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Research Article

TRANSMISSION ELECTRON-MICROSCOPIC STUDIES ON TONSIL OF SOFT PALATE OF THE BUFFALO (BUBALIS BUBALIS)

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ABSTRACT

Transmission electron microscopy was employed to examine tonsil procured from 06 heads of adult buffaloes of local mixed breed. The tonsil was lined by stratified squamous non-keratinised to partly keratinised epithelium. The cells of stratum basale oriented vertically were attached with each other by desmosomes. The stratum spinosum and superficiale had varying number of rows of cell layers and contained different cell organelles. The cell layers of stratum corneum were having serpentine course with very less concentration of organelles and their free ends presented spicule like pattern. The stratified epithelium was modified into reticular epithelium due to infiltration of lymphoid cells from underlying lymphoid tissue. The propria submucosa was having lymphoid aggregations constituted by lymphocytes of different size, plasma cells and macrophages. The high endothelial venules, present towards parafollicular areas presented the trafficking of lymphocytes by inter-endothelial and transendothelial routes.

Keywords: Tonsil of soft palate, microplicae, vesiculo-vacuolar organelles, buffalo

The tonsil of the soft palate constitutes part of lymphoid tissue of the Waldeyer's ring with a significant role in mucosal protection against alimentary and airborne pathogens (Perry and White, 1998). The tonsils are typical secondary lymphoid organs (Pabst, 2007) which lack afferent lymphatics but their surface area is greatly enlarged by the crypts being outlined by the reticular epithelium, which enables the uptake of particles and antigen (Chaker, 2015). In addition, the extrafollicular areas, the mantle zones of lymphoid follicles and the follicular germinal centers are specialized morphological compartments of the tonsils which contribute to immune functions (Brandtzaeg, 1998). The light microscopic features of the tonsil of soft palate have been studied in pigs (Ranjit*et al*., 2016) and buffaloes (Girgiri and Kumar, 2018). Thus, the present study was aimed at describing the basic ultrastructural features of the tonsil of soft palate in the buffalo by transmission electron microscopy.

MATERIALS AND METHODS

Fresh tissues were collected from 6 heads of local mixed breed of adult buffaloes and primarily fixed in 2.5% glutaraldehyde solution for 6-8 hours and post-fixed in 2% osmium tetraoxide for 1 hour at 4° C. The tissues were washed with chilled 0.1M phosphate buffer, dehydrated in grades of alcohol, infiltrated with resins and blocks were prepared with epoxy resin. Thin sections of 1µ were stained with toluidine blue to select the most appropriate area of the tissue. The ultrathin sections (50-70 nm) were taken on copper grids, stained with lead citrate and uranyl acetate and examined under transmission electron microscope (Technai G2) at AIIMS, New Delhi.

RESULTS AND DISCUSSION

The tonsil of the soft palate was having stratified squamous non-keratinized epithelium (Fig. 1A) except at few places where the keratinized type of epithelium was observed. The vertically oriented nuclei of stratum basale were electron-lucent which showed condensation of chromatin into smaller patches only towards the outer nuclear membrane (Fig. 1B) and contained one centric/eccentric electron-dense nucleolus. These nuclei presented irregular uneven surface and at places showed the nuclear indentations. The nuclei were wider in the proximal part and narrow tapering ends towards the basement membrane. The electronplasm of the cells contained the varying number of mitochondria, smooth and rough endoplasmic reticulum, Golgi bodies, ribosomes and filaments. The adjacent cells were attached at different places by desmosomes and their intercellular spaces presented the interdigitating villi which were closely associated with each other. At places, lymphocytes infiltrating in between epithelial cells of the basal strata were regular features as described in the horse (Kumar and Timoney, 2006) and ovine (Casteleyn *et al*., 2010).

The stratum spinosum also had varying number of rows of nuclei and their nuclei were comparatively more electron-lucent as compared to those of the stratum basale cells. These nuclei presented electron-dense chromatin in small concentration only towards the outer nuclear membrane (Fig. 1 C). These nuclei contained generally one nucleolus which was centric/eccentric in position. The outer surface of the nuclei was also irregular and at places showed nuclear indentations. The electronplasm of these cells had distribution of cell organelles similar to those of basale cells. However, the concentration of tonofilaments was comparatively more. These adjacent cells were also attached by numerous desmosomes. The concentration of these nuclei was comparatively lesser in the superficial layers however; ultrastructural features were same except

