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Post natal development of Caprine Tubal Tonsil: Histological, Immunohistochemical and Electronmicroscopical Studies

Ratnesh Kumar Choudhary[1*], P. Das[2] and R. K. Ghosh[2]

[1]Krishi Vigyan Kendra, Kishanganj-855107, Bihar

[2]Department of Veterinary Anatomy and Histology, Faculty of Veterinary and Animal Sciences
West Bengal University of Animal and Fishery Sciences, 37, K. B. Sarani, Kolkata 700037

*Corresponding Author E-mail: dr.ratneshchoudhary@gmail.com

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ABSTRACT

Tubal tonsil was found along the margin and around the associated flap of the slit like pharyngeal opening on either side the eustachian tube. In the caprine tubular tonsil several crypts were found. The frequency of CD4 and CD8+ cells proportionally were increased according to advancement of age but maximum cellular aggregation was noticed from 3rd month onward. IgM positive cells were mostly located towards the central portion of the follicle. APCs were identified immunohistochemically near the epithelium, parafollicular region, interfollicular region and also at the centre of the follicle. Transmission electron microscopy of the tonsillar crypt revealed the presence of epithelial cells with low microvillus. M cells were isolated with eccentric nucleus, numerous oval to elongated mitochondria which were predominant at the supra nuclear area.

Key words: *Caprine, Tubal Tonsil, Post natal, Immunohistochemical*

INTRODUCTION

Tubal tonsil is a nodular aggregation of lymphoid tissue, present in the mucosa of the orifice of the auditory tube. Tonsils and adenoids are strategically located in waldeyer's ring as effector organ of local systemic and mucosal adaptive immune to airborne and alimentary antigens[1]. The mucosa of the medial nasopharyngeal part of the auditory tube contained several primary and secondary lymph nodules, whereas the lateral mucosa contained less and mostly diffuse lymphoid tissue. The epitheliums covering the lymphoid tissue are sometimes infiltrated by lymphocytes[3]. The tubal tonsil of the horse surrounds the pharyngeal opening of the Eustachian tube and is lined by pseudostratified columnar ciliated epithelium interspersed with areas of follicle associated epithelium (FAE) heavily infiltrated by lymphocytes but devoid of goblet and ciliated cells[5]. The tubal tonsils are composed of individual lymphoid nodules with lymphoid follicles containing B lymphocytes and Follicular Dendritic Cell (FDC), parafollicular areas containing CD4+, CD8+ and γδ T lymphocytes and dome-like accumulation of lymphocytes[7].

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The lymphoepithelium (LE) with a mixture of ciliated and microvillous bearing cells interpreted to be M cells occurs in the center of the epithelium above domes[7]. The tubal tonsil of the horse are microvillous cells and cells with features characteristic of M cells such as reduced microvilli or depressed bare surface, more numerous mitochondria, small vesicles and lysosomes, as well as vimentin filaments and epitopes. M cells are also identified in areas of respiratory epithelium not associated with lymphoid follicles[5]. The present study is explores the post natal development of caprine Tubal Tonsil and its histological, immunohistochemical and ultrastructure features.

MATERIAL AND METHODS

Tubal tonsil were collected from clinically healthy zero day and month wise from one month to fifth months old Black Bengal goats (*Capra hircus*) from the farm maintained by AICRP (All India Coordinate Research project) on goat. The animals were maintained in the farm house according to the stipulated guideline and permission of Institutional Animal Ethical committee of faculty of Veterinary and Animal sciences; West Bengal University of Animal and fishery Sciences, Kolkata. Two goats of either sex were utilized for this experiment in each age group. The samples were harvested from mucosa of the auditory tube near the orifice and were fixed in 10 per cent neutral buffered formalin. The fixed samples were processed for paraffin sections and routine staining was done as per Luna, 1968.

