Lymphoglandular Complexes in Proximal Colon of Buffalo Calves (*Bubalus bubalis*)

Complejos Linfoglandulares en Colon Proximal de Terneros de Buffalo (*Bubalus bubalis*)

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SUMMARY: The present study was conducted on six healthy early neonatal and six prepubertal buffalo calves to study the location, gross morphology, histomorphology and histochemistry of lymphoglandular complexes in proximal colon. In very proximal part of colon of buffalo calves, an irregular oval mucosal lymphoid patch was found grossly as a proximal colon (PC) patch. Histologically, in proximal colon patch of early neonates (3-4 weeks), an extensive invasion of mucosal glands was observed towards lymphoid nodules that were present in submucosa. The structure as a whole thus formed a complex known as lymphoglandular complex (LGC). Large number of such complexes *i.e.*, LGCs were observed in submucosa of proximal colon at this age. At some places, invasion of mucosal glands into lymphoid tissue was restricted to superficial layer of complexes, with the lymphoglandular complexes opening directly into the lumen but some were deep seated. However, by the age of 6 months in buffalo calves *i.e.*, prepubertal period, LGCs were reduced and were present in single layer within the submucosa of the proximal colon. Moreover, some of LGCs were completely encapsulated by their own lamina muscularis mucosae. But some of the complexes still had their mucosal openings into lumen while others had lost their connection with tunica mucosa. Histochemically, the glands that were observed within LGCs contained mucosubstances, glycogen, mucopolysaccharides, and mucin. However, lipids were present around the lymphocytes observed towards the periphery of these LGCs.

KEY WORDS: Proximal colon; Lymphoglandular complex; Histomorphology; Histochemistry; Buffalo calves.

INTRODUCTION

The immune system is important for protection of body from several harmful exogenous and endogenous substances. So body has developed various primary and secondary lymphoid organs for generation of immune response. Mucosal lymphoid tissue, as a part of secondary lymphoid tissue, especially the gut associated lymphoid tissue (GALT), however, acts to prevent the pathogens to gain access to the surface epithelium and penetrate the underlying tissues. Gut, therefore, acts as first line of defense towards antigens entering through oral route. Lymphoid tissue aggregates in small intestine *i.e.*, Peyer’s patches functions to generate immune response against foreign antigens. Peyer’s patches has been extensively studied in sheep, cattle, pigs, rodents and man (Cornes, 1965; Faulk et al., 1970; Sobhon, 1971; Owen & Jones, 1974; Chu et al., 1979; Sminia et al., 1983; Reynolds & Morris, 1983; Liebler, 1985), but lymphoid aggregates in submucosa of large intestine that formed lymphoglandular complexes (LGCs) received much less attention. Although a few workers have worked on lymphoid aggregates in colon of man (Kealy, 1976), rats (Crouse et al., 1989), dogs (Atkins & Schofield, 1972) and pigs (Liebler et al., 1988) but work in the literature is deficient on lymphoglandular complexes in bovines, especially buffalo calves. Therefore, the present study was carried out with the aim to study the location, gross appearance, histomorphology and histochemistry of LGCs in proximal colon of buffalo calves.

MATERIAL AND METHOD

The present study was conducted on healthy six early neonatal and six prepubertal buffalo calves. The proximal colon tissues from large intestines were collected and processed for paraffin and frozen sectioning techniques. The sections were stained with Mayer’s haematoxylin and eosin stain for histomorphological studies (Luna, 1968), Masson's

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Trichrome stain for collagen fibers (Luna), Gridley’s stain for reticular fibres (Sheehan & Hrapchak, 1973), Verhoeff’s for elastic fibers (Sheehan & Hrapchak), Alcian blue for mucosubstances (pH 2.5), McManus’ PAS method for glycogen, Mayer’s mucicarmine method for mucin, and Sudan black B method for fats (Luna).

RESULTS AND DISCUSSION

In very proximal part of colon of 3-4 week old calves, an irregular oval elevated (2.0±0.1 cm in length) mucosal lymphoid patch was found grossly as proximal colon (PC) patch. Several small openings distributed randomly over the irregular mucosal folds were present. By the age of 6 months, this proximal colon patch significantly increased in size into a large grossly evident oval patch (3.5±0.1 cm in length) formed by several mucosal folds mixed with lymphoid tissue raised above mucosa. Small pores or openings, 15-20 in number, located randomly over the irregular mucosal folds of patch were present (Fig. 1). These pores were the openings of lymphoglandular complexes (LGCs) that were formed within the patch and were confirmed histologically. Atkins & Schofield and Abd-El-Hady et al. (2013) reported the similar observations in caecum and colon of adult dogs.

