

HISTOLOGICAL DIFFERENTIATION OF PAROTID LYMPH NODE IN BUFFALO FOETUSES

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ABSTRACT

The present study was conducted on prenatal development of parotid lymph node of buffalo. A total of 18 foetuses ranging from 17 cm CVRL (105 days) to 108 cm CVRL (317 days) were divided into three groups and parotid lymph nodes collected. The lymph nodes were fixed in 10% neutral buffered formalin (NBF) solution and processed as per acetone benzene schedule. At 17 cm CVRL (105 days), the lymph node parenchyma was clearly divided into primitive cortex (darker) and medulla (lighter). At 19 cm CVRL (114 days) numerous blood vessels were found in differentiating cortical area. The cortex was divided into diffused lymphoid tissue/ paracortical area (deeper area) and lymph nodules from 28 cm CVRL (137 days) onwards. Secondary lymphatic nodules were found at 108 cm CVRL (317 days) in cortex. The lymphocytes were round to oval in shape with irregular boundaries at 21 cm CVRL (121 days). Plasma cells were found in the cortex from 48 cm CVRL (182 days). The cortical thickness doubled in subsequent age groups. The endothelium of blood vessels was fusiform with short and rounded projections in 21 cm CVRL (121 days) buffalo foetus.

Key words: Histomorphology, cortex, buffalo, fetus, parotid lymphnode

Lymph nodes are the only lymphatic organs located in the course of lymphatic vessels with the characteristic function of filtering of lymph before it flows into venous system (Nishioka and Yoshino, 2001). The superficial lymph nodes are significant indications of certain disease processes in animals (Sarma *et al.*, 2003). Therefore, the histogenesis of lymph nodes is of great importance for diagnosis of various diseases during foetal and adult life. Most of the research on superficial lymph nodes has been reported on the postnatal life of goat (Sarma *et al.*, 2008), buffalo (Gadhawe *et al.*, 2011) and pig (Sarma *et al.*, 2006) and during prenatal life, much work has been reported in goat (Asha *et al.*, 2011). But work is very scant on histogenesis of superficial lymph nodes of buffalo fetuses, which prompted this study

MATERIALS AND METHODS

The study was conducted on parotid lymph nodes of 18 buffalo foetuses at different gestational ages which were obtained from pregnant buffaloes slaughtered at an abattoir, in Saharanpur and presented at Veterinary Clinics, GADVASU, Ludhiana. The approximate age of the foetuses was calculated by using the formula given by Soliman (1975).

$Y = 28.66 + 4.496 X$ (CVRL < 20 cm);

$Y = 73.544 + 2.256 X$ (CVRL ≥ 20 cm)

Where Y is age in days and X is CVRL in centimeters.

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Based on CVRL the foetuses were divided into three groups: Group I: Foetuses of CVRL between 0–20 cm; Group II: Foetuses of CVRL between 20 to 40 cm and Group III: Foetuses of CVRL above 40 cm. The parotid lymph nodes were collected, fixed in 10% neutral buffered formalin (NBF) and Bouin's fixatives. They were processed for paraffin sections of 5-7 μ thickness and stained with haematoxylin and eosin for routine morphology, Masson's trichrome for connective tissue, Verhoeff's stain for elastic fibers and Gridley's stain for reticular fibers (Luna, 1968).

RESULTS AND DISCUSSION

Cortex: At 17 cm CVRL (105 days) the lymph node parenchyma was clearly divided into primitive cortex (darker) and medulla (lighter). The outer zone showed condensation of mesenchymal cells with the appearance of lymphoblasts and lymphocytes which were evenly scattered among the differentiating reticular cells (Fig. 1). Krupal *et al.* (1969) reported that the lymph nodes lacked the differentiation into cortical and medullary regions even at 50th day of gestation in pig foetuses. Eikelenboom *et al.* (1978) observed that the primitive cortex and medulla came into existence by 22nd day of gestation in rat. With advancement of age, the number of lymphocytes increased greatly by direct transformation of mesenchymal cells. Our findings were correlated with observation of Bansal *et al.* (2009) in lymphnode of buffalo foetuses.

At 19 cm CVRL (114 days) numerous blood vessels were found in differentiating cortical area (Fig. 2). Each lymphatic nodule was comprised of large, small and medium sized lymphocytes and plasma cells at 20 cm CVRL (119 days) (Fig. 3). The cortex was divided into diffused lymphoid tissue/ paracortical area (deeper area) and lymph nodules from 28 cm CVRL (137 days) onwards (Fig. 3). The lymphatic nodules had more cellular density as compared to paracortical area (Fig. 3). Similarly, Singh *et al.*, (1994) observed that the superficial region of cortex contained varying number of primary nodules of tightly packed small lymphocytes in buffalo. However Sarma *et al.* (2008) observed that the cortex had thickly arranged cell population which formed primary and secondary lymphatic nodules in goat. Asha *et al.* (2011) stated dense cortex by 60 days in parotid lymphnode of goat fetuses.

Primary Lymphatic Nodules: Primary lymphatic nodules were comprised of small lymphocytes with few macrophages and plasma cells. Van Rees *et al.* (1990) stated that the primary follicles were seen for the first time in the outer cortex of the mesenteric lymph node by two weeks after birth in rats.

Secondary Lymphatic Nodules: Secondary lymphatic nodules with germinal centers were found at 108 cm CVRL (317 days) in cortex. Germinal center was composed of lymphoblasts in various stages of mitotic division few macrophages, plasma cells, reticular cells and occasional neutrophils. The lymphoblast population decreased towards the periphery of germinal center which was indicative of their migration after their maturation from corona (Banks, 1993). Krumal *et al.* (1969) also noticed that mesenteric and thoracic lymph nodes lacked germinal center up to 84th day of gestation in pig foetuses. However, Sarma *et al.* (2008) and Kalita *et al.* (2014) observed that the germinal center of the secondary lymphatic nodules revealed a diversified cell population mostly lymphoblast, few macrophages, plasma cell and reticular cells with occasional neutrophils.

