Histological Structure of the Lingual Tonsils of the Buffalo Calf (Bos bubalus)

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ABSTRACT

Key words: Buffalo calf, Lingual tonsils, Histology

Tonsils form Waldeyer's ring which guards all body orifices. They form the first line of defense against foreign antigens and play a key role in initiating immune responses. The lingual tonsils react to ingested antigen and are replication sites of some pathogens. The data about the lingual tonsils of buffalo are lacking, therefore the present work studied the lingual tonsils of 5 buffalo calves (40-60 days). Fresh lingual tonsils were examined macroscopically and used for microscopic and ultrastructural investigations. The lingual tonsils were formed from several elevated macroscopic spherical masses on the dorso-lateral surface of the tongue with central crypt. The surface and crypt was covered with stratified squamous non-keratinized epithelium. An incomplete capsule encloses all the tonsils except at the crypt. The parenchyma was formed mainly from lymphoid follicles and interfollicular diffused lymphocytes. The lymphoid follicles arranged as one layer around the crypts. The interfollicular regions were formed from diffused lymphocytes supported with reticular fibers. High endothelial venules were present among these lymphocytes. Groups of mucous secreting units were distributed among the tonsilar units enclosed within the connective tissue capsule.

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1. INTRODUCTION

The water buffalo represents an important source of animal products in Egypt. The last estimated number of buffalos in Egypt was about 5,317 million. There was an increase in number of water buffalo for next generation in Egypt and in the world (Ibrahim, 2011). The buffalo farming increased in the past decades in both Italy and overseas due to increase the mozzarella consumption (Borghese, 2013). Tonsils form a first line of defense against foreign antigens and form Waldeyer's ring (Zidan and Pabst, 2009) which play a key role in initiating immune responses against antigens entering the body through the mouth (Brandtzæg, 1984). The tonsils classified into two types according to the surface epithelium: tonsils without crypt (e.g., palatine tonsil of the dog and cat) and tonsils with crypt which have deep surface invaginations, referred to tonsillar fossulae (crypts) these invaginations allow for a high concentration of lymphatic tissue in a given area (e.g., lingual tonsil in the horse, ruminants and pig; palatine tonsil in the horse and ruminants) (Dellmann and Brown, 1976). Daniel and Chiego (2013) classified the tonsil according the covering epithelium into two types including: Tonsils covered with stratified squamous epithelium as both of palatine and lingual tonsils, and tonsils covered with pseudostratified columnar epithelium as pharyngeal tonsil. (Kamata, 1992) reported that, the human lingual tonsils was immunologically more active than both palatine and pharyngeal tonsils during middle-age. They were replication sites of some pathogens, among which prions causing bovine spongiform encephalopathy were of major importance in public health. (Liebler-Tenorio and Pabst, 2006) concluded that the tonsils played a significant role in the pathogenesis of several infectious diseases of cattle, and added that, the lingual tonsils are not only important for diagnosing overt diseases, but also for clinically detecting in apparent carriers. Understanding of the structure of lingual tonsils may be useful for adjusting programs of oral vaccination in spite of this, there is no data available about the histological structure and there is no available literature about the lingual tonsils of the Egyptian water buffalo. The present study is the first investigation to the best of our knowledge describe the histological structure of lingual tonsils of the Egyptian water buffalo.

2. MATERIALS AND METHODS

Samples:
Fresh lingual tonsils were obtained from five buffalo calves of 40-60 days old of both sexes slaughtered for human consumption according to the rules of the Egyptian Veterinary Authorities in the abattoir of
Faculty of Agriculture, Alexandria University, Alexandria, Egypt. The samples were prepared for histological and ultrastructure examination as follow:

**Light microscopy**

Each lingual tonsil was examined macroscopically and preserved for histological examination. Specimens from each tonsil were fixed in 10% phosphate-buffered formaldehyde. The fixed specimens were processed for paraffin sectioning. Serial sections (5µm) were prepared and stained as outlined by Bancroft and Steven (1979) using the following stains:

1. Mayer's hematoxylin and eosin stain for general studies (H&E) (Mayer,1937)
2. Crossman's trichrome, for demonstration of connective tissue tissue (Crossman,1937).
4. Periodic acid Schiff (PAS) technique for demonstration of neutral mucin and glycogen (McManus,1948).

