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Research Paper

Association between histological alterations in the thymus and sudden infant death syndrome

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ABSTRACT

Introduction: Sudden infant death syndrome (SIDS) involves the death of an infant during the first year of life and it is among the leading causes of infant mortality worldwide. One hypothesis regarding the pathogenesis of SIDS is that it results from a combination of three independent factors: endogenous vulnerability, a critical time window during postnatal development, and exogenous stressors. This hypothesis is known as the "triple-risk model".

Methods: In this study, we used an immunohistological approach to compare the cellular microenvironments of thymuses from 19 infants whose sudden death was classified as SIDS and a control group, which consisted of thymuses from age-matched children undergoing surgery for various congenital heart defects. We hypothesized that morphological signs of stress-related thymic involution would be present.

Results: Based on our observations, we found evidence that the proliferation and maturation of T-lymphocytes in the thymuses of infants with SIDS were suppressed. We observed enhanced macrophage activity, suggesting an increase in the apoptosis of lymphocytes and decrease in number of thymic dendritic cells and myoid cells. Significant apoptosis of thymic lymphocytes without cell regeneration typically leads to atrophy of the thymus. All cellular events we observed resemble the initial stage of stress-related thymic involution.

Conclusion: These results support the "triple-risk model," suggesting that certain exogenous stressors might be involved in the pathogenesis of SIDS. This was probably not recognized during the autopsies of infants who died suddenly.

1. Introduction

Sudden infant death syndrome (SIDS) causes the death of an infant during the first year of life. It arises abruptly without any apparent preceding medical complications and it typically occurs during sleep. As of 2017, its exact cause still remains unexplained,[1] despite the fact that it was first described in the medical literature almost 50 years ago.[2] With only small epidemiological differences among countries, SIDS is among the leading cause of infant mortality.[3] SIDS meets the definition of *diagnosis per exclusionem* because there are many historically documented cases of misdiagnosis. Thus, it is critical to develop firm criteria to prevent these medical errors.[4] When all possible causes of death are ruled out and there are no other medical explanations available after conducting a thorough death scene investigation and a detailed autopsy, the diagnosis of SIDS is made.[5] Based on these findings, the recent and most complex definition of SIDS is: "The sudden unexpected death of an infant under 1 year of age, with onset of the fatal episode apparently occurring during sleep, that remains unexplained after a thorough investigation, including performance of a complete autopsy and review of the circumstances of death and the clinical history".[2]

Risk factors for SIDS are believed to include low birth weight, low Apgar score, sleeping on the stomach, socioeconomic status of parents, tobacco smoke exposure, improper ambient temperature, and alcohol consumption during pregnancy. The multifactorial pathogenesis of SIDS is underscored by the fact that authors typically link all these factors by summarizing the overarching risk factors as follows: endogenous vulnerability, a critical time window during postnatal development, and exogenous stressors. This is known as the "triple-risk model," which was first described in the scientific literature in 1971.[6,7] The nature of SIDS remains obscure, despite the fact that many attempts have been made to clarify the underlying pathological mechanisms. Currently, one of the organs of particular interest for its association with SIDS is the

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thymus.

The thymus is a primary lymphoid organ with an important endocrine function. It is the site of the proliferation, differentiation, and maturation of T lymphocytes. It attains its maximum size between the time of birth and the first few months of life. During this period, the thymus has its greatest weight relative to body size. Age-related thymic involution begins during or soon after the first postnatal year and continues progressively throughout an individual's life.[8,9] From a functional point of view, the thymus is sometimes metaphorically described as the "university for developing T-lymphocytes" because its principal role is to "teach" these cells how to distinguish self-antigens of the human body from non-self-antigens found in invading pathogens or cancer cells. During the process of T-lymphocytes acquiring immunocompetence, a large proportion (95–98%) of developing lymphocytes with altered reactivity to self-antigens are eliminated through apoptosis. Subsequently, dead lymphocytes are quickly removed by thymic macrophages.[10,11] The differentiation of T-lymphocytes is a carefully orchestrated process supported by thymic epithelial cells (often called nurse cells), fibroblasts, myoid cells, and bone marrow-derived accessory cells, such as B- lymphocytes, macrophages, and interdigitating dendritic cells. All of these aforementioned cells together form the unique microenvironment of the thymus.[12,13]

In children, the thymus is particularly sensitive to the effects of stress (endogenous corticosteroids). The accidental or stress-related involution of the thymus is a very sensitive "barometer" of exposure to any type of chronic or acute stress, such as infections, malnutrition, and side effects from medications.[14–17] In this study, we performed a histological and immunohistochemical examination of the cellular microenvironment of thymuses from infants whose sudden death was classified as SIDS. We compared these microenvironments to a control group, which consisted of thymuses from age-matched children undergoing surgery for various congenital heart defects. We hypothesized that we might find morphological signs of stress-related thymic involution. If this hypothesis were true, our morphological study would therefore support the "triple-risk model," demonstrating the involvement of some type of as-yet unknown exogenous stressor in the pathogenesis of SIDS.