Histologically, in proximal colon patch of early neonates at the age of 3-4 weeks, an extensive invasion of mucosal glands of the crypt area was observed towards lymphoid nodules present in submucosa. The structure as a whole thus formed lymphoglandular complex, located on antimesenteric side of the intestinal wall (Fig. 2). Many other workers have also observed that the lymphoglandular complexes were present in the proximal colon and were oriented towards the antimesenteric border in mouse (Deasy et al., 1983) and porcine (Liebler et al.). There was disintegration of lamina muscularis mucosae where mucosal glands of the crypt region invaded the submucosal lymphoid tissue. This view was similar to that expressed in caecum and colon of adult dogs (Atkins & Schofield; Abd-El-Hady et al.). At some places, invasion of mucosal glands into the lymphoid tissue was restricted to its superficial layer, with lymphoglandular complexes opening directly into the lumen. Some of the deeply seated LGCs also had openings into lumen while others had no connection (Fig. 2). Abundant...
collagen and reticular fibers were observed in between the lymphoid tissue and were encapsulating the lymphoglandular complexes. Similarly Liebler et al. reported in calves that in the lymphoglandular complexes, epithelial diverticulum extended from the intestinal lumen through muscularis mucosae, branched further into the lymphoid tissue and in the center an asymmetrically shaped transverse fold of lymphoid tissue protruded from the bottom. Moreover, the openings of these LGCs opened on elevations formed by mucosal folds of proximal colon patch visible grossly (Fig. 1). This was in accordance with the observations of Atkins & Schofield in dogs. Moreover, at some locations the lymphoid nodules without association with the glandular epithelium was also present.

However, by the age of 6 months, in buffalo calves LGCs were reduced to be present in single layer in proximal colon. Some of the complexes still had their mucosal openings into the lumen while others had lost their connection with tunica mucosa (Fig. 4). Moreover, some of LGCs were completely encapsulated by their own lamina muscularis mucosae (Fig. 5). The center of the LGC presented overlying mucous membrane that formed an irre-

Fig. 4. Photomicrograph of proximal colon in 6 month old buffalo calf showing lymphoglandular complex (LGC) with their mucosal openings (arrows), various lymphoid nodules (LN) in submucosa (Haematoxylin and Eosin x 20). [Boxed: Inset showing follicle associated epithelium (FAE) lined by columnar cells having lymphocytes (arrow)] (Haematoxylin and Eosin x 400).

Fig. 5. Photomicrograph showing mucosal glands entering lymphoid tissue of proximal colon of 6 month old buffalo calf by disintegration of lamina muscularis mucosae, lymphoid nodules with germinal center (GC), without GC (stars), lymphoglandular complex (LGC) also without GC and is enclosed by its own lamina muscularis mucosae (dotted arrow) (Haematoxylin and Eosin X40).

Fig. 6. Photomicrograph of proximal colon in 6 month old buffalo calf showing very strong PAS positive substance in mucosal glands entering lymphoglandular complex (LGC) and in glands just above the lymphoid tissue (arrows) i.e., away from the center of LGC (McManus’ Method x 40).

Fig. 7. Photomicrograph of proximal colon in 6 month old buffalo calf showing small amount of phospholipids, distributed especially around the peripheral cytoplasmic rims of lymphocytes (red arrows) located at the periphery of Lymphoglandular complex (LGC). Acid Haematin X400.

RESUMEN: El presente estudio se llevó a cabo en seis terneros de búfalo neonatos sanos y seis terneros prepuberales para estudiar la ubicación, morfología macroscópica, histomorfología e histoquímica de los complejos linfoglandulares en el colon proximal. Se observó en un área del colon proximal (AP) de los terneros de búfalo un óvalo linfoide de mucosa irregular en la parte más proximal de éste. Histológicamente, en el área proximal del colon de los terneros neonatos (3-4 semanas), se observó una invasión extensa de las glándulas mucosas hacia los nódulos linfáticos presentes en la submucosa. La estructura en su totalidad formaba un complejo conocido como complejo linfoglandular (CLG). A esta edad se observó un gran número de estos complejos es decir, se observaron CLGs en la submucosa del colon proximal. La invasión de las glándulas mucosas en el tejido linfoide, se limita a la capa superficial de los complejos, los complejos linfoglandulares distribuidos directamente en el lumen, sin embargo otros se encontraban arreglados de manera profunda. En búfalo a los 6 meses de edad, es decir en el período prepuberal, se observó un número reducido de CLGs presentes en una sola capa dentro de la submucosa del colon proximal. Por otra parte, algunos de CLGs estaban completamente encapsulados por su propia lamina muscularis mucosae. Algunos de los complejos mantenían abertura de las mucosas en el lumen, mientras que otros habían perdido su conexión con la mucosa. En análisis histoquímico, las glándulas que se observaron dentro del CLGs contenían mucosustancias, glucógeno, mucopolisacáridos y mucina. Sin embargo, se encontraron lípidos presentes alrededor de los linfocitos hacia la periferia de los CLGs.

PALABRAS CLAVE: Colon proximal; Complejo linfoglandular; Histomorfología; Histoquímica; Terneros búfalo.
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