Original article

Morphological study of thymus in anencephalic and normal fetus

Vijaya, R.[1], Rao, TMS.[3] and Kumar, PA.[2]*

[1]Department of Anatomy, Meenakshi Medical College & Research Institute, 631552, Kanchipuram, Índia
[2]Department of Anatomical Sciences and Neurobiology, Oman Medical College, PC 321, Sohar, Oman
[3]Department of Pathology, PSG Institute of Medical Sciences and Research, 641004, Coimbatore, Índia
*E-mail: drpakumar@yahoo.com

Abstract

Anencephaly is the commonest congenital anomaly of nervous system where the rostral end of neural tube is malformed. In the present study, the thymus gland from an anencephalic fetus is compared with that of a normal stillborn term fetus. Thymus glands were fixed in neutral buffered formalin and processed for routine histological study. In addition to routine histological features, the study included characteristic features of the reticular epithelial cells, dendritic cells, immature T cells and the process of apoptosis of the thymocytes, using an array of markers including cytokeratin, S100, terminal nucleotidyl transferase and CD99. Cytokeratin in the anencephalic fetus was diffusely distributed all over the lobule, compared to normal thymus which showed positivity in medulla & reticular epithelial cells of Hassall's corpuscles. Nature and distribution of dendritic cells of anencephalic thymus did not reveal noticeable difference from the thymus of the normal still born fetus. Immature T cells in anencephalic fetus were well pronounced around Hassall's corpuscles in medulla. Degree of apoptosis of thymocytes was far less compared to the thymus in normal fetus. Decreased degree of apoptosis might have led to thymic hyperplasia as reflected by reduced expression of CD99 in the anencephalic fetus.

Keywords: fetal thymus, anencephaly, reticular cells, dendritic cells, T cells, thymocytes, apoptosis.

1 Introduction

Meroanencephaly is a form of neural tube defect characterized by defective cranial vault, through which malformed derivatives of the cranial end of the neural tube protrude out. The material protruding out is formed by an admixture of vascular and glial tissue. Just like in any other form of neural tube defect, anencephaly could present with structural derangement in other organs as well (LEMIRE, 1988). Review of the literature reveals that reports on morphological & histological features of endocrine glands in general and thymus in particular in anencephalic fetuses are rather scanty.

In a study of a small series of anencephalic fetuses, MITRA (1973), reported that thymus gland showed hyperplasia with large cortical areas packed with thymocytes in three of the specimens, while in two other specimens thymus appeared to be hypoplastic with large Hassall's corpuscles (HC). In yet another report, thymus was found to be enlarged and extended upto the diaphragm in a case of holoanencepahlic fetus (LEVY, 1976). Thymus of an anencephalic fetus was seen with hyperplasia and increase in the number of cortical thymocytes (SHIN and LEE, 1999). Association of thymomegaly and anencephaly, at least in isolated cases has been confirmed more recently by Mazzitelli, Vauthay, Grandi et al. (2002).

Immunological functions of thymus gland, including its role in the maturation of T Lymphocytes are well documented. Thymus is also known to secrete proteins that act within it as paracrine mediators and function as growth factors to bring in stimulation and proliferation of T lymphocytes (JUNQUERIA and CARNEIRO, 2010). Thymic structure could reflect on its function and have varied effects on the developing fetus. In this context, it is possible to surmise that thymus of anencephalic fetus could exhibit features that deviate from the normal and this might help in the understanding of the thymic functions better.

2 Materials and Methods

Thymus from a meroanencephalic male fetus, delivered in the teaching hospital of Meenakshi Medical College, Kanchipuram; India which was handed over to the Department of Anatomy was dissected out and studied. Similarly, thymus from a still born female fetus was also dissected out and studied histologically. Consent was obtained from parents of both the fetuses to conduct this study. The gross morphological features like weight of the fetus, weight of the isolated thymus, the base breadth, height and thickness of thymus were measured in both the cases, as per the protocol used by an earlier study (AJITHA, 2006). Thymic index in both the cases and estimated weight for the anencephalic fetus were also calculated (Table 1).

