Original Research

Enzyme Histochemistry of Hemal Nodes of Indian Buffalo (*Bubalus bubalis*)

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Abstract

The present study was conducted on hemal nodes of buffalo to study the histoenzymic distribution of phosphatases and oxidoreductases. The fresh unfixed buffalo hemal nodes were immediately collected after sacrifice and were subjected to cryostat sectioning at -20^0 C with cryostat microtome. The sections were incubated with substrates for demonstration of various enzymes. The study revealed that in both the groups, weak alkaline phosphatase (AKPase) activity was observed in capsule of the hemal node. The AKPase activity was moderate in subcapsular sinus, periphery of lymphoid follicles and diffused lymphocytes. Uniform weak Glucose 6-phosphate activity was observed in entire hemal node. Succinic dehydrogenase (SDH) activity was weak in capsule and lymphoid follicles and weak to moderate in subcapsular sinuses. Lactic dehydrogenase activity was moderate in capsule, sub-capsular sinus and peripheral area of lymphoid follicles. Nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-D) and reduced Nicotinamide adenine dinucleotide diaphorase (NADH-D) was weak to moderate in the capsule and strong to intense in lymphoid follicles. The presence of variable activity of different enzymes in hemal nodes was correlated with maturation of lymphocytes and development of different metabolic pathways.

Key words: Buffalo, Enzyme Histochemistry, Hemal Nodes, Oxidoreductases, Phosphatases

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Introduction

The hemal node is a hemato-poietic and lymphoid organ found in some mammals such as ruminants, humans and rats. In ruminants, hemal nodes are located in the subcutaneous region of the head, mesenteric region and along the large blood vessels such as the aorta in the thorax and abdomen; however, the distribution and number of hemal nodes in these regions seem to be diverse (Casteleyn *et al*., 2008). The functions of hemal nodes had not been fully elucidated. Postulated functions included erythrophagocytosis, erythropoiesis, platelet formation, blood storage and filtration and immune functions in bovines (Cerutti

and Guerrero 2008, Zidan *et al*., 2012). Keeping in view the diverse views expressed about the histophysiology of hemal nodes, the present study was planned to study distribution of various enzymes in hemal nodes of buffalo.

Materials and Methods

The present study was conducted on hemal nodes of buffalo (n=12) of different age groups. The fresh unfixed hemal nodes from buffalo were immediately collected after sacrifice and stored in liquid nitrogen. These tissues were subjected to cryostat sectioning at -20 ^{0}C with cryostat microtome. The sections of 10 - 12 µm thickness were obtained on clean glass slides and were incubated for demonstration of various enzymes as alkaline phosphatase (AKPase), glucose-6-phosphatase (G-6-pase) by coupling azodye method (Barka and Anderson,1963), succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), reduced nicotinamide adenine dinucleotide diaphorase (NADH-diaphorase), reduced nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-diaphorase) and glucose-6-phosphate dehydrogenase (G-6-PD) by Nitro BT method (Pearse , 1972).

Result and Discussion

The histoenzymic activity of different phosphatases and oxidoreductases of hemal nodes in buffalo is detailed below.

Phosphatases

Alkaline phosphatase (AKPase)

The alkaline phosphatase activity was constantly weak in the capsule of the hemal node. The subcapsular sinus showed weak (Fig. 1) to moderate activity whereas stroma of the hemal nodes showed weak activity for AKPase. The activity was moderate to strong in the lymphoid follicles at the periphery (Fig. 2), however weak activity was observed in the germinal centers of the lymphoid follicles (Fig. 3). Lymphoid follicles devoid of germinal centers showed moderate to strong activity. The diffused lymphocytes present loosely in the stroma /parenchyma of hemal nodes were moderately positive for alkaline phosphatase (Fig. 2). Similar observations were made by (Zidan and Pabst, 2004) in endothelium of the sinuses and lymphoid follicles. The frequent association of the enzyme alkaline phosphatase with monosaccharides in the tissue was observed and may be associated with the transport of nutrients (Kumar *et al*., 2017).

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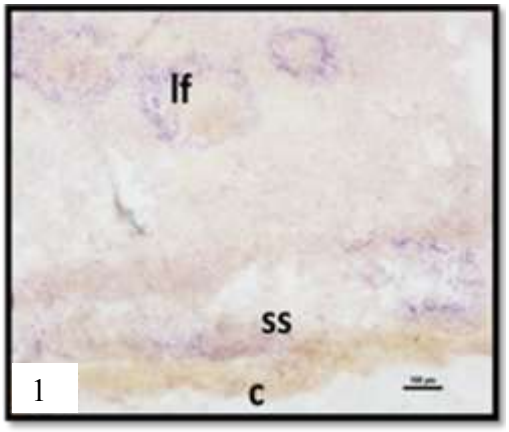

Fig. 1: Cryosection of the hemal node of buffalo calf showing weak activity for AKPase in capsule (c), subcapsular sinus (SS), whereas secondary lymphoid follicle (lf) showed moderate activity in peripheral area. Azodye method X100.

