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Prenatal development of palatine tonsil in sheep (Ovis aries)

RAJU, N. K. B.[1]*, RAMESH, G.[2], BASHA, S. H.[2] and USHAKUMARY, S.[2]

[1]Departament of Veterinary Anatomy, College of Veterinary Science, Tirupati - 517 502, Chittoor (Dt), Andhra Pradesh, India

[2]Departament of Veterinary Anatomy, Madras Veterinary College, Chennai - 600 007, Tamilnadu, India

*E-mail: nkbraju@gmail.com

Abstract

Introduction: The palatine tonsil play a key role in initiating immune responses against the antigenic material entering the mouth. **Materials and Methods:** Tissue pieces of oropharynx for the palatine tonsilwere collected from different prenatal age groups of sheep. These tissue pieces were fixed and processed for routine paraffin embedding technique to get 3-5µm thick sections. The paraffin sections were subjected to routine haematoxylin and eosin and some other special staining methods. **Results:** In three months foetal age of sheep, the palatine tonsils appeared as diffused lymphocytic infiltration in the propria submucosa overlaid by the mucosa of the oropharynx. The surface epithelium of the mucosa of the oropharynx formed primary crypts. The primary crypts in turn invaginated to form secondary crypts. The collagen fibres from the submucosa appeared to enter at the base of the developing tonsil. In the fourth month of foetus, the tonsillar surface epithelium became thicker and many crypts arose from that of primary crypts. Organized lymphocytic infiltration as isolated units was noticed near or around the secondary crypts. In five months of foetal age, the crypt epithelium was found to be infiltrated with lymphocytes, macrophages, plasma cells and mesenchymal cells. The connective tissue capsule and septa were completely formed. The lymphocytic tissue was characterized by an increased population of lymphocytes in the primary follicles without germinal centres. **Conclusion:** The results were compared with the literature and the palatine tonsil showed the gradual changes of histoarchitecture from third to fifth month of foetal age.

Keywords: prenatal, palatine tonsil, sheep.

1 Introduction

Palatine tonsils occupy a significant position at the beginning of the gastrointestinal tract allowing intimate contact with bacteria and other antigens that are ingested. Banks (1993) and Dellmann and Eurell (1998) stated that the aggregated lymphatic nodules in the pharynx or caudal oral cavity are referred to as tonsils in domestic animals. Cooper, Gabrielsen, Peterson et al. (1967) stated that the palatine tonsil is a representative of gut-associated lymphoid tissue. All the tonsils (palatine tonsil, lingual tonsil, tonsil of the soft palate, pharyngeal tonsil, tubal tonsil and paraepiglottic tonsil) together form a ring of lymphoid tissue in the pharyngeal wall called the "Waldeyer ring" (NICKEL, SCHUMMER and SEIFERLE, 1979; PERRY and WHYTE, 1998).

The palatine tonsil play a key role in initiating immune responses against the antigenic material entering the mouth and their lymphatic pathways are important in disseminating immunological information to the lymph nodes and other mucosal surfaces (BRANDTZAEG, 1984; BELZ and HEATH, 1995) in dogs.The tonsils are often the site of an early encounter with infectious agents and other antigens, and the local production of antibodies is important in a rapid initial response and the subsequent elaboration of a generalized immune response in domestic animals (DELLMANN and EURELL, 1998).

A thorough knowledge of the histogenesis of the palatine tonsil is very essential to gain a comprehensive knowledge on the gut immunology and to form a basis for the interpretation of various pathological conditions of the gut. Hence, the present work has been undertaken to explore the prenatal development of the palatine tonsil in sheep.

2 Materials and Methods

Tissue pieces in the area of oropharynx for the palatine tonsil were collected from sheep. The tissues from six animals each from different age groups viz. three months, four months and five months in prenatal were procured from the Corporation slaughter house, Perambur, Chennai. The determination of age ascertained as described by Richardson, Hebert and Terlecki (1976) in prenatal age groups.

Tissue pieces collected were fixed in different fixatives viz., 10 per cent neutral buffered formalin, Bouin's fluid and Zenker's fluid. The fixed tissues were processed for routine paraffin embedding technique and sections of 3-5µm thickness were cut. The paraffin sections were subjected to routine Haematoxylin and Eosin and special histological staining methods viz.Masson's trichrome and Gomori's reticulum.