2. Materials and methods

In this study, we examined human thymic tissue samples from 19 infants, who died with a post-mortem diagnosis of SIDS. Samples were acquired from the Department of Forensic Medicine in Prešov, Slovakia. The mean age of the infants at the time of death was 3.1 months (12 boys and 7 girls) with mean weight at the time of death 4153 g. The control group consisted of thymus specimens from 14 newborns and small babies of a similar age. These control samples were perioperatively obtained from children when they underwent median sternotomy and corrective cardiovascular surgery at the Children's Cardiac Center in Bratislava, Slovakia following the diagnosis of a congenital heart anomaly.

Formalin-fixed and paraffin-embedded tissues were processed at the Institute of Histology and Embryology, Faculty of Medicine, at the Comenius University in Bratislava, Slovakia using routine histological techniques. Immunohistochemical characterization of different thymic cells was performed using a panel of six antibodies. A short description of each antibody is presented in Table 1. Briefly, sections were stained to detect the expression of cell type-specific antigens that might contribute to the formation of the thymic microenvironment, such as desmin, actin, S100 protein, and CD68. We also detected expression of the proliferation marker Ki67 and the oncoprotein BCL2, which inhibits apoptosis. For antigen retrieval and visualization, the EnVision™ + Dual Link System HPR (DAKO, Denmark) was used with diaminobenzidine as a brown chromogen. To orient samples on the slide, cell nuclei were stained dark blue with Mayer's hematoxylin. For visualization of the histological sections by light microscopy, the LEICA DM2500 microscope was used, and images were captured using the LEICA DFC290HD digital camera. Slides were evaluated semi-quantitatively.

3. Results

Compared to the controls, all of the thymuses from infants with a post-mortem diagnosis of SIDS displayed a normal histological structure, and both the cortex and medulla were well-developed. The Hassall's corpuscles in the thymic medulla of SIDS patients varied in both size and number. However, this variation was typical also of thymuses from children without SIDS. By comparing the thymuses of infants with SIDS to those of the controls, we concluded that the thymuses of SIDS patients have the following features:

- fewer lymphocytes and a "starry sky" appearance of the thymic cortex in hematoxylin and eosin staining (Fig. 1) due to numerous tingible body macrophages.
- a greater number, larger diameter, and increased activity of CD68-positive macrophages (Fig. 2). Inside the cytoplasm of macrophages, we observed numerous tingible bodies, which represented apoptotic bodies, and stainable cellular debris.
- fewer S100-positive antigen-presenting dendritic cells within the thymic medulla (Fig. 3).
- fewer Ki67-positive proliferating lymphocytes in the subcapsular portion of the thymus (Fig. 4), which was a sign of decreased lymphocytopoiesis.
- fewer BCL2-positive mature and long-lived lymphocytes within the thymic medulla (Fig. 5), another sign of decreased lymphocytopoiesis.
- slightly fewer desmin and smooth muscle actin positive thymic myoid cells within the medulla (Fig. 6).

Based on our observations, we suspect that the proliferation and maturation of T-lymphocytes in the thymuses of infants with SIDS might be suppressed. Moreover, the increased activity of macrophages indicates enhanced apoptosis of lymphocytes (the process of lymphocyte death is definitely not via necrosis because necrosis is associated with inflammation and not with tingible body macrophages). Significant apoptosis of thymic lymphocytes without cell regeneration leads to thymic atrophy. All of the cellular events we observed resemble the initial stage of accidental, stress-related thymic involution. We suggest that even though the coroner or forensic doctor could not determine the causes of the infants' deaths, the changes in the microscopic

Table 1
Monoclonal antibodies used in this study (in alphabetical order). Researched cells/cellular events within thymic microenvironment are highlighted.

Antibodies against	Short description
BCL2 oncoprotein	Plays a central role in apoptosis. **Blocker of apoptotic cell death.**
CD68	Glycoprotein highly expressed by monocytes and tissue **macrophages.**
Desmin	Forms a cytoskeletal network across the muscle fibers. Specific for smooth muscle cells, striated muscle fibers, and **thymic myoid cells.**
Ki67	A marker of **cell proliferation**. A nuclear protein preferentially expressed during all phases of the cell cycle, but absent in quiescent cells.
Smooth muscle actin	Belongs to the microfilament system of smooth muscle cells, myofibroblasts, myoepithelial cells, and **thymic myoid cells.**
S100 protein	Present in cells derived from the neural crest (e.g., Schwann cells), but also present in **antigen-presenting dendritic cells.**