were equally distributed in the sheep (Kumar and Singh, 2014). The glandular acini also showed strong positive reaction for colloidal iron and Mayer’s mucicarmine methods (Figs. 10, 11).

Scanning electron microscopy revealed that surface of the tonsil of the soft palate had longitudinally oriented small folds of mucosa (Figs. 12-14) whereas; the corresponding tonsillar surface of the horse appeared irregular with circular and polygonal markings (Kumar and Timoney, 2006). Holes or small crypts with oval or elongated openings were irregularly distributed (Figs. 12, 13) as reported earlier in the pigs (Belz and Heath, 1996) and horse (Kumar and Timoney, 2006). Higher magnification of the surface epithelium revealed patterns of micropiciae (Figs. 15, 16) as reported in horse (Kumar and Timoney, 2006). Microvillus and goblet cells reported in the tonsil of the soft palate of the pig (Belz and Heath, 1996) could not be demonstrated during present study.

4. Conclusion

The structure of the tonsil of the soft palate of pigs revealed reticular epithelium towards the crypts and large amount of lymphoid tissue which is suggestive of mucosa associated lymphoid tissue. The tonsil of the soft palate may play role in early pathogenesis of different diseases of the pigs.
References