**Transmission electron microscopy**

Fresh specimens, about 1mm³ in size, were obtained from the buffalo lingual tonsils and immediately fixed in 4 FIG (2% formaldehyde, 1.25% gluteraldehyde in 0.1 M sodium cacodylate, PH 7.2) and stored at 4 °C, then processed in electron microscope unite, Faculty of Science, Alexandria university. After fixation, the tissues were washed in several changes of cold (4 °C) 0.1 M phosphate every 15 minutes for 2 hours. Then the tissues were post-fixed in 1% solution of phosphate buffered osmium tetroxide (2% osmic acid 5 ml and phosphate buffer 5 ml) for 2 hours at room temperature. Then they were rapidly dehydrated through ascending grades of ethyl alcohol series (30, 50, 70, 90 and 100% for 2 changes) for 30 minutes in each. Then transferred to propylene oxide and placed over night in a 1:1 mixture of propylene and epoxy araldite. Then they were embedded in epoxy araldite (Hayat, 1986). Polymerization of embedding mixture and the tissue blocks was done in an oven for 5 days as following: at 35 °C for 24 hrs, at 45 °C for 24hrs, and lastly at 60 °C for 3 days. Semithin sections (1µm) were cut firstly and stained with toluidine blue and examined with light microscope to select the suitable areas for the electron microscopic examination. Then the ultrathin sections (60-100nms) were cut by a glass knife, then they were stained with uranyl acetate followed by lead citrate (Hayat, 1986). The sections were examined with Joel transmission electron microscope working at 100 cx 80 KVS.

**3. RESULTS**

The lingual tonsils were formed from several elevated macroscopic spherical masses close to each other on the dorso-lateral surface of the tongue just caudal to the last circumvallate papillae and extended near to the epiglottis. Each spherical mass had a central macroscopic apical opening or crypt (Fig1). The outer surface of these tonsils was lined by stratified squamous non-keratinized epithelium of about 18 cell layers (Fig.2) and composed of: 1.Stratum basale resting on a basement membrane and consisted of cuboidal to columnar cells with spherical to oval deeply basophilic vertically oriented nuclei. Their cytoplasm was basophilic (Fig.3). 2. Stratum spinosum consisted of several layers of polyhedral cells with variable number of rows of lightly basophilic spherical to oval nuclei. These cells were intermingled with those of the stratum superficiale. 3. Stratum superficiale consisted of squamous cells with pale elongated nuclei and pale eosinophilic cytoplasm (Fig.2). Each tonsil had a single unbranched crypt (Fig.4). Each crypt was lined with stratified squamous epithelium, non-keratinized continuous with the surface epithelium (Fig.5). A variable number of lymphocytes were observed among the cell layers of the crypt epithelium and rarely plasma cells (Figs.6-8). The parenchyma of the lingual tonsils was formed mainly lymphoid follicles and interfollicular diffused lymphocytes. The lymphoid follicles were mainly primary or secondary with ill developed germinal center surrounded with corona facing the crypt. The lymphoid follicles arranged as one layer around the crypts and enclosed within an incomplete capsule extended to the epithelial surface (Fig.9). Lymphoid follicles were formed mainly from lymphocytes, Follicular dendritic cells and reticular cells. A few macrophage and few Plasma cells were also observed. The follicular dendritic cells had an irregular, stellate shape with delicate branching cytoplasmatic process and a central, nuclei. The reticular cells had ovoid nuclei and thin cytoplasmic process (Figs.10-11). Each follicle was enclosed within a reticular fiber network (Fig.12). Interfollicular regions were formed from diffused lymphocytes supported with reticular fibers (Fig.13). High endothelial venules were present among these lymphocytes. These venules were lined by cuboidal endothelial cells attached with desmosome. Their nuclei were polyhedral. Several migrating lymphocytes were observed in the wall of the high endothelial venules (Figs.14-16). The cytoplasm of
these cells contained the grape-like clusters forming the vesiculo-vascular organelle (Fig. 15-16). The lingual tonsils were partially enclosed by a thin connective tissue capsule formed from collagen fibers (Fig. 17). Groups of mucous secreting units were distributed among the tonsillar units enclosed within the connective tissue capsule (Fig. 19). Excretory ducts of these glands opened through the surface epithelium and at the top of the crypt epithelium (Fig. 19). The glandular acini were formed of pale cuboidal cells with foamy cytoplasm and flat nuclei rested at the basal surface (Fig. 20).

