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Histoenzymic Localization of Oxidoreductases and Phosphatases in the Tonsils of Naso-pharyngeal region of Buffaloes

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ABSTRACT

The present study was aimed to explore the localization of oxidoreductases and phosphatases enzymes in the nasopharyngeal, tubal and paraepiglottic tonsils of six adult healthy buffaloes. The succinic dehydrogenase, lactic dehydrogenase, glucose-6-phosphate dehydrogenase and glutamic dehydrogenase showed a strong positive activity in the pseudostratified columnar ciliated epithelium and stratum basale layer of the stratified squamous epithelium of the tonsils. The diffuse lymphoid tissue and the striated muscles showed moderate to strong positive reactions. The mucous secretory acini did not show any affinity for these enzymes, however, a uniform moderate to strong positive reaction was observed towards their basement membranes. The acid and glucose-6-phosphatases showed a moderate activity in the respiratory epithelium and the deepest layer of cells next to the basement membrane of the stratified squamous epithelium. The associated submucosal lymphoid tissue and the glandular tissue of the tonsils showed positive reactions like those of the oxidoreductases group of enzymes. A comparison of the pattern of localization of these enzymes in healthy and infected animals may play a significant role in disease diagnosis, if any.

Key words: Buffalo, Oxidoreductases, Phosphatases, Tonsils

INTRODUCTION

Tonsils are located at defined mucosal sites and form a first line of defense against foreign antigens. Histological features like active germinal centres, the presence of plasma cells, follicular dendritic cells, and acid phosphatase-positive antigen-presenting cells indicate the immune competency of the lymphoid organs. The tonsils acted as replication sites for some pathogens and played significant role in the pathogenesis of several infectious diseases of cattle especially prions causing bovine spongiform encephalopathy (Liebler and Pabst, 2006; Donaldson *et al*., 2012). Enzyme histochemistry serves as a link between biochemistry and morphology, and their varying levels may be used as biochemical markers indicating the health status of the animal. Oxidoreductases, a group of enzymes, are actively involved in the physiological process of respiratory chain pathway due to their capability of oxidoreduction reactions. Similarly, phosphatases enzymes play key role in cellular functions due to phosphorylation and dephosphorylation. The present study is the first of its type to demonstrate the qualitative distribution of oxidoreductases and phosphatases group of enzymes in the nasopharyngeal, tubal, and paraepiglottic tonsils of buffaloes.

A detailed light histological, histochemical, and electron microscopic study has been carried out on the tonsils of buffaloes (Girgiri and Kumar, 2019; 2020; 2021). The research work was also done on histoenzymology of the tonsils of oro-pharyngeal region in buffaloes (Kumar *et al.*, 2023), but there is scanty literature available on the histoenzymology of the tonsils of naso-pharyngeal region, which was the main limitation to compare and discuss the present findings with those of previous reports.

However, these specific localizations in healthy animals can be later utilized as markers in different disease conditions. The localization of these enzymes in different tonsils of buffalo may be correlated with physiological functions and may be useful for planning research projects associated with oral vaccination programs.

MATERIALS AND METHODS

The fresh tissues from the nasopharyngeal,tubal, and paraepiglottic tonsils

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of six adult healthy buffaloes of either sex of 5-6 years age of local mixed breed were collected immediately after their sacrifice by captive bolt stunning gun method at the slaughterhouse, New Delhi. An approval of the Institutional Animal Ethical Committee was not required as the collection was done after the death of the buffaloes. The tissues collected in liquid nitrogen were stored at - 20°C, and later blocks were made using tissue freezing medium. The frozen sections of 10-12 µ thickness cut with cryostat microtome at - 20°C were mounted on clean glass slides coated with 3-aminopropyltriethoxy-silane. The sections defrosted at room temperature were circled with a PAP pen slide marker and incubated with different substrates at 37°C to study the distribution pattern of different oxidoreductases and phosphatases enzymes (Table 1).

