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MORPHOLOGICAL STUDY

Thymic Hassall's bodies of children with congenital heart defects

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Abstract: The development of the thymus and heart are closely related while in both, the neural crest cells play an important role. In our preliminary study, the thymic microscopic structures of the infant's thymuses with the congenital heart defects were observed. The study was conducted on 36 specimens of newborn thymuses removed due to surgery for cardiovascular malformations. Standard formalin-fixed paraffin-embedded tissue technique was used. Five-µm-thick sections were stained with hematoxylin and eosin and the microscopic examination was focused on the structure of Hassall's bodies. The Hassall's bodies showed considerable variations in size as well as in quantity. In most cases, the Hassall's bodies were large with the heterogeneous amorphous material enclosed in cystic dilatations. This type of Hassall's bodies is typical for adult thymuses. The most conspicuous changes (huge Hassall's bodies with the cystic dilatation filled up with cell detritus) were observed in patients with ventricular septal defect, atrioventricular septal defect and tetralogy of Fallot. Small-sized Hassall's bodies corresponding with infant age, were observed in cases with pulmonary valve atresia, atrial septal defect and in some cases of transposition of great arteries. We assume that the changes of microenvironment of the thymic medulla are associated with disrupted migration of the neural crest cells which are essential in the normogenesis of both heart and thymus *(Tab. 1, Fig. 12, Ref. 33)*. Full Text in free PDF *www.bmj.sk.*
Key words: thymus, Hassall´s bodies, congenital heart defects, neural crest cells.

The thymus is a primary lymphoid organ found in all vertebrates, with the exception of jawless fish. The thymus has a unique capacity to support the development of self-tolerant T cells expressing a diverse repertoire of antigen receptors. Thymopoiesis involves reciprocal tissue interactions between the epithelial cells derived from the endoderm of the embryonic pharynx (branchial region) and neural crest-derived mesenchyme. However, the contribution of mesenchymal cells to thymic epithelial cell proliferation and creation of the thymic microenvironment are still unclear (1 – 3).

The general microscopic description of human thymus is complicated because the thymus is a very dynamic organ rapidly changing under exogenous influence and involuting with age (4). Individual thymic lobules are variable in shape, size, and orientation. Each lobule contains central and peripheral parts representing the medulla and cortex, respectively. These are easily distinguished by their histomorphological features such as lymphocyte density, which is more pronounced in the cortex. Between cortex and medulla, the cortico-medullary junction is recognized as an area rich in blood vessels and a site where connective-tissue septa reach the medulla region (5, 6). Since 1849, when a physician Arthur Hill Hassall, who lived in London, described acidophilic squamous spherical structures in the thymic medulla, these corpuscles have been thought to be specific for this organ (7). During thymic ontogenesis, the Hassall's bodies (corpuscles) appear when lymphopoiesis has been already established and the cortex, medulla and the cortico-medullary junction are capable of conducting the positive and negative selection of T cells (8). Hassall's bodies have been the object of only few studies focused on both, morphological and functional aspects. Classical studies describe them as "onion-like" structures, variable in number and size, often displaying degenerative changes in the central area, such as necrosis, cellular detritus, sometimes extensive calcification, cystic alterations, and foamy macrophages (9, 10). But the importance of Hassall's bodies residing in ensuring proper functioning of the immune system is also evident from the data relating to the effects of immunosuppressive treatment on the morphology of thymus (number of Hassall's bodies decreased) (11, 12), and interesting associations between human immunodeficiency virus and Hassall's bodies. A viral antigen (gp120) was predominantly located in and around the thymic epithelial cells in Hassall's bodies. The thymic epithelial cells of the Hassall's bodies can be a target and/or reservoir in an early stage of HIV infection (13).

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Tab. 1. Cardiac defect causes classified into groups.

Cardiac defect	Number of newborns
Ventricular septal defect	12
Atrial septal defect	2
Atrioventricular septal defect	1
Discordant ventriculoarterial connection (transposition of great arteries)	6
Tetralogy of Fallot	4
Double-outlet right ventricle	2
Pulmonary valve atresia	1
Congenital malformations of pulmonary valve	2
Congenital malformations of tricuspid valve	2
Hypoplastic left heart syndrome	3
Congenital stenosis of aortic valve	1

The most accepted definition of Hassall's corpuscles is according to Bodey et al (8) who define it as a unique, antigenically distinct, functionally active, multicellular component of the non-lymphocytic cellular microenvironment of the thymic medulla. Hassall's bodies participate in physiological activities of thymus in both, prenatal and adult phases.