Table 1. Gross features of the thymus gland.

Parameters	Thymus of normal stillborn	Thymus of anencephalic fetus
Fetus weight	1750 gm	1500 gm (Estimated wt 1650 gms)
Thymus weight	5 gm	7 gm
Thymic index*	0.29	0.47
Base width X Ht	1 × 1 cm	4 × 2 cm
Greatest thickness	0.5 cm	1 cm

*Thymic index = weight of thymus/weight of the fetus × 100

These organs were quickly fixed in neutral buffered formalin. After 24 hours of fixation, tissue samples were processed for routine histological examination. These paraffin embedded sections were stained with haematoxylin & eosin. The morphology and appearance of epithelial reticular cells, dendritic cells, immature T cells and the thymocytes undergoing apoptosis were studied using a battery of immunoprobes, consisting of antihuman cytokeratin cocktail (pancytokeratin), anti S100 protein, terminal deoxynucleotidyl tranferase (Tdt) and CD99, according to the Streptavidin Biotin Complex (SBC) technique (BUCHWALOW and BOCKER, 2010). The immunostains and the chemicals used were from DAKO. The stained slides were studied by light microscopy using Nikon microscope. Relevant fields of interest were photographed for analysis.

3 Results

Gross features of the thymus glands are summarized in Table 1.

Light microscopy using haematoxylin and eosin (H&E) stain of the thymus from the normal stillborn fetus revealed the clear demarcation of cortex & medulla. Lobules were well separated by connective tissue septa. HC were present in the medulla (Figure 1A). Thymus gland of anencephalic fetus showed ill defined lobular architecture, and cortex and medulla were not well differentiated. The medulla showed an abundance of HC (Figure 1B).

Epithelial reticular cells within the normal fetal thymus as revealed by the expression of cytokeratin showed that they were restricted to medulla (Figure 2A), whereas in the case of the anencephalic fetus, the staining was diffuse all over the lobule, both within the cortex and medulla. Staining was strongly positive within the HC and the cells surrounding them Figure 2B).

S100, meant to show the dendritic cells within the corticomedullary junction exhibited positivity both in the normal thymus and anencephalic thymus, thus exhibiting equivocal results (Figures 3A and B).

Tdt meant for identifying the immature T cells expressed poorly, showed a few scattered cells in the normal thymus, while its expression was markedly pronounced particularly around the HC within the thymus of the anencephalic fetus (Figures 4A and B).

CD99, the marker for apoptosis was well expressed in the cortex of normal thymus. These cells were faintly positive for CD99, in the anencephalic thymus and were seen to be seen dispersed both within the cortex and medulla, resulting in loss of demarcation between the two regions (Figures 5A and B).

The results are summarized in Table 2.

4 Conclusion

The present finding that thymus of the anencephalic fetus was larger in terms of size, weight and thymic index, when compared to the thymus from a still born baby support of the earlier reports on these parameters (BIZZARD and MARION, 1956; CHI and PARK, 1982). The observation that the demarcation of cortex and medulla was not clear in the case of the thymus of the anencephalic fetus when compared to that of a normal fetus also complies with earlier observations (SHIN, 1999). Similarly, an abundance of HC in the anencephalic thymus has also been reported earlier (MITRA, 1973).

The present study has demonstrated the profile of a few of the interesting cytological features with the aid of immunocytochemical markers. Expression of cytokeratin has been demonstrated earlier in the normal thymus within the subcapsular epithelium, cortex, medulla and the areas surrounding the HC (SHEZEN, OKONE, BENHUR et al., 1995). There have been no reports on the expression of cytokeratin in the anencephalic fetus. Although thymus from the normal fetus exhibited well marked expression of cytokeratin, conforming to the earlier observations (SHEZEN, OKONE, BENHUR et al., 1995) thymus from the anencephalic fetus exhibited diffusely distributed cytokeratin. It is known that cytokeratin represents the presence of reticular epithelial cells. The significance if any, of the difference in the pattern of the expression of cytokeratin seen in the anencephalic fetus and the implications on the population of reticular epithelial cells has to be explored further.