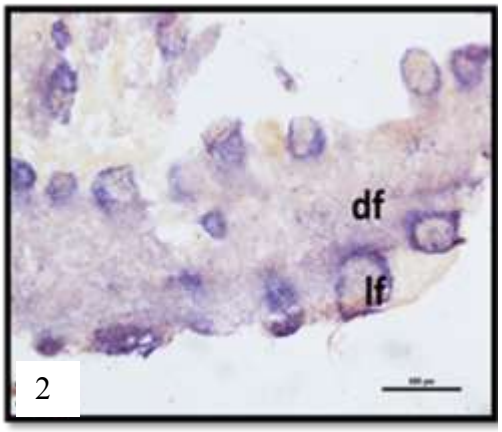

Fig. 2: Cryosection of the hemal node of buffalo showing moderate to strong AKPase activity in diffused lymphocytes (df) and lymphoid follicles (lf). Azodye method X40.

Glucose-6-Phosphatase (G-6-Pase)

Weak to mild activity for Glucose-6-phosphatase was observed in entire hemal node (Fig. 4). Glucose-6-phosphatase consisted of amino acids, anchored to the endoplasmic reticulum (ER) and is involved in the release of glucose into the circulation.

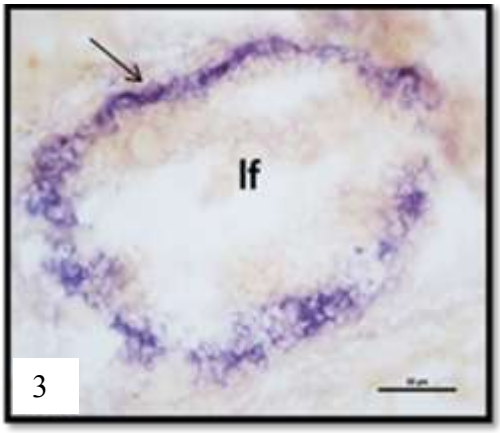

Fig. 3: Cryosection of the hemal node of buffalo showing strong AKPase activity at the periphery (arrow) and weak activity in the germinal centers of the lymphoid follicle (lf). Azodye X100.

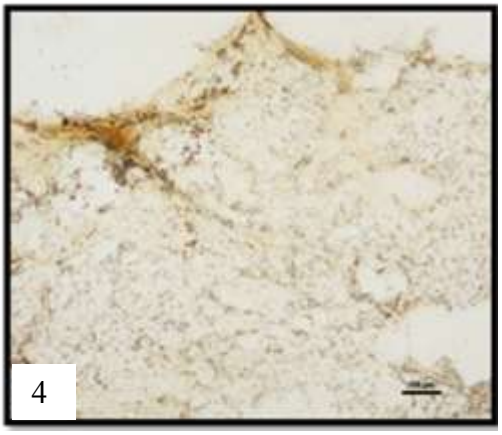

Fig. 4: Cryosection of hemal node of buffalo showing weak activity for Glucose-6-phosphatase (G-6-Pase). Lead nitrate method X100

Oxidoreductases

Dehydrogenase

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Succinic Dehydrogenase (SDH)

In the present study weak SDH activity was noted in the capsule of the hemal node whereas subcapsular sinus showed weak to moderate activity for SDH (Fig. 5). The stroma of the hemal node was weakly positive for SDH. SDH is a mitochondrial enzyme that is involved in generation of energy by oxidation-reduction reaction in the cell (Smith, 1969). This enzyme might be associated with oxidation of fatty acids that lead to formation of lipid pigment granules (Smith, 1969).

Glucose 6 Phosphate Dehydrogenase (G-6-PD)

In the present study, glucose 6 dehydrogenase activity was weak in the capsule and stroma. The cortex showed. Weak G-6-PD activity and lymphoid follicles showed weak activity (Fig. 6). The glucose 6 phosphate dehydrogenase enzyme activity is associated with the pentose phosphate shunt (Fennel and Pearse, 1961). These pentose phosphates might be utilized for nucleic acid synthesis during development. Turkoglu and Aldemir (2003) also reported the Glucose 6-phosphate dehydrogenase (G-6-PD) enzyme activity in all mammalian tissues especially in cytosol and mitochondria.