3 Results

By the third month of foetal life in sheep, diffused lymphocytic infiltration as the representative of future palatine tonsil was noticed in the propria submucosa of the oropharynx. The surface epithelium which overlaid the developing tonsil was found to be non-cornified stratified squamous epithelium and at these places, the epithelium was thicker than that of the oropharynx. At this age, the invagination of surface epithelium

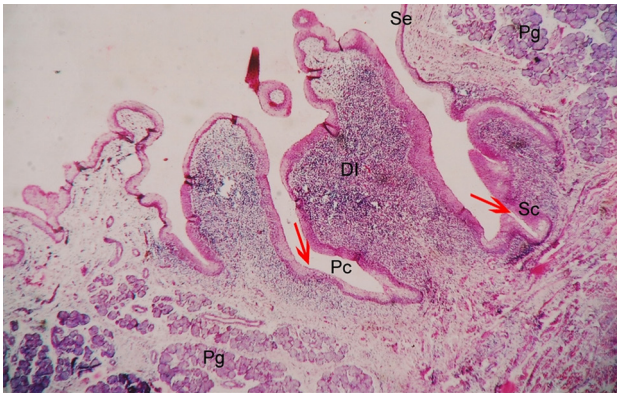

Figure 1. Photomicrograph of palatine tonsil of the three month-old foetus of sheep showing diffused lymphocytic infiltration in the propria submucosa of the oropharynx. Dl- Diffused lymphocytic infiltration; Pc- Primary crypt; Sc- Secondary crypt; Se- Surface epithelium; Pg- Palatine glands; Haematoxylin and Eosin x 40.

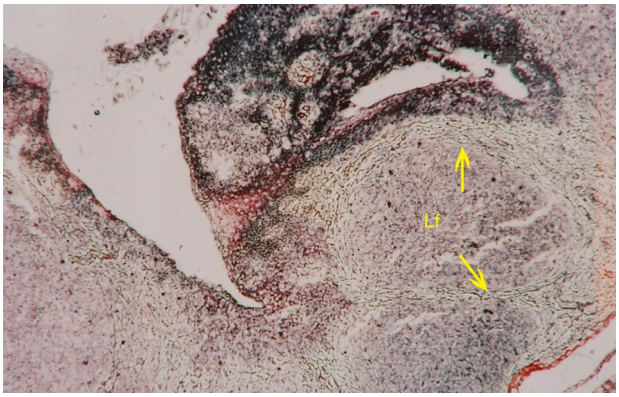

Figure 3. Photomicrograph of palatine tonsil of the four month-old foetus of sheep showing lymphoid follicles and parenchyma mainly supported by reticular fibres (arrows). Lf- Lymphoid follicle; Gomori's reticulum x 100.

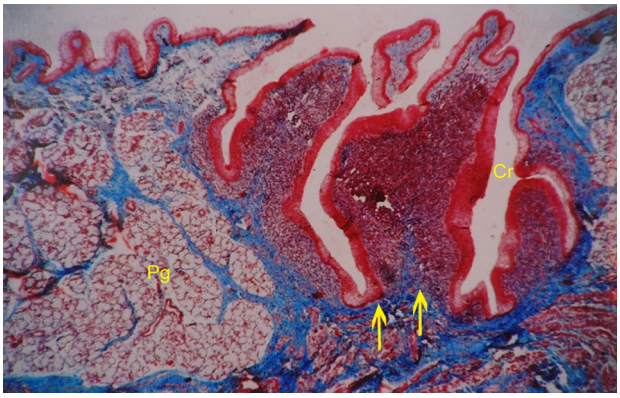

Figure 2. Photomicrograph of palatine tonsil of the three month-old foetus of sheep showing submucosal collagen fibres (arrows) entering at the base of the tonsil. Cr- Crypt; Pg- Palatine glands; Masson's trichrome x 40.