For immunohistochemical examination the collected samples were fixed in 10 % neutral buffer formalin (NBF) and were dehydrated through graded alcohols before being embedded in paraffin wax. The sections (horizontal & Vertical) of 5µm thickness were obtained from each specimen. The different lymphocyte subpopulations were visualized using the avidin-biotin peroxidase complex technique (ABC; Vector Laboratories, U.S.A). Paraffin-embedded samples were dewaxed with xylene and hydrated. Then dip into antigen unmasking (Vector Laboratories, U.S.A) solution for 15 min at 95°C. After washing the slides in PBS for 5 min, the samples were pre-treated with normal rabbit serum (Dako, denmark) diluted 1:100 in PBS for 60 min at room temperature to block the non-specific binding sites. They were then incubated with the ready-to-use of each primary antibody (Table 1) in a moist chamber for 120 min at room temperature. Next, the slides were again washed in PBS and then incubated with a biotinylated rabbit antimouse IgG or horse antirabbit IgG (Vector Laboratories, U.S.A) according to primary antibody diluted 1:250 for 60 min. Before being washed in PBS and incubated again with ABC for 40 min, according to the instructions of the maker (Vectastain ABC Elit® Kit, Vectror Laboratories). Color was developed by a final incubation with 3.3' diaminobenzidine tetrahydrochloride (DAB, Peroxidase substrate kit Vector Laboratories) in buffer with hydrogen peroxide in distilled water for 5 min at room temperature. The reaction was stopped by rinsing the slides in water. The some slides were counterstained with haematoxylin, dehydrated and mounted. Most slides were mounted without counterstain.

Tissue samples for electron microscopy were fixed in 2.5% glutaraldehyde in PBS (0.1M; pH7.0). The standard protocol was followed for TEM (Transmission electron microscopy) and SEM (Scanning electron microscopy). The present study was carried out partly in the department of Anatomy, Histology and Embryology, West Bengal University of Animal and Fishery Sciences and partly at Electron- Microscopic facility, All India Institute of Medical Science, Ansari Nagar New Delhi.

Table 1. Primary Antibodies (ready- to- use) used in the Immunohistochemical Technique

Antibodies	***Specificity***	***Isotype***	***Supplier***
4B12	CD4*	IgG1	Dako Denmark
C8/144B	CD8*	IgG1	Dako Denmark
	IgM**		Dako Denmark
TB01	CD57*	IgM	Dako Denmark

*****Monoclonal Antibody, ******Polyclonal Antibody

RESULTS

Histological study

Tubal tonsil was found along the margin and around the associated flap of the slit like pharyngeal opening on either side the eustachian tube right from the day old age till the 5th months of age (Fig.1). The tonsillar surface was lined by stratified squamous non keratinized epithelium with numerous folds (Fig. 2). The thickness of epithelium was made up of 12- 14 layers of cellular aggregations. The epithelium was comprised of stratum basale, spinosum and superficiale. The cell of stratum basale was rested on a uniform basement membrane. The nucleus was oval to elongate in outline and was strongly basophilic and the cytoplasm was lightly eosinophilic. An appreciable number of cellular rows of lightly basophilic nuclei of varied shape formed the next layer that was stratum spinosum. At the stratum superficiale the size of the nuclei were smaller as compared to the previous layer and was found mostly round to oval, vacuolated towards the surface. Their cytoplasm was eosinophilic and finally granular. The nuclei of the later part of the stratum superficiale were mostly elongated, basophilic revealed picknosis towards their free surfaces and chromatin material was finally granular and homogenously distributed.

The lamina propria was made up of connective tissue fibres with scatteredly distributed fibroblast and lymphocytes. Within the lamina propria lymphocytes were distributed. Surface epithelium in some places become thin and invaded into the depth in the form of crypts with free luminal surfaces.

At the base of the epithelium there was numerous invagination of the surface epithelium. The epithelial tissue was modified within the crypts into lymphoepithelium (Fig.4). Within the lumen of the crypt, the number of rows was found to be reduced as compared to the other adjacent area of the outer most layers. It was found that some cells having round to oval nuclei with depressed surface area in association with lymphocytes were probably designated as M cells (Fig.5).