Fig. (1): Lingual tonsil of a buffalo calf is formed of spherical macroscopic masses with macroscopic crypts (arrows heads).
Fig. (2): Photomicrograph of lingual tonsil of a buffalo calf showing the stratified squamous non keratinized epithelium, stratum basale (SB), stratum spinosum (SP), stratum superficial (SS) and connective tissue (CT). H&E stain (Mic.Mag.X400).

Fig. (3): Photomicrograph of lingual tonsil of a buffalo calf showing the basement membrane (b) underlines the stratum basale. PAS stain. (Mic.Mag.X1000).
Fig. (4): Photomicrograph of lingual tonsil of a buffalo calf showing deep crypt (C) lined with stratified squamous epithelium (CE), lymphoid follicles (F), capsule (Ca) and mucous gland (G) between the tonsillar unit. H&E stain. (Mic.Mag.X32).
Fig. (5): Higher magnification of previous photomicrograph of lingual tonsil a buffalo calf showing the stratified squamous non keratinized cryptal epithelium is infiltrated with a number of lymphocytes (L). H&E stain. (Mic. Mag. X400).

Fig. (6): Transmission electron micrograph of cryptal epithelium, showing the cells of stratum basale (SB) stratum spinosum (SP), stratum superficial (SS) and some lymphocytes (L) among them. (Mic. Mag. X 500).

Fig. (7): Transmission electron micrograph showing the epithelial cells of the superficial layer (SS) of the cryptal epithelium are infiltrated with lymphocytes (L). (Mic. Mag. X1000).

Fig. (8): Transmission electron micrograph of the crypt epithelium showing plasma cell (P) with dilated rough endoplasmic reticulum. Lymphocyte (L) and stratum spinosum (SP).X (Mic. Mag. 2000).

Fig. (9): Photomicrograph of the lingual tonsil of a buffalo calf showing the lymphoid nodules (F) located between the capsule (Ca) and the crypt (C). Mucous glands (G) are found between the tonsillar units. H&E stain. (Mic. Mag. X32).

Fig. (10): Transmission electron micrograph of lymphoid nodules showing numerous lymphocytes (L), follicular dendritic cells(FD), reticular cells (RC) and plasma cell(P) (Mic. Mag. X1000).

Fig. (11): Transmission electron micrograph of lymphoid nodule showing follicular dendritic cell (FD) between lymphocytes (L). (Mic. Mag. X2000).
Fig. (12): Photomicrograph of lingual tonsil of buffalo calf showing the reticular fibers (R) in the interfollicular area (I) and lymphoid follicles (F). Reticulin stain (Mic. Mag. X 100).

Fig. (13): The interfollicular regions supported with reticular fibers (R) and F = lymphoid follicles. Reticulin stain (Mic. Mag. X 400).

Fig. (14): Photomicrograph of the interfollicular area of the lingual tonsil a buffalo calf showing the high endothelial venules lined with cuboidal cells resting on basement membrane. AS stain (Mic. Mag. X 400).

Fig. (15): Transmission electron micrograph showing high endothelial venule (H) and lymphocyte (L). (Mic. Mag. X20000).

Fig. (16): Higher magnification of high endothelial venule showing vesiculo-vascular organelle (VVO), rough endoplasmic reticulum (arrows heads), free ribosome(r), lysosomes (l) and desmosom (arrow). (Mic. Mag. X50000).