Table 1: Different substrates used for demonstration of respective enzymes

Enzyme	Substrate	Method
Oxidoreductases		
Succinic dehydrogenase (SDH)	Di-Na-succinate	Standard method of bound enzyme by nitro BT method (Pearse, 1972)
Lactic dehydrogenase (LDH)	Na-DL-lactate	(Pearse, 1972)
Glutamic dehydrogenase (GLD)	Na-L-glutamate	(Pearse, 1972)
Glucose-6-phosphate dehydrogenase (G-6-PD)	Di-Na-glucose-6-phosphate	(Pearse, 1972)
Phosphatases		
Acid phosphatase (ACPase)	Naphthol AS-TR phosphate disodium salt in combination with fast blue RR	Simultaneous coupling azo dye method using substituted naphthols (Barka and Anderson, 1963)
Alkaline phosphatase (AKPase)	Naphthol AS-MX phosphate disodium salt in combination with fast blue RR	Simultaneous coupling azo dye method using substituted naphthols (Barka and Anderson, 1963)
Glucose-6 phosphatase	Glucose-6-phosphate and lead nitrate	Lead nitrate method (Barka and Anderson, 1963)

RESULTS AND DISCUSSION

The nasopharyngeal and tubal tonsils were lined with pseudostratified columnar ciliated epithelium became modified irregularly into follicle associated epithelium due to intimate association with underlying lymphoid tissue. Similarly, the stratified squamous epithelium of paraepiglottic tonsil changed into reticular epithelium due to heavy infiltration of the lymphoid tissue of propria submucosa.

Succinic dehydrogenase (SDH)

A strong activity of the enzyme was observed in the pseudostratified columnar ciliated epithelium of nasopharyngeal tonsil (Fig. 1); whereas moderate to strong reaction in the tubal tonsil (Fig. 2). The stratified squamous epithelium of the paraepiglottic tonsil showed mild to moderate reaction for the enzyme except stratum basale where the activity was strong as compared to other strata (Fig. 3). The lymphoid tissue beneath the epithelium was showing uniform mild to moderate reaction for the enzyme (Figs. 1, 2). The lymphoid tissue, blood vessels of different dimensions and glandular ducts exhibited moderate reaction whereas; no activity was observed in the glandular acini except their peripheral basal portions (Fig. 3). The SDH enzyme found in all the aerobic cells is an essential part of the Kreb's cycle. This enzyme, a mitochondrial enzyme, related to mitotic and secretory activity is involved in oxidation (Pearse, 1972; Barka and Anderson, 1963). The muscle fibres were showing moderate to strong presence of enzyme. The rest of the connective tissue was non-reactive towards the enzyme. The cartilaginous tissue of paraepiglottic tonsil also showed negative activity.

Lactic dehydrogenase (LDH)

The respiratory epithelium of the nasopharyngeal and tubal tonsils showed intense reaction for lactic dehydrogenase (Fig. 4). Similarly, the stratified squamous epithelium of PET had strong affinity for the enzyme towards the stratum basale. The lymphoid aggregates present in propria submucosa showed mild to moderate activity. The reactivity for the enzymes was slightly stronger towards periphery as compared to central part of the lymphoid tissue (Fig. 4). The glandular acini and the associated ducts showed no activity towards the enzyme, however basement membrane of the acini showed weaker reaction for presence of the enzyme. The muscular tissue showed moderate to strong

reaction. The cartilage of paraepiglottic tonsil also showed no reaction. LDH is a membrane bound enzyme present in almost all the organs released after injury in response to the inflammation and may be considered as nasopharyngeal secretions during upper respiratory affections and otitis media (Mansbach *et al.*, 2012). Higher concentration of the enzyme has been reported in cases affected with adeno and rhinoviruses and showed a positive correlation with an increased level of cytokines (Ede *et al.*, 2013). The presence of enzyme indicated an anaerobic cellular respiration and involvement in an increased glycolytic pathway protein synthesis in active cells (Raekallio, 1970).

Glutamic dehydrogenase (GLD)

The reaction was very strong in the respiratory epithelium of the nasopharyngeal and tubal tonsils (Fig. 5). The reaction was strong specifically towards the outermost layers and basal layer of the stratified squamous epithelium of the PET. The lymphoid aggregates also showed moderate to strong reaction for enzyme although the germinal centre showed mild reaction. The muscle fibres exhibited mild reaction. The glandular acini did not show enzymic activity except their peripheral portions. The cartilage of the PET was devoid of activity (Fig. 6).