Lymphoid tissue and loose irregular connective tissue formed by sparsely distributed reticular and collagen fibres were scattered throughout sub epithelial lamina propria mucosae. It was observed that tonsil was composed of lymphoid follicles; glandular acini's and dense connective tissue network (Fig. 3). The lymphoid follicle was distributed scatteredly. At birth the lymphoid follicle was less and the number increased gradually. The follicles were mostly filled up with lymphocytes beside the macrophages, follicular dendritic cells and plasma cells (Fig. 6 & 7). Lymphocytes were also observed within the interfollicular zone. Glandular tissue was also observed and glands were mostly mucous secreting glands.

Immmunohistochemistry

Immmunohistochemistry was conducted for identification of CD4 and CD8 positive cells in the tubal tonsils. Both CD4 and CD8 positive cells were identified in the epithelium, lamina propria, parafollicular region and in the interfollicular zone (Fig. 8 - 9). The CD4 cells were predominant in the parafollicular area as compared to the CD8 cells. The population of CD4 and CD8 positive cells was almost same in the interfollicular area and the epithelium. CD4 and CD8+ cells were less frequent and distributed scatterdly. CD4and CD8+ cells were identified since day old to 5th month of age but only difference was identified in term of their morphology and frequency. The frequency of CD4 and CD8+ cells proportionally were increased according to advancement of age but maximum cellular aggregation was noticed from 3rd month onward. Morphologically large and small lymphocytes revealed immunoreactivity against cell markers.

IgM positive cells which were common marker for B cells were used to identify B cells in the tonsil. IgM positive cells were mostly located towards the central portion of the follicle (fig. 10). B lymphocytes were also identified in the interfollicular region but the numbers were very few. B cell population was almost static from day old to 2nd month but

third month onward number of B cells were increased with the increment of age.

MHC-II antibody was used to identify Antigen presenting cells (APC). APCs were identified immunohistochemically within the follicle. The cells were distributed in the epithelium, parafollicular region, interfollicular region and also at the centre of the follicle (Fig. 11). The number of APC increased from day old to 5th month of age. MHC-II cells revealed similar morphological characteristics throughout the experimental period. The only noticeable feature was that MHC-II cell number increased from 4th months onwards.

Electron microscopy

Scanning electron microscopy

The cut surface of tubal tonsil revealed an irregular surface with small and large foci of lymphocytes and M cells (Fig. 12).

Transmission electron microscopy (TEM)

Transmission electron microscopy of the tonsillar crypt revealed the presence of epithelial cells with low microvillus. The shape of nucleus of epithelium was round with numerous mitochondria, golgi bodies and some secretory vesicles also observed within the cell. Smooth endoplasmic reticulum and glycogen granules also noticed in this area (Fig. 13-17). M cells were isolated with eccentric nucleus, numerous oval to elongated mitochondria which were predominant at the supra nuclear area (Fig. 14). Huge numbers of small size vesicles were revealed within the cytoplasm. Smooth and rough endoplasmic reticulum, Golgi body, glycogen granules were also identified. The free surface of the cell revealed small microvilli in the Para follicular region of tubal tonsil. The TEM revealed a large round to oval nucleus with clumped heterochromatin condensed at the nuclear membrane. The cytoplasm was granular and there were numerous small vesicles and mitochondria were observed.

DISCUSSION

Histological study

In the present investigation the occurrence of tubal tonsil around the pharyngeal opening of the eustachian tube cannot be supported by any available literature on goat. However Cocquyt *et al.*[3], reported similar occurrence in case of ovine and Kumar and Timoney[5] reported in case of equine. Chen *et al.*[2], reported a nodular aggregation at the opening of the auditory tube. But macroscopically such type of nodule was not observed. Thome[8] also reported similar finding. Regarding the location of Tubal Tonsil Stanley *et al.*[7], reported similar finding.