Lymphatic Vessels: From 21 cm CVRL (121 days) onwards (Fig. 3) the wall of lymphatic vessels was lined by flat endothelial cells. At 108 cm CVRL (317 days), the thin layer of connective tissue surrounded the flat endothelial cells of lymph vessels and lymph capillaries as also reported by Schmid-Schonbein (1990). The valves were reported in lymphatic vessels at 28 cm CVRL (137 days). Bloom and Fawcett (1969) found that valves appeared in the lymphatic vessels several weeks later than their appearance in the blood vascular system. Takada (1971) reported that valves were present in

lymphatic vessels and not in lymphatic capillaries in mice and rabbits.

Different Cell Types: The lymphocytes were round to oval in shape with irregular boundaries at 21 cm CVRL (121 days). The cytoplasm was in the form of narrow ring around the nucleus. Nucleus was large irregular and oval (Fig. 2). Reticular cells were found in cortical nodules, subcapsular sinus, trabecular sinus, medullary sinus and medullary cords in all the age groups (Fig. 3).

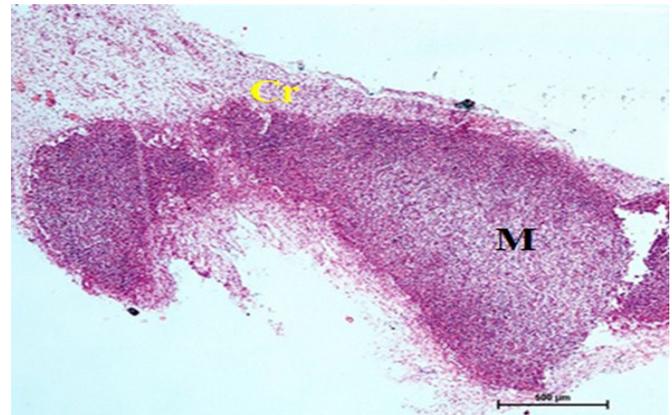


Fig 1. Darker outer cortex (Cr) and inner medulla (M) in prescapular lymph node at 17 cm CVRL (105 days) H&E×400

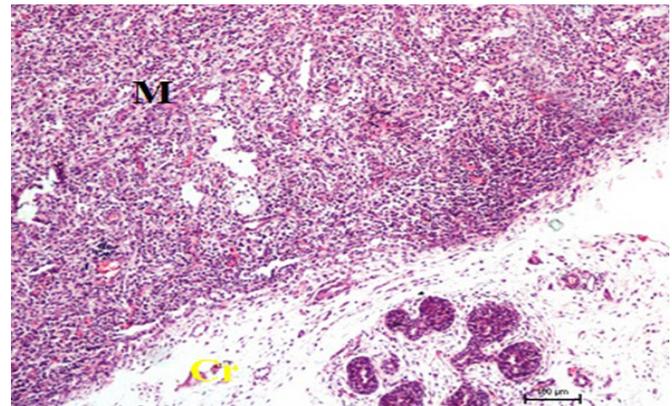


Fig 2. Presence of Paracortical area and sinuses in cortex (Cr) and medulla (M) in parotid lymph node at 20 cm CVRL (119 days) H&E×100

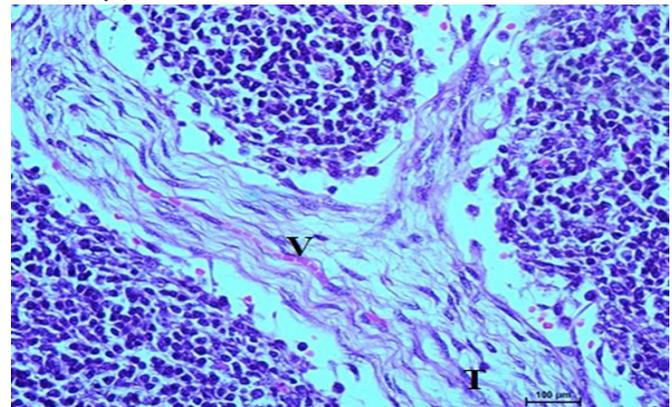


Fig 3. Parotid lymphnode showing single endothelial lining of lymphatic vessels (LV) at 44 cm CVRL (173 days) H&E×400

Cells became active macrophages and were endowed with ability to turn into all the cell types and connective tissue. These cells were thickly populated in the sub capsular and the trabecular sinuses as also confirmed by Ackerman and Knouff (2005) in chick. Similarly, Baishya *et al.* (2003) reported that the primary lymphatic medulla presented few cells with mitotic figures and abundant reticular cells during all gestational weeks in pigs. However, Sarma *et al.* (2008) found that the reticular cells formed a mesh work in the subcapsular sinus as well as in the trabecular sinus, the latter being wider. Plasma cells were found in the cortex from 48 cm CVRL (182 days).

Micrometry: The cortical thickness of the parotid lymph nodes of group I varied from 118.41 to 192.92 μ with mean value of $145.867 \pm 23.636 \mu$. From 25 cm CVRL (130 days) to 35 cm CVRL (152 days), the cortical thickness varied from 228.36 to 324.89 μ with a mean of $283.96 \pm 28.814 \mu$ where as in group III, the cortical thickness varied from 398.12 to 590.28 μ with the mean value of $466.188 \pm 62.144 \mu$. The micrometrical data revealed that the cortical thickness became double from group I to group II and from group II to group III.

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