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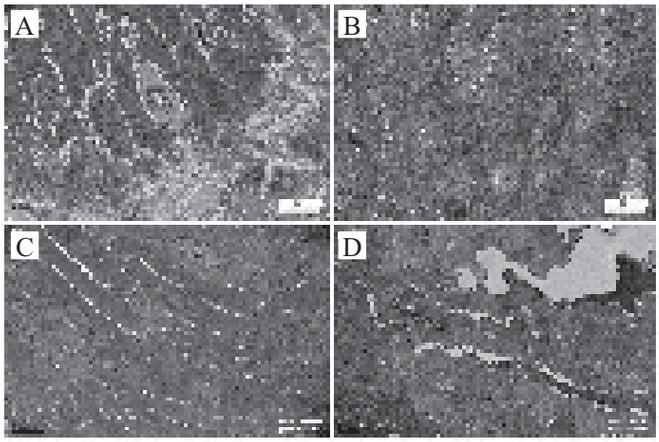

Fig. 1. Transmission electron micrograph of the non-keratinized epithelium showing (A) The cells of stratabasale and spinosum.x 570; (B) The cell of stratum basale at higher magnification.x2550; (C) Higher magnification of the cell of stratum spinosum. x2550; (D) The cells of stratum superficiale with small microvilli towards free surface. x 2550

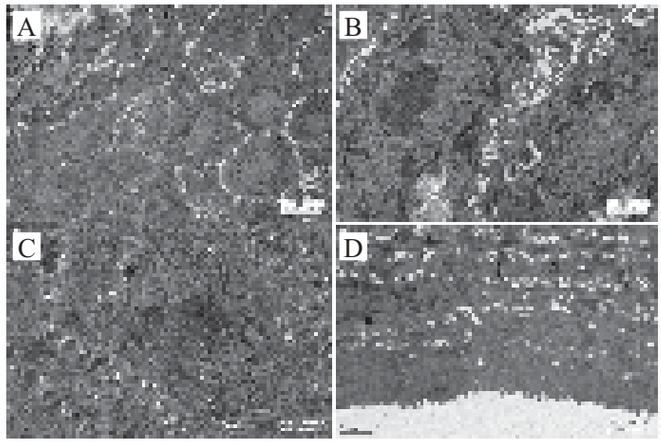

Fig. 2. Transmission electron micrograph of stratified squamous keratinized epithelium showing (A) Cells of stratum basale. Note the infiltration of lymphocyte. x 830; (B)Distribution of cell organelles in the cells ofstratum spinosum. x 2550; (C) The cells of stratified epithelium below the cell layers of stratum corneum. x 830; (D) The stratum corneum of stratified keratinized epithelium. x 830

more distribution of large sized mitochondria and distinctly visible arrangement of tonofilaments.

The cells of the stratum superficiale had the nuclei which were comparatively lesser in number and smaller in dimensions. These nuclei were also electron-lucent because of condensation of very fine chromatin material in smaller aggregates. These cells contained generally one nucleolus which was centric/eccentric in position and these were not distinctly visible in many of the cells (Fig. 1 D). Their nuclei were also irregular in shape with few nuclear indentations. Generally these cells in the deeper layer appeared round to oval in shape and their interdigitating villi were closely associated with each other and the adjacent cells showed numerous desmosomes attachments. The superficial layer of the stratum superficiale presented varying patterns towards the luminal surface and only a few cells showed the nuclei. The cell organelles were very less and the free surface presented microvilli like arrangement. In between these cell layers, the penetrating lymphocytes were also observed at different heights of the epithelium. The luminal surface of most superficially placed cells presented small sized microvilli (Fig. 1 D). In the porcine, the epithelial cells were packed tightly by tight junctions and desmosomes and the thick layer of collagen fibres underneath was frequently infiltrated by lymphoid cells (Liu et al., 2012).

At some places, the stratified squamous keratinised epithelium was also observed (Fig. 2 A).The cells of stratum basale and stratum spinosum were similar to those of non-keratinized epithelium (Fig. 2 B, C). The stratum granulosum was not distinctly visible however; the superficially placed cells below the stratum corneum had large sized tapering ends and electron-lucent nucleus with distinct nucleolus (Fig. 2 C). Their intercellular spaces had small sized microvilli. The cells of stratum corneum were mainly the electron-lucent and possessed very large tapering ends and only a few cells showed the presence of electron-dense nuclei which were pyknotic having degenerative stages (Fig. 2 D). The cells were mainly undulating with the serpentine pattern with very less intercellular spaces. The electronplasm of these cells was devoid of the cell organelles routinely present in the cells. Some of the cells were very electron-dense and were interposed in between these electron-lucent cells.