The lining epithelium was found be stratified squamous non keratinized epithelium. Cocquyt *et al.*[3], in their statement did not mention the type of epithelium in case of ovine. However Kumar and Timoney stated that the surface was lined by pseudostratified coloumnar ciliated epithelium in case of equine which was not accordance with our present finding. In the caprine tubular tonsil several crypts were found. Similar observation was mention by Koch[4] and Kumar and Timoney[5]. Lymphoepethelial and M cells were found in the crypts of the caprine lymphnode. Lymphoid follicles were aggregated in the propria. Lymphocytes & macrophage were identified within the follicle. Cocquyt *et al.*[3], & Kumar and Timoney reported similar observation in case of ovine and equine tubal tonsil respectively.

Caprine tubal tonsil comes under Mucosa associated lymphoid tissue (MALT) and is the most anatomically and histologically organized lymph node. The compactness of follicle was found from date of birth till 5th month and presence of lymphoepithelial cells in the crypts and reduction of epithelial layer in crypts indicate the active participation of Tubal Tonsil against the nasopharyngeal antigen. In comparison to lingual tonsil, it was more organized. Age wise changes were noticed and remarkably from 2nd month onward the number of lymphocyte increased.

Immunohistochemistry

Both CD4 positive and CD8 positive cells were identified in the epithelium, propria, para follicular and interfollicular area of tubal tonsil. This is in accordance with Kumar and Timoney[5] in case of equine. Stanley *et al.*[7], document the presence of CD4 and CD8

positive cells in the tubal tonsil. The number of immunocompetent cells increased from 3rd month onward. But regarding the post natal changes no supportive paper is available. Presence of B lymphocytes in the tubal tonsil was evident by Stanley *et al.*[7]. Regarding the occurrence of B cell similar finding was reported by Kumar and Timoney[5] in case of equine. Regarding the increment of B cells in different age group was not recorded anywhere.

APC was immunohistologically identified in all age groups. The number was static up to third month and from 4th month the number was increased. This may be due to more exposure of environmental antigen prevailing with advancement of age.

Electron microscopy

Scanning and transmission electron microscopical findings confirmed the presence of M cell and lymphoepithelial cells which are the most predominating features of the Tubal Tonsil. This observation was in accordance with Stanley *et al.*[7], and Kumar & Timoney[5].

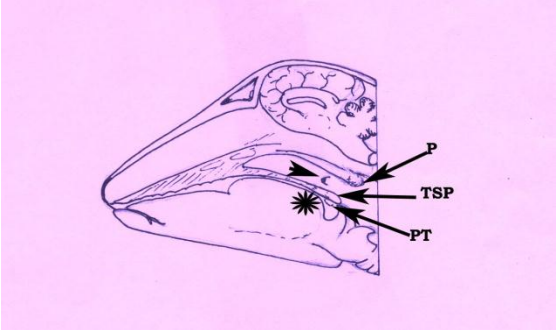

Fig.1: Schematic drawing of median section of a Black Bangal Goat showing location of pharyngeal tonsil (P), tubal tonsil (arrow), tonsil of the soft palate (TSP), palatine tonsil (PT) and lingual tonsil (aster)