Approximately 0.4 % up to 0.6 % of newborn infants are delivered with a moderate or severe congenital heart defects (14). These congenital heart defects are etiologically heterogeneous, and the genetic and environmental causes have been proposed for many specific defects (15). More then twenty-five years ago, the first paper showed the relationship of neural crest-cells with heart development (16). The region of neural crest cells migrating to the heart was documented extensively using quail chick chimeras and experimental partial ablation of neural crest, and was called "cardiac neural crest", not because the cells migrated exclusively to the heart but because they were found to be critical for normal heart development (17). Pluripotent neural crest cells originating from the hindbrain migrate to the caudal three pharyngeal arches and become condensed in the mesenchymal subendocardial folds, known as the aorticopulmonary septation complex (18). The cardiac neural crest ablation phenotype includes three distinct components (19, 2):

- Defective development of the cardiac outflow tract,
- Abnormal myocardial function,
- Defective development of the derivates of the caudal pharynx including arch arteries, parathyroid and thyroid glands, thymus and the secondary heart field.

Ablation of a smaller area within the cardiac neural crest is thought to contribute to conotruncal anomalies including tetralogy of Fallot and double-outlet right ventricle (20). The transposition of large arteries occurs infrequently after cardiac neural crest ablation (21).

Since the development of thymus and heart are closely related, the association between the thymic microscopic structure of infants and congenital heart defects was observed. The changes in the structure, size and number of the Hassall's bodies were noticed and therefore our preliminary morphological study is focused on these characteristic structures of the thymus.

Patients and methods

The study group consists of thirty-six patients whose thymuses were removed (partial thymectomy) during surgery performed for various congenital heart defects (Tab. 1). In the group, none of the patients suffered from DiGeorge syndrome. The age of patients ranged between one month up to nine years (more than 50 % before the age of six months), with a mean value of 11.5 months. Fragments of the thymic parenchyma were fixed in formalin for 24 hours, embedded in paraffin, and five-µm-thick sections were stained with the hematoxylin and eosin. Histological examination was performed by Nikon Eclipse 80i microscope and images were captured with Nikon DS-Fi1 digital camera.

Results

All observed thymuses were of normal structure, including the well-developed cortex as well as medulla. An exception was only in one case of a hypoplastic thymus of a three-month-old

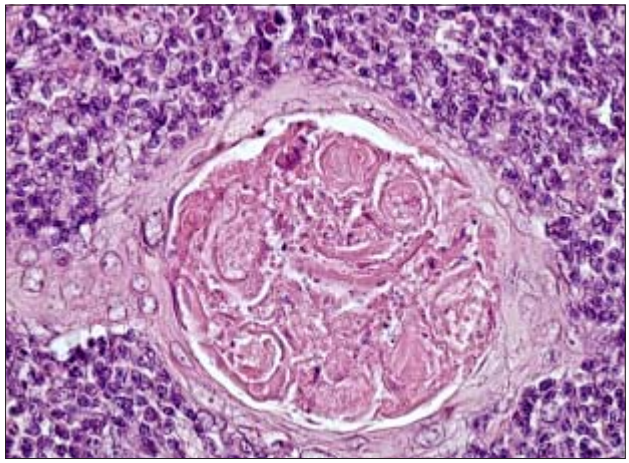

Fig. 1. Thymic medulla with large cystic Hassall's corpuscle of a nine-month-old boy with ventricular septal defect (HE, magn. 400x).

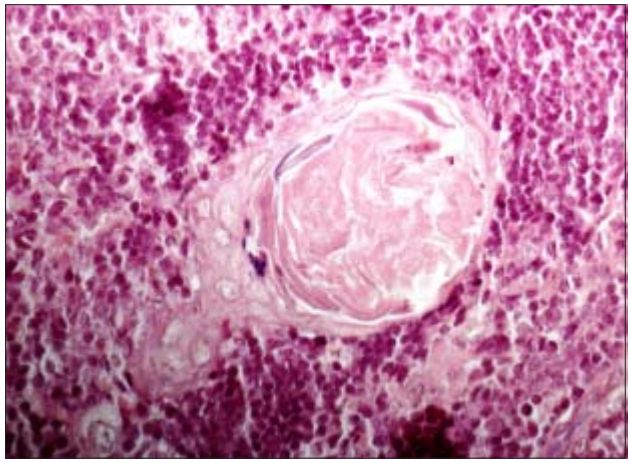

Fig. 2. Thymic medulla with large cystic Hassall's corpuscle of a three-year-old boy with ventricular septal defect (HE, magn. 400x).