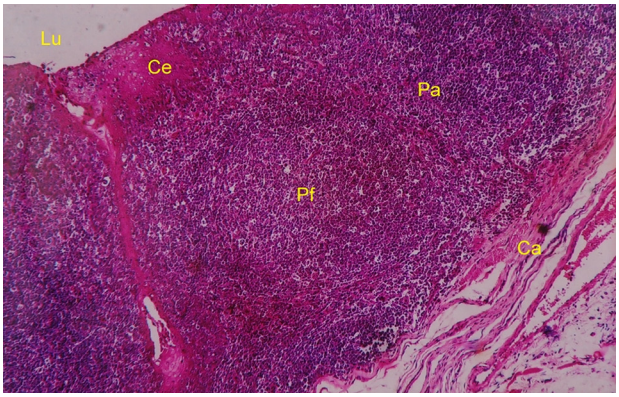

Figure 4. Photomicrograph of palatine tonsil of the five month-old foetus of sheep showing primary lymphoid follicles (without germinal centres) and parafollicular areas. Pf- Primary lymphoid follicle; Ca- Capsule; Pa- Parafollicular area; Ce- Crypt epithelium; Lu- Crypt lumen; Haematoxylin and Eosin x 100.

to form primary crypts as well as the formation of secondary crypts by the invagination of the former were recorded in the present study (Figure 1). The epithelium of both primary and secondary crypts of palatine tonsils of foetal sheep was found to be non-keratinized stratified squamous epithelium in the present observation.

In the three month-old foetus, the crypt epithelium was composed of a stratum basale with densely packed columnar cells. The middle layer was made up of polyhedral cells where the larger cells were noticed in the lower row. Outer layer of the crypt epithelium was made up of a single layer of flattened squamous cells. The lymphocytic aggregation around the crypts was noticed by three months of foetal age in sheep. Lymphocytes of various sizes, reticular epithelial cells with their processes were the major components of these aggregation. Erythrocytes and lymph vessels were also noticed among these cellular population. By third month of prenatal age, collagen fibres from the submucosa appeared to enter at the base of developing tonsil which may be the indicative feature of future capsule around the palatine tonsil (Figure 2).

At the fourth month of foetal age, there was a difference noticed in the middle layer of crypt epithelium where polyhedral cells were found to be uniform and also, the outer squamous cell layer was made up of one to two rows. Organized lymphocytic infiltration as isolated units was recorded near or around the secondary crypts in the present study. Further, parenchyma was found to be well supported with reticular and collagen fibres (Figure 3).

By fifth month of foetal age, the infiltration of crypt epithelium with lymphocytes, macrophages, plasma cells and mesenchymal cells was observed in the present study. The connective tissue capsule and septa were present. Primary follicles without germinal centres were also found. Parafollicular areas were observed (Figure 4) in the same period along with a synchronous development of reticulocytes.

4 Conclusion

The appearance of lymphocytic infiltration is said to occur by the age of 22nd day of gestation in rabbits by Leene (1971), but in human beings, the anlage of palatine tonsil was reported

to occur by nine weeks of foetal development by Khlystova and Baryshev (1979). The thickening of embryonic oropharynx at the places of future palatine tonsil was in total agreement with the report of Noussios, Xanthopoulos, Zaraboukas et al. (2003) in human beings where it is said to be noticed by 14-15th week of gestational period.

However, formation of crypt has been reported by the ninth week of gestation in human embryos by Khlystova and Baryshev (1979). The epithelium of both primary and secondary crypts of palatine tonsils of foetal sheep was found to be non-keratinized stratified squamous epithelium in the present observation. Similar report has been recorded by Khlystova and Baryshev (1979) in human foetuses but by the age of 16th week of gestational period.

The lymphocytic aggregation around the crypts was noticed by three months of foetal age in sheep. Lymphocytes of various sizes, reticular epithelial cells with their processes were the major components of these aggregation. Erythrocytes and lymph vessels were also noticed among these cellular population. But, in human embryos, only by 13th week of foetal age, lymphocytic infiltration of the connective tissue around the crypt branches was reported by Slipka and Slipka Junior (1996).

By fifth month of foetal age, the infiltration of crypt epithelium with lymphocytes, macrophages, plasma cells and mesenchymal cells was observed in the present study. However, von Gaudecker and Muller-Hermelink (1982) observed that the lymphocytes, plasma cells, macrophages and interdigitating cells were present in the crypt epithelium as early as 15th week of gestation in human. According to Vyborne (1999) these lymphoid cells and macrophages were said to migrate through the microcrypts near the crypt epithelial micropores on the surface of the reticularized epithelium.