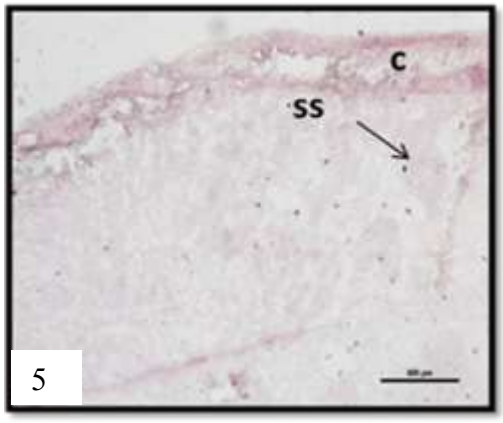

Fig. 5: Cryosection of hemal node of buffalo showing weak to moderate activity of succinic dehyrogenase (SDH) in capsule (c), subcapsular sinus (SS) and lymphoid follicle (arrow). Nitro BT method X40.

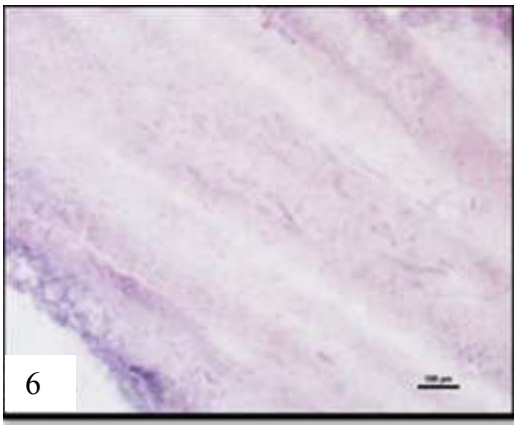

Fig. 6: Cryosection of hemal node of buffalo showing weak activity for Glucose-6-phosphate dehydrogenase (G-6-PD). Nitro BT method X 100.

Lactate Dehydrogenase (LDH)

A moderate activity to lactate dehydrogenase was observed in the capsule of hemal node and lymphoid follicles. The subcapsular sinus showed weak to moderate activity for LDH. The diffused lymphocytes arranged loosely in the parenchyma showed weak to moderate LDH activity (Fig. 7). LDH is an NAD dependent enzyme found in cells in which glycolytic pathway is active. It catalyses the formation of lactate in anaerobic glycolysis and pyruvate in aerobic respiration. The presence of LDH enzyme in hemal nodes

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suggested the presence of glycolytic pathway in cellular elements especially in the capsule and lymphoid follicles.

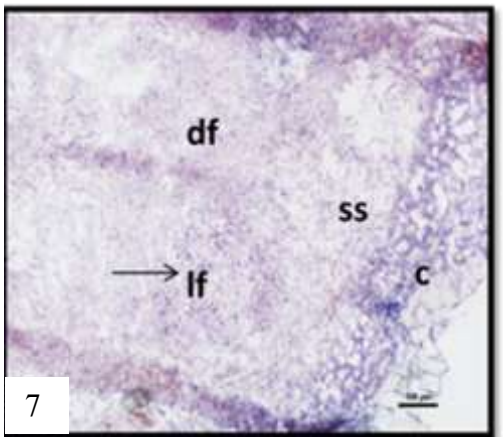

Fig. 7: Cryosection of hemal node of buffalo showing moderate activity for Lactate dehydrogenase (LDH) in lymphoid follicles (lf, arrow), capsule (c), weak activity at subcapsular sinus (ss) and weak to moderate activity at diffused lymphocytes (df). Nitro BT method X 100.

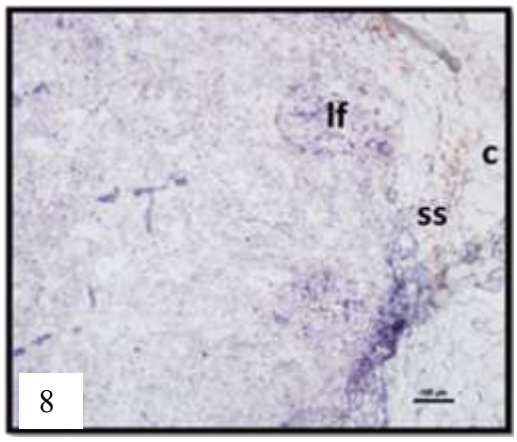

Fig. 8: Cryosection of hemal node of buffalo showing weak activity for Nicotinamide adenine dinucleotide diaphorase (NADH-D) in capsule (c), subcapsular sinus (ss) and moderate activity at lymphoid follicle (lf). Nitro BT method X 100.