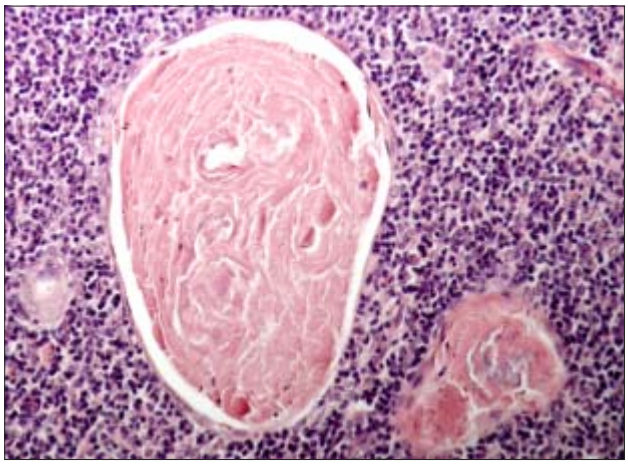

Fig. 3. Thymic medulla with extremely large cystic Hassall's corpuscle of a two-year-old boy with ventricular septal defect (HE, magn. 400x).

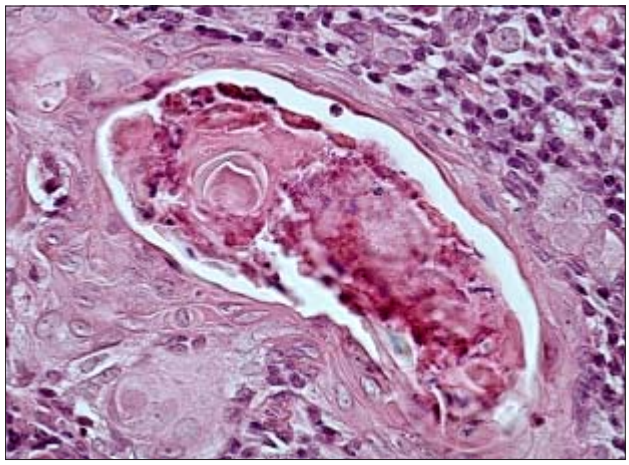

Fig. 6. Hypoplastic thymus with larger cystic Hassall's corpuscle with various cell detritus of a three-month-old girl with dextro-transposition of great vessels (HE, magn. 400x).

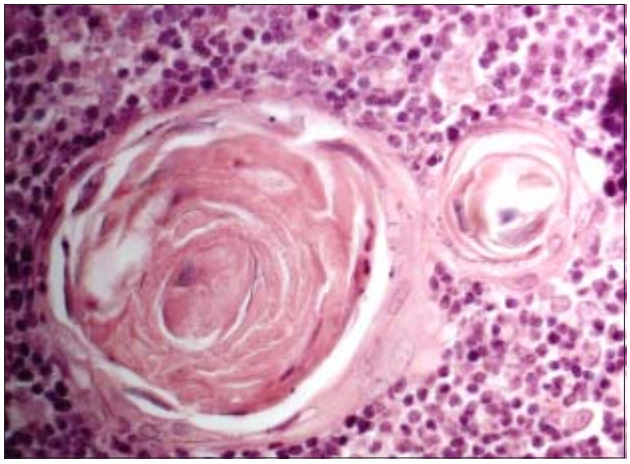

Fig. 4. Thymic medulla with large cystic Hassall's corpuscle of a six--month-old boy with tetralogy of Fallot (HE, magn. 400x).

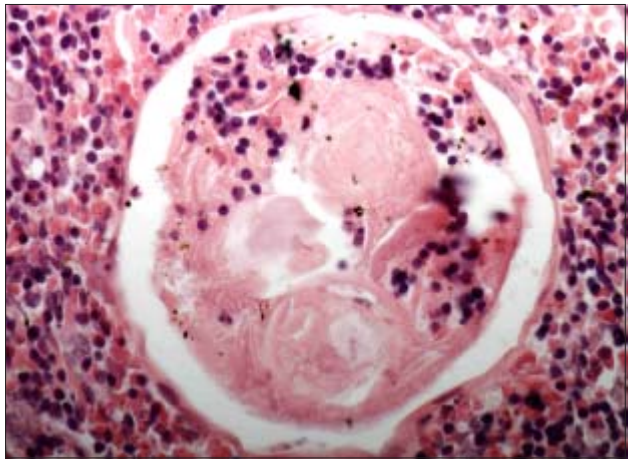

Fig. 7. Thymic medulla with extremely large lymphocyte-rich Hassall's corpuscle of a two-month-old girl with hypoplastic left heart syndrome and ventricular septal defect (HE, magn. 400x).