The connective tissue capsule and septa were present. Primary follicles without germinal centres were also found which is in accordance with the statement of Khlystova and Baryshev (1979) and Noussios, Xanthopoulos, Zaraboukas et al. (2003) in human foetus. Parafollicular areas were observed (Figure 4) in the same period along with a synchronous development of reticulocytes. However in human foetus, the occurrence of follicular and parafollicular areas was observed by 19-22nd week by Noussios, Xanthopoulos, Zaraboukas et al. (2003). The same authors also had recorded the synchronous development of reticulocytes with an increased population of lymphocytes in primary follicle by the age of 29-35th week of human foetus.

References

BANKS, WJ. *Applied veterinary histology*. 3rd ed. Missouri: Mosby Yearbook, 1993. p. 277-279.

BELZ, GT. and HEATH, TJ. Intercellular and lymphatic pathways of the canine palatine tonsils. *Journal of Anatomy*, 1995, vol. 187, n. 1, p. 93-105. PMid:7591989.

BRANDTZAEG, P. Immune functions of human nasal mucosa and tonsils in health and disease. In BINENSTOCK, J. (Ed.). *Immunology of the lung and upper respiratory tract*. New York: McGraw Hill Book, 1984. p. 28-95.

COOPER, MD., GABRIELSEN, AE., PETERSON, RDA. and GOOD, RA. Ontogenic development of the germinal centres and their function- relationship to the bursa of Fabricius. In COTTIER, H., ODARTCHENKO, N., SCHINDLER, R. and CONGDON, CC. (Eds.). *Germinal centres in immune responses*. New York: Springer-Verlag, 1967. p. 28-33.

DELLMANN, HD. and EURELL, JA. *Textbook of veterinary histology*. 5th ed. Baltimore: Williams and Wilkins, 1998. p. 137-191.

KHLYSTOVA, ZS. and BARYSHEV, BB. Differentiation of the palatine tonsillar tissues of the human fetus. *Arkhiv Anatomii, Gistologii I Émbriologii*, 1979, vol. 77, n. 12, p. 59-63.

LEENE, W. Origin and fate of lymphoid cells in the developing palatine tonsil of the rabbit. *Zeitschrift für Zellforschung und Mikroskopische Anatomie*, 1971, vol. 116, n. 4, p. 502-522. http://dx.doi.org/10.1007/BF00335055. PMid:5103855.

NICKEL, RA., SCHUMMER, E. and SEIFERLE, E. *The viscera of the domestic animals*. Berlin: Verlag Paul Parley, 1979. p. 52-56.

NOUSSIOS, G., XANTHOPOULOS, J., ZARABOUKAS, T., VITAL, V. and KONSANTINIDIS, I. Morphological study of development and functional activity of palatine tonsils in embryonic age. *Acta Otorhinolaryngol*, 2003, vol. 23, p. 98-101.

PERRY, ME. and WHYTE, A. Immunology of tonsils. *Immunology Today*, 1998, vol. 19, n. 9, p. 414-421. http://dx.doi.org/10.1016/S0167-5699(98)01307-3. PMid:9745205.

RICHARDSON, C., HEBERT, C. and TERLECKI, S. Estimation of the developmental age of ovine foetus and lamb. *The Veterinary Record*, 1976, vol. 99, p. 22-26. http://dx.doi.org/10.1136/vr.99.2.22. PMid:951923.

SLIPKA, J. and SLIPKA JUNIOR, J. The palatine tonsil as an evolutionary novelty. *Acta Oto-Laryngologica. Supplementum*, 1996, vol. 523, p. 8-11. PMid:9082817.

VON GAUDECKER, B. and MULLER-HERMELINK, HK. Ultrastructural analysis of lymphoid subpopulations and their relation to stationary cells in the epithelial crypts and the follicles during early ontogeny of the human tonsilla palatina. *Advances in Experimental Medicine and Biology*, 1982, vol. 149, p. 485-490. http://dx.doi.org/10.1007/978-1-4684-9066-4_68. PMid:6983228.

VYBORNE, E. The sequence of reticularisation of epithelium of human palatine tonsil: scanning electron microscopic study. *Acta Universitatis Palackianae Olomucensis Facultatis Medicae*, 1999, vol. 142, p. 139-142. PMid:10743744.

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