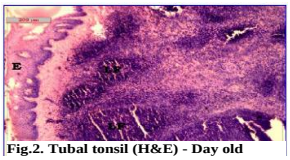

Fig.2. Tubal tonsil (H&E) - Day old

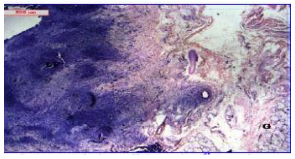

Fig.3. Tubal tonsil (H&E) – 1st Month

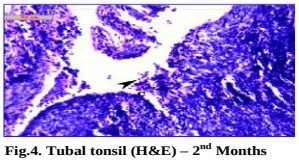

Fig.4. Tubal tonsil (H&E) – 2nd Months

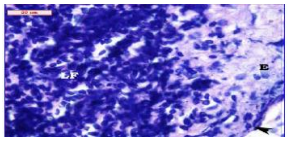

Fig.5. Tubal tonsil (H&E) – 4th Months

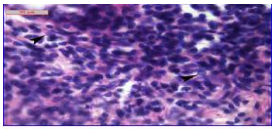

Fig.6. Tubal tonsil (H&E) – 3rd Months

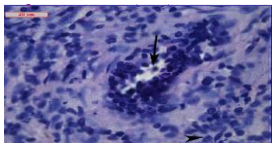

Fig.7. Tubal tonsil (H&E) – 5th Months

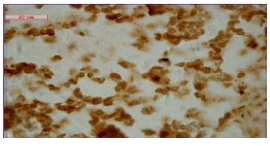

Fig.8. CD4 - Day old

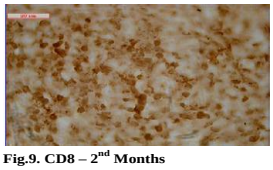

Fig.9. CD8 – 2nd Months

Fig. 2-7 Photomicrographs showing the histoarchitecture of tubal tonsil in different age groups and magnifications. E= Epithelium, LF= Lymphoid follicle, G= Gland, C= Crypt, Arrow head= Lymphoepithelium (Fig.4), M cell in (Fig.5) and Macrophage in (Fig.6 & 7), Arrow= Lymphocytic infiltration.

CONCLUSION

The lining epithelium of tubal tonsil was found be stratified squamous non keratinized epithelium instead of pseudostratified coloumnar ciliated epithelium in case of other animals. Lymphoepethelial and M cells were found in the crypts of the caprine lymphnode. Caprine tubal tonsil comes under MALT and is the most anatomically and histologically organized lymph node. The compactness of follicle was found from date of birth till 5th month and presence of lymphoepithelial cells in the crypts and reduction of epithelial layer in crypts indicate the active participation of Tubal Tonsil against the nasopharyngeal antigen. In comparison to lingual tonsil, it was more organized. Age wise changes were noticed and remarkably from 2nd month onward the number of lymphocyte increased. The number of CD4 and CD8 + cells increased from 3rd month onward. APC was immunohistologically identified in all age groups. The number was static up to third month and from 4th month the number was increased. This may be due to more exposure of environmental antigen prevailing with advancement of age. Scanning and transmission electron microscopical findings confirmed the presence of M cell and lymphoepithelial cells which are the most predominating features of the Tubal Tonsil.

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REFERENCES

1. Brandtzaeg, P., Immunology of tonsils and adenoids: everything the ENT surgeon needs to know. *Int. J. Pediatr. Otorhinolaryngol*. **67S1:** S69–S76 (2003).
2. Chen W., Alley M.R. and Manktelow B.W., Respiratory tract associated lymphoid tissue in conventionally raised sheep, *J. Comp. Pathol*. **101:** 327–340 (1989).
3. Cocquyt, G., Baten, T., Simoens, P. and Broeck, W.V.D., Anatomical localisation and histology of the ovine tonsils *Veterinary Immunology and Immunopathology*. **107:** 79–86 (2005).
4. Koch, T., Einrichtungen zum Abschlucken der Nahrung. In: Koch, T. (Ed.), Lehrbuch der Veterina¨r Anatomie, Band II Eingeweidelehre, Zweite Auflage. *VEB Gustav Fisher Verlag Jena, Berlin*. 88–107 (1970).
5. Kumar, P. and Timoney, J.F., Histology, Immunohistochemistry and Ultrastructure of the Equine Palatine Tonsil. *Anat. Histol. Embryol*. **34:** 192–198 (2005).
6. Luna, L.G., Manual of histologic staining method of the Armed Force Institute of pathology. 3rd Edn. Mac. *Graw-Hill Book Company*, Ny. (1968).
7. Stanley, A.C., Huntley, J.F., Jeffrey, M. and Buxton, D., Characterization of ovine nasal-associated lymphoid tissue and identification of M-cells in the overlying follicle-associated epithelium. *J. Comp. Pathol*. **125:** 262–270 (2001).
8. Thome, H., Mundho¨hle und Schlundkopf. *In*: Nickel, R. Schummer, A. and Seiferle, E., (Eds.) (1999).