Fig. (17): Photomicrograph of a lingual tonsil of a buffalo calf showing that a capsule of collagen fibers (arrows) encloses the tonsil except at the site of the crypt (C), G = gland and surface epithelium (arrow heads). Crossman Trichrome stain (Mic. Mag. X 100).

Fig. (18): Photomicrograph of lingual tonsil of buffalo calf showing lymphoid follicles (F), the mucous gland (G) beneath the capsule (Ca) and between the tonsillar unit. H&E stain. (Mic. Mag. X32).
4. DISCUSSION

The present study showed that the buffalo lingual tonsils were macroscopic several spherical elevated masses close to each other on the caudal surface of the tongue with single apical crypt. This structure is similar to that observed in human (Gray et al., 1973) and horse (Kumar and Timoney, 2005a). This location provided an immunological response against ingested antigens (Brandtzaeg, 1984). Each lingual tonsil was covered with the non-keratinized stratified squamous surface epithelium of the tongue which was continuous with the lining epithelium of the crypt and enclosed with an incomplete capsule encircling the tonsils from all sides except that facing the crypt epithelium. The capsule isolate the tonsilar tissue from the surrounding structure, therefore the antigen can be introduced to the tonsil only through the crypt epithelium to be different from the palatine tonsil where the surface epithelium does this function (Brandtzaeg and Pabst, 2004). The presence of crypts expands the epithelial surface area exposed to antigen (Casteleyn et al., 2008; Palmer et al., 2009). The present study indicates a large number of crypts for the tonsils which bring all the lymphoid nodules into direct contact with the crypts and magnifying the immune response of the lymph nodes to the antigens. Thus, the buffalo lingual tonsils play a main immunological role due to the nature of food (milk) as the cryptal epithelium act as a barrier which samples and translocates antigens to the underlying lymphoid tissue (Perry and Whyte, 1998). Perry (1994) concluded that, the degree of leukocytic infiltration of the crypt epithelium related to the degree of antigen exposure. The lymphoid tissue constitutes the majority of tonsillar structure and is organized into lymphoid follicles and interfollcularly diffusely scattered lymphoid cells. This is similar to equine lingual tonsils (Kumar and Timoney, 2005b) and also palatine tonsils of other species as equine (Kumar and Timoney, 2005c), camel (Zidan and Pabst, 2009) and buffalo (Zidan and Pabst, 2011). The lymph follicles have ill developed germinal centers and a small corona facing the crypt epithelium. Follicular dendritic cells extend among the lymphoblasts in the germinal centers. These follicular dendritic cells bind antigen-antibody complexes to their surface for long periods and are essential for humoral immune responses (Heinen et al.,1995). The ill developed germinal centers may be attributed to that the calves depend in their nutrition on suckling pathogen free milk from healthy udder. The presence of immune response indicates the active role of buffalo lingual tonsils in immune responses. Similar to other species the interfollcular regions were rich in high endothelial venules. Several migrating lymphocytes were observed among their lining endothelium. The high endothelial venules are specialized vessels that support active lymphocyte transmigration from peripheral blood to secondary lymphoid organs depending on molecules on the lymphocytes and corresponding receptors on the endothelial cells (Zidan et al., 2000). The lining epithelium of high endothelial venules was cuboidal with polyhedral nuclei and had vesiculo-vacuolar organelle in the
form of grape-like clusters in the peripheral cytoplasm. These findings are in agreement of the findings of (Zidan et al., 2000) where they showed that vesiculo-vacuolar organelle provided a major mode of extravasation of macromolecules at sites of vascular permeability induced by vascular permeability factors as vascular endothelial growth factor and cytokines. Clusters of mucous glandular acini were observed among the lingual tonsils. Kumar and Timoney (2005a) described similar glands in equine lingual tonsils. These glands discharge their secretion into the crypt or surface epithelium, the secretion of the gland into the crypt was mixed with the cellular contents of the crypt, forming the white discharge observed in buffalo lingual tonsils.

5. REFERENCES


Crossman, G. 1937 A modification of Malloy’s connective tissue stain with a discussion of principles involved.