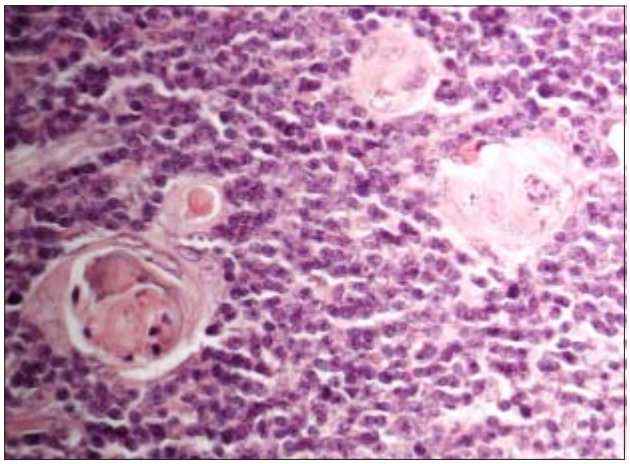

Fig. 5. Thymic medulla with larger Hassall's corpuscle of a three--week-old boy with transposition of great vessels (HE, magn. 400x).

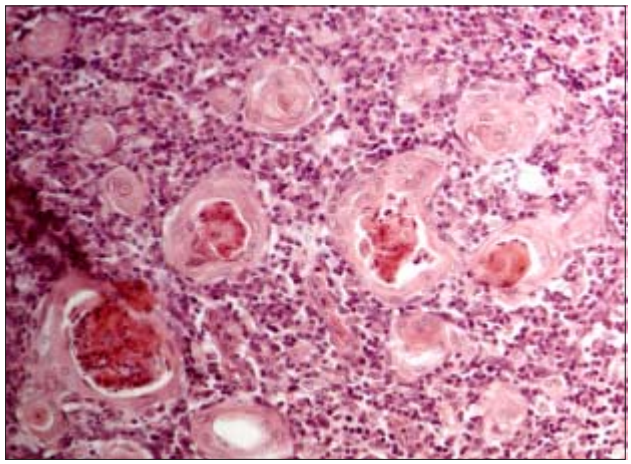

Fig. 8. Thymic medulla with numerous large Hassall's corpuscles of a two-month-old boy with congenital malformations of pulmonary valve (HE, magn. 200x).

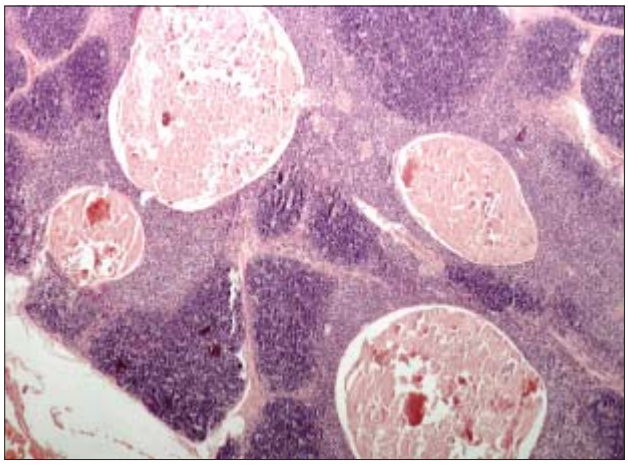

Fig. 9. Thymic medulla with enormous large Hassall's corpuscles of a nine-year-old girl with atrioventricular defect (HE, magn. 40x).

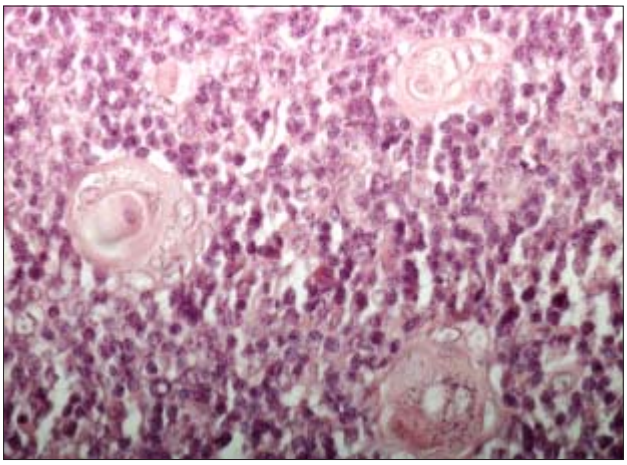

Fig. 11. Thymic medulla with Hassall's corpuscles of normal size and structure of a one-year-old boy with pulmonary valve atresia (HE, magn. 400x).