The results from the S100 marker meant to show the morphology of dendritic cells did not differentiate between the normal and the anencephalic thymus, because of the equivocal results. Phenomenon of the uptake of S100 by the dendritic cells has been shown earlier (NAKAHAMA, MOHRI and MORI, 1989) similar studies have not been reported in the anencephalic fetuses. Findings from this study, in addition to supporting the earlier findings on the nature of the dendritic cells have also brought out the suggestion that these cells do not seem to show alteration in morphology and distribution in the anencephalic fetus.

Immature T cells have earlier been studied using immunoflourescence (SCHNEIDEK and GREGOIRE, 1977). In this study, these cells have been studied using their histochemical properties, using terminal deoxynucleotidyl tranferase (Tdt). Immature T cells have been seen to be increased in the anencephalic fetus, which were seen more pronounced around the HC, as indicated by the expression of Tdt. Increase in the number of T cell precursors could be interpreted as an attempt by the thymocytes for differentiating more number of T lymphocytes for immunological sequencing.

Hassall's corpuscles (HC), first described over 150 years exhibits different morphology in the mammalian species. Varied functions have been ascribed to HC. Among other functions, HCs have also been linked with the function of removal of apoptotic thymocytes and the maturation of thymocytes. These structures have been proposed as the source of thymic stromal lymphoprotein (TSLP) which are capable of activating some of the dendritic cells (WATANABE, WANG, LEE et al., 2005).

It is interesting to find that the thymus from the anencephalic fetus exhibited an increased number of HC, epithelial reticular cells and immature T cells, which were seen to be concentrated mainly around the HC. Thus, HC seems to be a center of increased activity in the anencephalic fetus. At the same time, the degree of apoptosis of the thymocytes has been at a slower rate, as indicated by the reflection of results of CD99 marker. It could be surmised that lack of apoptosis has led to thymic hyperplasia, as reflected by reduced expression of CD99 in the anencephalic fetus. It is possible that the decreased level of apoptosis could account for an increase in the number of immature T cells.