Glucose-6-phosphate dehydrogenase (G-6- PD)

The enzymic activity was strong in the tonsillar epithelium of the nasopharyngeal and tubal tonsils. The most superficial layers and stratum basale of the PET showed strong reaction for enzyme activity. The lymphoid tissue also showed moderate to strong reaction; however, the peripheral zone of lymphoid mass showed comparatively more positive reaction for the enzyme than the central zone. The muscle fibres showed mild to moderate reaction but rest of the connective tissue was negative for the enzyme activity (Figs. 7, 8). The glandular acini showed no reaction except their basement membranes showing moderate positive reaction (Figs. 7, 8). The reactivity was negative in cartilaginous tissue of paraepiglottic tonsil. Higher concentration of the enzyme was suggestive of more utilization of glucose or an increased protein synthesis (Darzi *et al.*, 1998).

Acid phosphatase

The reactivity was moderate in the respiratory epithelium of the nasopharyngeal and tubal tonsils. A moderate activity was observed in the strata basale and corneum of the stratified squamous epithelium of the PET. Glandular acini were negative for the reaction except a few where fine granular moderate reaction was present (Fig. 9). Lymphoid tissue, blood vessels and the glandular ducts presented strong positive reaction. The muscular tissue was devoid of activity. The acid phosphatase enzyme, in tonsillar tissues, characteristic of the follicular dendritic cells and macrophages may be considered as a marker for the latter adjacent to the T cells (Jesic *et al*., 2006).

Alkaline phosphatase

The enzyme could not be demonstrated in any structural component of all the tonsils except small blood capillaries.

Glucose-6-phosphatase (G-6-Pase)

A moderate reactivity was observed in the epithelium of nasopharyngeal and tubal tonsils; however, the reaction was more at the apical surface of the epithelium. The epithelium of the PET tonsil showed very weak reaction, except the stratum basale where it was showing moderate reaction (Fig. 10). The lymphoid tissue was showing weak reaction in the centre but peripheral area showed moderate positive activity for the enzyme. The G-6-Pase enzyme is a membrane-bound enzyme and has been found to be firmly associated with endoplasmic reticulum and related to carbohydrate metabolism (Chayen *et al.*, 1969). The glandular population showed no reaction for the enzyme. The muscle fibres were also negative for the enzyme activity. The cartilaginous tissue of paraepiglottic tonsil showed negative reaction but connective tissue around its periphery showed some mild activity. A strong G-6-Pase positive reaction in the active cells has been correlated with a higher concentration of the endoplasmic reticulum (Shugyo *et al.*, 1986). It was concluded that a strong affinity of the basal layers of the epithelium for the enzymes indicated their importance in the maintenance and the growth of the epithelial cells. The present qualitative localization of these enzymes in different structural components of the tonsils may be a benchmark in health and disease conditions.

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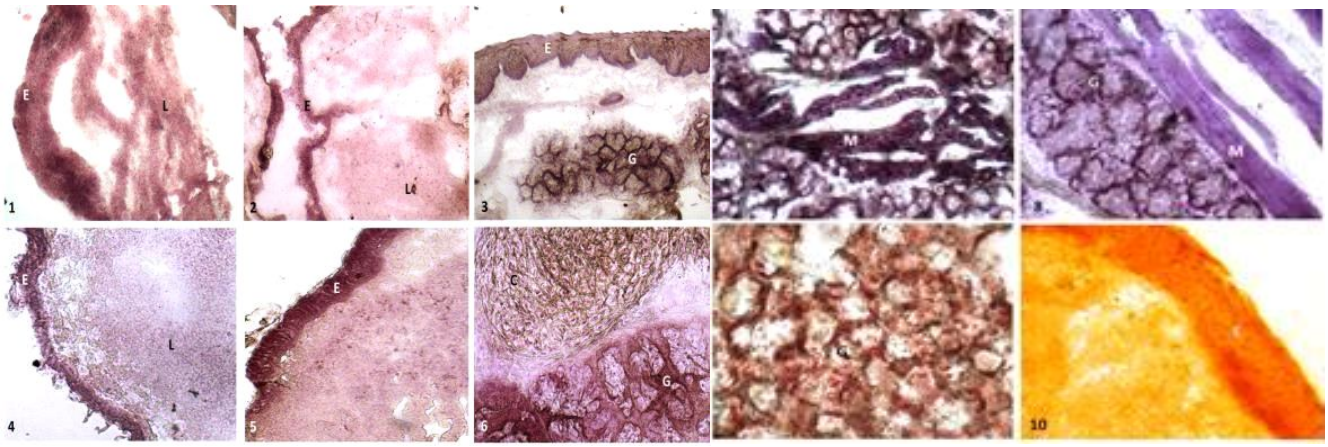