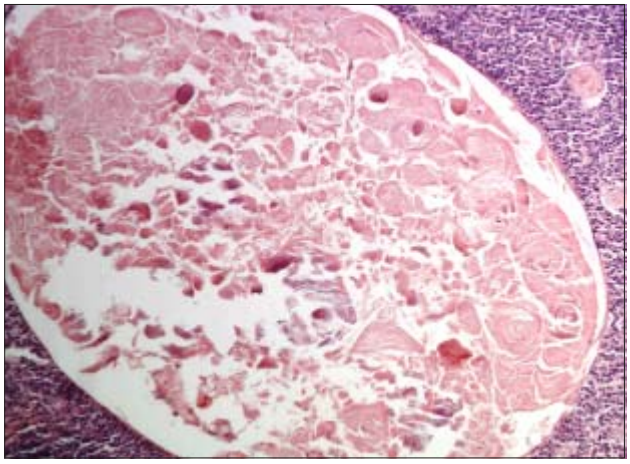

Fig. 10. Thymic medulla with enormous large Hassall's corpuscle of a nine-year-old girl with atrioventricular defect. In the right upper quadrant a Hassall's corpuscle of normal size (HE, magn. 100x).

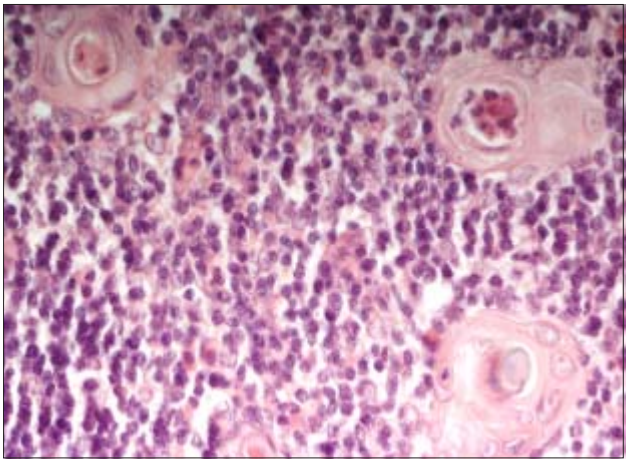

Fig. 12. Thymic medulla with Hassall's corpuscles of normal size and structure of a seven-month-old girl with atrial septal defect (HE, magn. 400x).

girl with dextro-transposition of great arteries. In this case neither cortex nor medulla was distinguishable. The Hassall's bodies showed considerable divergences in size as well as in quantity. In the thymuses of patients with the diagnosis of ventricular septal defects, sparse but giant Hassall's bodies were found in the medulla (Figs 1 – 3). The smaller corpuscles included more layers of squamous thymic epithelial cells on the surface (Figs 1 and 2), whereas the outer layer of the biggest ones was formed by one layer of extremely flattened epithelial cells (Fig. 3). Either more flattened cells were present inside of the bodies or in case of the larger bodies, the inside area was formed by the amorphous acidophilic material, probably the cell detritus. Similar finding was seen in the thymic medulla in cases of tetralogy of Fallot (Fig. 4). Extremely large Hassall's bodies with amorphous content were not observed in a case of ventricular septal defect. Cystic Hassall's bodies were found in a case of transposition of great arteries (Fig. 5) while, even larger Hassall's bodies were developed in a case of hypoplastic thymus related to this congenital heart defect (Fig. 6).

In one case (two-month-old girl with hypoplastic left heart syndrome and ventricular septal defect), Hassall's bodies possess the clusters of lymphocytes scattered between areas of cell detritus (Fig. 7). In a case of thymus of a patient with congenital malformations of pulmonary valve, higher number of the Hassall's bodies in the medulla was observed (Fig. 8). Extremely large Hassall's bodies were found in a case of a nine-year-old girl with atrioventricular septal defect. The medulla of each lobule was filled up with huge Hassall's bodies (Fig. 9). Amorphous acidophilic material was present inside the body (probably necrotic material or cellular detritus) which was separated from the surrounding medullar parenchyma by one layer of flattened cells (Fig. 10).