The stratified squamous non-keratinised epithelium was modified into reticular epithelium (Fig. 3 A) because of infiltration of lymphocytes from the underlying lymphoid tissue present in propria submucosa.

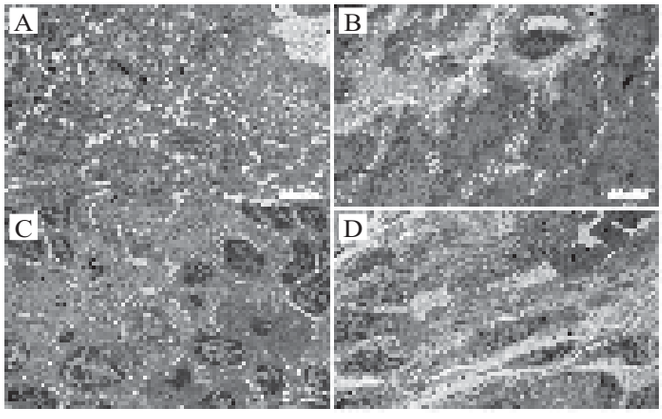

Fig.3. Transmission electron micrograph showing (A) Modification of stratifiedsquamous non-keratinized epithelium into reticular epithelium having a few cell layers. x1100; (B) A regular arrangement of collagen fibres just below the epithelium.x1100; (C) Distribution of lymphoid cells in the propria submucosa. x830; (D) Presence of high endothelial venule, reticular cell and blood capillary in the propria submucosa. x 830

The epithelium was characterized by presence of only a few cell layers without any distinct strata. The cells towards free surface were of varying shapes and contained different cell organelles like those of stratum basale cells especially the mitochondria. In contrast to surface epithelial cells, their comparatively large sized nuclei were electron-lucent having fine distribution of chromatin throughout the nucleoplasm and had distinct one-two centric/eccentric nucleoli. The adjacent cells were attached by desmosomes and presented large sized interdigitating microvilli like arrangement. Large number of lymphocytes was infiltrated in between these cells and occasionally granulocytes especially neutrophils were also observed. The luminal surface of these cells presented large sized knob shaped microvilli like arrangement.

The propria submucosa was comprised of fine blood capillaries, a few reticular cells and aggregates of the lymphoid tissue especially the lymphocytes of varying size and shapes, few plasma cells, granulocytes and macrophages (Fig. 3 B, C) as reported in the horse (Kumar and Timoney, 2006). In addition, interdigitating reticulum cells and follicular dendritic cells were observed in the interfollicular and germinal centres of the lymphoid follicles in the horse (Kumar and Timoney, 2006). A continuous layer of collagen fibres in subepithelial portion (Fig. 3 B) was a characteristic feature as observed in light microscopy (Girgiri and Kumar, 2018). A few high endothelial venules (HEV's) were also observed towards the periphery of the lymphoid tissue or the interfollicular regions (Fig. 3 D). These HEV's had high cuboidal type of endothelial cells and presented the cytoplasmic processes of varying size projecting towards the lumen. These processes presented electron-dense electronplasm and at places some granules and small vesicles like structures arranged in different patterns were observed which were considered as equivalent tovesiculo-vacuolar organelles. In the horse, the endothelial cells of the HEVs contained more glycogen granules with much fewer mitochondria, less endoplasmic reticulum and fewer vesiculo-vacuolar organelles (Kumar and Timoney, 2006).The HEVs are specialized vessels that support abundant lymphocyte migration from peripheral blood into secondary lymphoid organs (Indrasingh et al., 2002). Adhesion molecules on lymphocytes and endothelial cells of the HEV interact in multistep fashion, resulting in rolling, adhesion activation and transmigration of lymphocytes (Zidan et al., 2000; Schaerli et al., 2000). In addition, few lymphocytes were observed trafficking in the form of inter-endothelial migration or transvascular migration as reported in the horse (Kumar and Timoney, 2006). Transvascular passage of lymphocytes via the HEVs is an intercellular process which is significant for selective migration of lymphocyte from the circulation into the lymphatic parenchyma (Marchesi and Gowans, 1964) and may be an essential element in the regulation of lymphocytes level in the peripheral circulation (Vincent and Gunz, 1970).

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