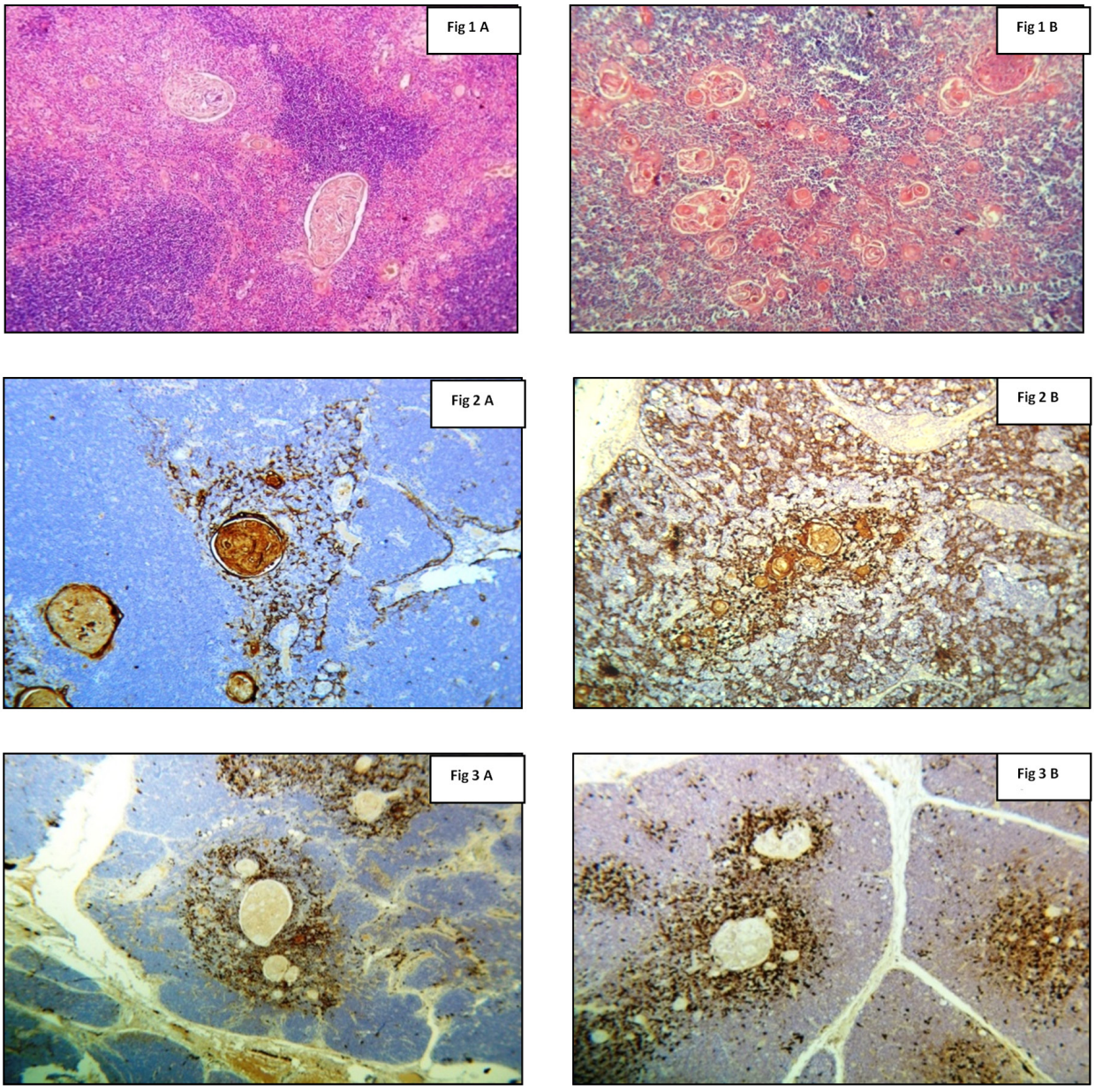

Plate 1. Photomicrographs showing differences in the microscopic structure of thymus in a still born normal fetus and the anencephalic fetus. Figure 1A. × 100. Thymus from normal still born fetus. Stained with H and E. Lobular architecture of cortex and medulla well differentiated. Figure 1B. × 100. Thymus from the anencephalic fetus. Stained with H and E. Shows abundance of HC and ill-defined lobular architecture. Figure 2A. × 40. Thymus of the normal still born fetus, showing expression of cytokeratin limited to the medulla. Figure 2B. ×40. Thymus of ananecephalic fetus. Note the diffuse expression of cytokeratin within cortex, medulla and in HC. Figure 3A × 40. Thymus of the normal still born fetus, showing equivocal expression of S 100. Figure 3B. × 40. Thymus of the anencephalic fetus, showing equivocal expression of S 100.

Table 2. Summary of the immunocytochemical findings.

S No	Marker	Significance of the marker	Result	
			Normal stillbirth	Anencephalic fetus
1	Cytokeratin	Epithelial reticular cells	Restricted to medulla	Diffuse all over the lobule; markedly pronounced around HC
2	S100	Shows Dendritic cells	Positive	Positive
3	Tdt	Identifies immature T cells	Poorly expressed	Pronounced mainly around HC
4	CD99	Marker for apoptosis	Well expressed in the cortex	Faintly positive, dispersed within both cortex and medulla