Figs.1-6. Photomicrograph showing xidoreductases activity by Nitro BT method in the tonsils of the buffalo. 1. Succinic dehydrogenase in NPT. x 100; 2. Succinic dehydrogenase in TT. x 100; 3. Succinic dehydrogenase in PET. x 100; 4. Lactic dehydrogenase in TT. x 100; 5. Glutamic dehydrogenase in TT. x 100; 6. Glutamic dehydrogenase in PET. x 100. Epithelium (E), glands (G), lymphoid tissue (L) and cartilage (C). **Figs.7-10.** Photomicrograph showing oxidoreductases activity by Nitro BT method in the tonsils of the buffalo. 7. Glucose-6-P-dehydrogenase in NPT. x 100; 8. Glucose-6-P-dehydrogenase in NPT. x 100; 9. Photomicrograph showing glucose-6-phosphatase activity by simultaneous coupling azo dye method in NPT x 100; 10. PET x 100. Muscles (M), glands (G) and epithelium (E).

REFERENCES

Barka T. and Anderson, P.J. 1963. *Histochemistry : Theory, Practice and*media. *Pediatric Research* 73(3): 349-354. *Bibliography*. 1st edn., Harper and Row Publishers Inc, New York.

Chayen J., Butcher, R.G., Bitensky, L. and Poulter, L.W. 1969. *A Guide to Practical Histochemistry*. Oliver and Boyl, Edinburg, England.

Darzi, M.M., Roy, K.S., Sood, N. and Gupta, P.P. 1998. Histochemical and histoenzymic studies on caprine mammary gland following induced Mycoplasma F38 mastitis. *Indian Journal of Animal Sciences* 68(4): 313-316.

Donaldson, L.J., Hayes, J.H., Barton, A.G., Howel, D. and Hawthorne, M. 2012. Impact of clinical practice guidelines on clinicians' behaviour tonsillectomy. *Journal of Otolaryngology* 28: 24-30.

Ede, L.C., O'Brien, J., Chonmaitree, T., Han,Y. and Patel, J.A. 2013. Lactate dehydrogenase as a marker of nasopharyngeal inflammatory injury during viral upper respiratory infection: implications for acute otitis media. *Pediatr Res.* 73(3): 349-54.

Girgiri, I.A. and Kumar, P. 2019. Histology, histochemistry and ultrastructure of the nasopharyngeal tonsil of the buffalo (*Bubalus bubalis*). *Anatomia Histologia Embryologia* 48: 375-383.

Girgiri, I.A. and Kumar, P. 2020. Light and electron-microscopic studies on the tubal tonsil of the buffalo (*Bubalus bubalis*). *Journal of Buffalo Science* 9: 60-70.

Girgiri, Ibrahim Alhaji and Kumar, Pawan. 2021. Light and electron-microscopic studies on the paraepiglottic tonsil of the buffalo (*Bubalus bubalis*). *Journal of Buffalo Science* 10: 85-98.

Jesic, S., Stojiljkovic, L., Petrovic, Z., Djordjevic, V., Nesic, V. and Stosic, S. 2006. Alteration of adenoid tissue alkaline and acid phosphatase in children with secretory otitis media. *International Journal of Pediatric Otorhinolaryngology* 70: 1069-1076.

Kumar, P., Singh, A., Gahlot, P. and Prakash,T. 2023. Histoenzymic localization of phosphatases and oxidoreductases in the tonsils of oro-pharyngeal region of buffaloes (*Bubalus bubalis*). *Journal of Dairy Veterinary Science* 15(5): 555923.

Liebler-Tenorio and Pabst, R. 2006. MALT structure and function in farm animals. *Veterinary Research* 37: 257-280.

Mansbach, J.M., Piedra, P.A., Laham, F.R.,McAdam, A.J. and Clark, S. 2012. Nasopharyngeal lactate dehydrogenase concentrations predict bronchiolitis severity in a prospective multicenter emergency department study. *Pediatric Infectious Disease* 31(7): 767-769.

Pearse, A.G.E. 1972. *Histochemistry*: *Theoretical and Applied*. 3rd edn., Churchill Livingstone, London.

Raekallio, J. 1970. Enzyme histochemistry of wound healing. *Progress in Histochemistry and Cytochemistry* 1(2): 1-92.

Shugyo, Y., Watanabe, J., Kanamura, S. and Kanai, K. 1986. Glucose-6- phosphatase activity in pregnant and lactating mammary glands of the mouse. *Anatomical Record* 214(4): 383-388.