Diaphorases

Reduced Nicotinamide Adenine Dinucleotide Diaphorase (NADH-D) and Reduced Nicotinamide Adenine Dinucleotide Phosphate Diaphorase (NADPH-D)

In the present study, weak nicotinamide adenine dinucleotide diaphorase was observed in the capsule of the hemal node (Fig. 8). The activity was weak to moderate in the stroma of the hemal node. In cortex, strong activity was noticed. The activity of this enzyme was intense in the lymphoid follicle (Fig .10 & 11). The periphery of the lymphoid follicle showed moderate activity to this enzyme (Fig. 9). The subcapsular sinus of hemal node showed weak enzyme activity. Connective tissue fibers showed moderate reaction to this enzyme. The cellular elements showed moderate to strong activity. However, intense activity was present in lymphoid follicles. Moderate activity to nicotinamide adenine dinucleotide phosphate diaphorase was present in the capsule of the hemal node (Fig. 9). The subcapsular sinus showed weak to moderate activity to NADPH. In stroma moderate activity of this enzyme was observed. The activity of this enzyme was strong to intense in lymphoid follicles. NADH and NADPH diaphorases are co-enzyme dehydrogenases and acts in the cell as a part of hydrogen transport chain. The enzyme intensity indicated metabolic activity of the cell.

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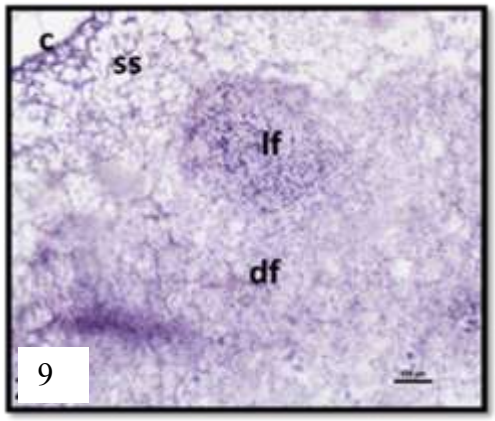

Fig. 9: Cryosection of hemal node of buffalo showing moderate activity for Nicotinamide adenine dinucleotide diaphorase (NADH-D) at capsule (c), lymphoid follicles (lf), and weak activity at diffused lymphocytes (df) and subcapsular sinus (ss) in adult buffalo. Nitro BT method X 100.

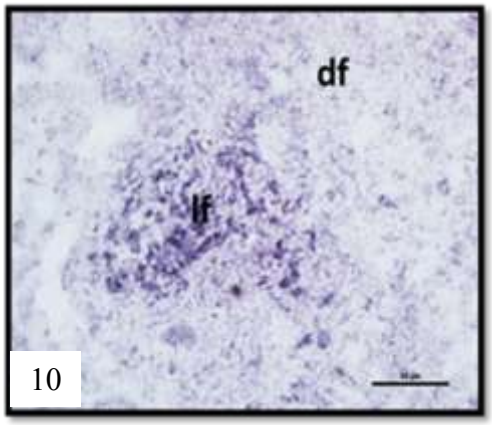

Fig. 10: Cryosection of hemal node showing intense activity for Nicotinamide adenine dinucleotide diaphorase (NADH-D) in lymphoid follicles (lf) in adult buffalo. Nitro BT method X 400.

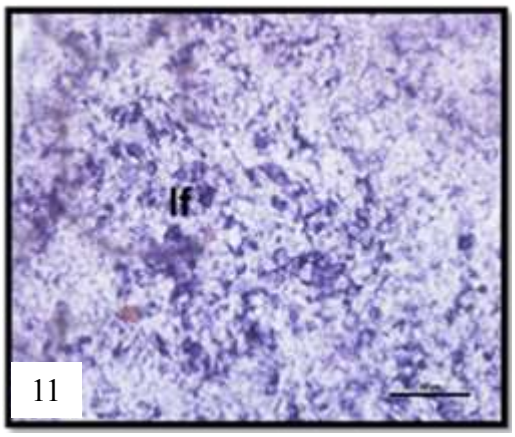

Fig. 11: Cryosection of hemal node showing intense activity for Nicotinamide adenine dinucleotide diaphorase (NADH-D) in lymphoid follicles (lf). Nitro BT method X 400.

Conclusion

The variable activity of phosphatases and oxidoreductases in hemal nodes of different age groups of buffalo reflected the development of metabolic pathways and maturation of cellular elements especially the lymphocytes.

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