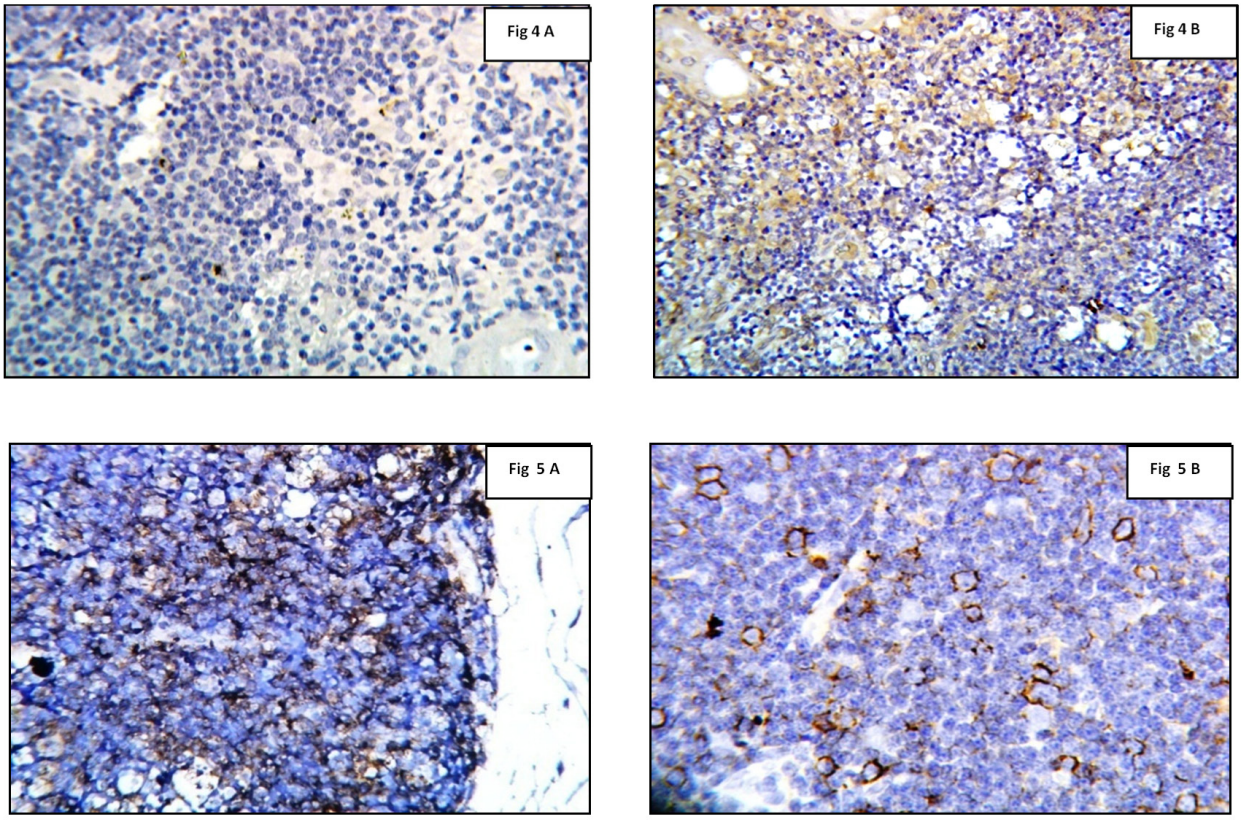

Plate 2. Photomicrogrpahs showing differences in the microscopic structure of thymus in a still born normal fetus and the anencephalic fetus. Figure 4A. × 400. Thymus of still born fetus, showing randomly scattered cells showing positivity for Tdt. Figure 4B. Thymus of anencephalic fetus, showing increased number of cells around HC, showing positivity for Tdt. Figure 5A. Photomicrograph of thymus in the normal still born fetus, showing many cortical thymocytes showing positivity for CD 99. Figure 5B. Photomicrograph of thymus in the anencephalic fetus. Note that the thymocytes, which show positivity for CD 99 are scattered.

However, the possible cause for an increase in the number of HC and epithelial reticular cells need to be studied further.