In most cases, the Hassall's bodies were large with the heterogeneous amorphous material enclosed in a cystic dilatation.

This type of Hassall's bodies is typical for adult thymuses. In infants and small children, the Hassall's bodies are rather small, formed by ovoid or irregular congestions of epithelial cells without prominent acidophilia, necrosis or cellular detritus. Small Hassall's bodies corresponding with infant age were observed in cases with pulmonary valve atresia (Fig. 11), atrial septal defect (Fig. 12), and in some cases of transposition of great arteries.

Discussion

Hassall's bodies are structurally organized from thymic epithelial cells, usually undergoing hypertrophy prior to their inclusion in the outer cell layer of the corpuscles (8). Hassall's bodies have a secretory function (cytokines and growth factors) (22), as well as a function in communication between antigen-presenting cells and T cells (23). Their number, size and morphological features depend mostly on age of the individual. Raica et al. (9) classified Hassall's corpuscles according to age, structure and immuno-histochemical features into four groups, namely juvenile, immature, mature and senescent types. The "mature" and "senescent" types are found only in patients aged over six years (9). Contrary to this study, in most of cases of patients with ventricular septal defect, atrioventricular septal defect, tetralogy of Fallot and other defects, we found Hassall's bodies with cystic dilatation filled with abundant cellular debris and acidophilic necrotic material. These morphologic features are typical for "senescent" Hassall's bodies according to Raica et al. (9). In one case, we described a "lymphocyte-rich" type of Hassall's bodies, originally depicted by Raica et al. (24). It is possible that this type of Hassall's bodies could reflect a rapid involution of the thymus induced by preoperative stress (24). In the review of literature, we found only one hypothesis about the origin of "cystic dilatations" in thymuses, which we noticed in most of our cases. In this hypothesis, Ors et al (25) suggested that the various types of cystic structures might represent maturational stages of classical Hassall's corpuscles. In other vertebrates, for example reptiles and amphibians, degenerative cysts were described by more research groups (26 – 28).

Changes of the thymic microscopic structure in infants with congenital heart defects are probably related to the embryonic development of both organs. Thymic organogenesis depends on the interactions between cells of all three embryonic germ layers, namely endoderm-derived epithelium of pharyngeal pouches, neuroectoderm-derived neural crest mesenchyme and mesoderm-derived hematopoietic cells (29). The normal development of heart outflow tracts, development of great arteries and secondary heart-field development are also induced by neuroectoderm-derived neural crest (30). It is important to take into consideration that the changes in microscopic structure of thymus may reflect the stress caused by changes in circulation and oxygenation of blood or by repeating preoperative examination of the patient. This hypothesis seems to be less presumable. In cases of heart defect with none or minimal influence of the migration of neural crest cells (for example atrial septal defect), structurally normal Hassall's bodies were found. The children were hospitalized at the same clinic; this excludes the possible impact of preoperative stress on morphology of Hassall's bodies.

Congenital heart disease is one of the most common phenotypic manifestations of chromosome 22q11 deletion syndrome. More than 75 % of patients with a chromosome 22q11 deletion express some forms of cardiovascular anomalies. E. g., in our ventricular septal defect cases found to yield the most prominent changes in thymic Hassall's corpuscles, 14 % up to 18 % of this deletion, is present (31). About 10 % of infants had chromosome 22q11 deletions with tetralogy of Fallot defect (32). Chromosome 22q11 deletions are often associated with extracardiac anomalies, typically affecting the neck and head and resulting in apparently different clinical syndromes including DiGeorge syndrome. These syndromes have been grouped together under an acronym "CATCH 22" (Cardiac defects, Abnormal facial features, Thymus aplasia or hypoplasia, Cleft palate, Hypocalcaemia while 22 denotes the deletion on chromosome 22). The normal development and structure of thymus has important clinical implications, e. g. Chaoui et al (33) stated that an absent or hypoplastic fetal thymus in ultrasound assessment can be a marker for cardiac defects and deletion 22q11.2.

Conclusion

In our preliminary morphological study, we described the changes of the Hassall's bodies in infant patients with congenital heart defects. The prominent changes such as large Hassall's bodies with cystic dilatation filled up with cell detritus were observed in patients with ventricular septal defect, atrioventricular septal defect, tetralogy of Fallot and some cases of transposition of great arteries. In conclusion, we assume that the changes in microenvironment of the thymic medulla are associated with disrupted migration of neural crest cells while the latter changes are essential in the normogenesis of heart.

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