Histology of thymus is being reviewed with renewed interest because of the growing concern about the varied functions of thymus. Possibility of using thymic tissue from anencephalic fetuses for organ transplants is one such area of interest. Inquiry into the histological features of thymus of anencephalic fetus and similar anomalies may help in bringing out more features of thymus and could possibly help in the understanding the functions of thymus and the role of its histological components.

Acknowledgements: The authors wish to thank Dr Usha Kothandaraman, Professor of Anatomy for permitting to use the laboratory facilities to do this project.

References

AJITHA, RK. An insight into the structure of the thymus in human foetus-a histological approach. *Journal of Anatomical Society of India*, 2006, vol. 55, n. 1, p. 45-49.

BIZZARD, RM. and MARION, A. Hypopituitarism, hypoadrenalism and hypogonadism in the newborn infant. *Journal of Paediatrics*, 1956, vol. 48, n. 6, p. 782-792. http://dx.doi.org/10.1016/S0022-3476(56)80210-2

BUCHWALOW, IB. and BOCKER, W. *Immunohistochemistry*: Basics and Methods. Springer Publications, 2010. p. 52.

CHI, JG. and PARK, KS. Pathological study on anencephaly (10 autopsy cases). *The Seoul Journal of Medicine*, 1982, vol. 23, n. 3, p. 392-405.

JUNQUERIA, LC. and CARNEIRO, J. *Basic histology* Text & atlas. New York: McGraw Hill, 2010. p. 266-267.

LEMIRE, RJ. Neural tube defect. *Journal of Anatomy*, 1988, vol. 259, p. 558-562.

LEVY, HA. Full term foetus with holocranial anencephaly & other abnormalities. *South African Medical Journal*, 1976, p. 178-181. PMid:1257854.

MAZZITELLI, N., VAUTHAY, L., GRANDI, C., FUKSMAN, R. and RITTLER, M. Reviewing old concepts at the start of a new millennium: Growth restriction, adrenal hypoplasia and thymomegaly in human anencephaly. *Teratology*, 2002, vol. 66, n. 3, p. 105. PMid:12210471. http://dx.doi.org/10.1002/tera.10053

MITRA, SC. Endocrine glands in anencephaly. *Indian Journal of Paediatrics*, 1973, vol. 40, p. 246-251. PMid:4770906. http://dx.doi.org/10.1007/BF02817863

NAKAHAMA, M., MOHRI, N. and MORI, S. Immunohistochemical & histometrical studies of human thymus with special emphasis on age related changes in medullary epithelial & dendritic cells. *Virchow's Archive B cell Pathology, Zell Pathologie*, 1989, *vol.* 58, n. 1, p. 245-257.

SCHNEIDEK, IG. and GREGOIRE, KE. Demonstration of Tdt in thymocytes by immunofluorescence. *Proceedings of National Academy of Sciences*, 1977, vol. 74, n. 2, p. 734-738. PMid:322141 PMCid:392368. http://dx.doi.org/10.1073/pnas.74.2.734

SHEZEN, E., OKONE, BH., BENHUR, H. and ABRAMSKY, O. Cytokeratin exp. in human thymus-immunohistochemical mapping. *Cell and Tissue Research,* 1995, vol. 279, n. 1, p. 221-231. PMid:7534649. http://dx.doi.org/10.1007/BF00300707

SHIN, YK. and LEE, GK. Reduced exp. of CD99 and functional disturbance in anencephalic cortical thymocytes. *Virchows Archive,* 1999, vol. 434, p. 443-449. PMid:10389628. http://dx.doi.org/10.1007/s004280050364

WATANBE, N., WANG, YH., LEE, HK., ITO, T., WANG, YH., CAO, W. and LIU, YJ. Hassall's corpuscles instruct dendritic cells to induce CD4+CD25+ regulatory T cells in human thymus. *Nature,* 2005, vol. 436, n. 7054, p. 1181-1185. PMid:16121185. http://dx.doi.org/10.1038/nature03886

Received June 6, 2012
Accepted May 12, 2013