MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL FEATURES OF THE LUNG TISSUE IN SUDDEN INFANT DEATH SYNDROME (SIDS)

Morphological and immunohistochemical features of the lung tissue from 50 corpses of infants aged 1 month to 1 year were studied to determine their place in the structure of causes of death. Immunohistochemistry revealed decreased expression of surfactant protein B in most cases of sudden infant death syndrome (SIDS), which is probably due to qualitative changes in pulmonary surfactant. The data obtained suggest that the comprehensive studies of lung tissue of infants may contribute to clarifying the degree of risk and more accurately determining the causes of death in some cases of sudden infant death. This will help reduce the risk of diagnostic errors, i.e., hypo- or over-diagnosis SIDS and is of practical importance for forensic examination. This is especially true for those cases that require the need for differential diagnosis of SIDS with different types of pneumonia or mechanical asphyxia. Further investigations in this area should focus on the study of the molecular and genetic mechanisms of synthesis and transport of surfactant proteins, and to develop simple inexpensive methods for detection of the lungs surfactant deficiency in infants.

Keywords: Sudden infant death syndrome (SIDS), lung tissue, surfactant, morphology, immunohistochemistry

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Introduction

Sudden infant death syndrome (SIDS) includes sudden, non-violent deaths of infants aged 1 month to 1 year. In this syndrome results of scene investigation, review of the clinical history, autopsy, morphological and other laboratory studies do not provide clear evidence about the cause of infant death. According data by Giyasov and Khalmatova (2003) the rate of SIDS in the city of Tashkent on 1000 live births in the range 1.2 - 1.4, and of all infant deaths in the home more 41% are SIDS. These data are roughly comparable to the literature data obtained in different countries with different levels of development. However, in the study of the frequency and the relation with the overall infant mortality has revealed some heterogeneity for different countries and regions (Kelmanson and Adulas, 2006). These differences in terms of epidemiology of SIDS associated with many factors. First of all, this is due to the lack of a unified approach to the verification of the diagnosis SIDS, inadequate or improper use of techniques that would help pinpoint the reasons for the death of an infant. The important role played by public policies aimed at reducing or eliminating risk factors for the development of SIDS. In 1992 the American Association of Pediatrics (AAP) recommended “non prone” (no prone, not over the abdomen) position the baby to sleep as a strategy to reduce the risk of SIDS. This allowed the reduction of the frequency of SIDS in the USA from 1.2 deaths per 1,000 live births in 1992 to 0.56 deaths in 2001, i.e. was reduced on 53% for 10 years. Subsequently, from 2001 to 2006 the SIDS rate has stabilized. Although indicators syndrome decreased by more than 50% since beginning of the 1990s, the SIDS in USA remains the third leading cause of all infant mortality and the leading cause of post-neonatal (28 days to 1 year of age) mortality (Moon, 2011).

This issue is of great interest not only for pediatricians but for pathologists, forensic scientists and other professionals (Sharma, 2007). In the last decade have been published
numerous review and research articles on various aspects of SIDS. They analyzed the risk factors, pathological physiology, genetic, epidemiological, immunological and other aspects of the pathogenesis of SIDS, consider possible options for the classification and differential diagnosis of this syndrome (Guntheroth and Spiers, 2002; Krous, Beckwith and Byard, 2004; Prandota, 2004; Hunt and Hauck, 2006; Leiter and Bohm, 2007). However, based on a critical analysis of all existing hypotheses, P.N. Goldwater (2011) rightly points out that there is not such a hypothesis, which would meet all the requirements of pediatricians, pathologists, lawyers and etc. He believes that every hypothesis must consider pathological and epidemiological risk factors. Many authors agree that SIDS is a complex syndrome that may have multiple etiologies with a lot number of possible risk factors. In their view, great prospects in this area are new approaches to identifying predisposing to SIDS risk factors disclosure molecular genetic mechanisms of this syndrome (Mitchell, 2009; Goldwater, 2011; Emura and Usuda, 2011).

Available clinical and epidemiological evidence suggests that the mechanisms underlying SIDS, largely due to the abnormal phenomena observed during sleep, as well as the structure of the sleep disturbances in infants (Kelmanson, 2010). There is a sufficiently close relationship between the structure of the infants sleep and activity of respiratory system. In the rapid phase of sleep is expressed irregular breathing, including short episodes of hypo- and apnea. It is believed that sleep apnea of infant is pathological if its duration is more 20 seconds. Hypoxia in infants and children during the first months of life in the rapid phase of sleep does not only suppress the ventilation, but does not contribute to the awakening of the child. In infants rapid phase of sleep is defined by a set of changes of respiratory characteristics predisposing to pathological disorders of respiratory function. The most important risk factors for sudden infant death, such as poor organization of life, lack of stimulation of the child's development, premature and / or low body weight at birth, maternal smoking during pregnancy, sharing the bed with parent(s) often associated with sleep disturbances in infants aged 2 to 6 months (Kelmanson, 2010). It is this period of life characterized by a maximum peak of SIDS, usually the ensuing in night while baby is sleeping.

At present, a number of countries in cases of sudden unexpected infant death are conducting thorough macro- and microscopic studies of various vital organs, especially the lungs, heart and kidneys, in order to establish the cause of death and verification of the final diagnosis. A retrospective analysis by M. Weber, J. Pryce, M. Ashworth, M. Malone, and N. Sebire (2012), showed that microscopic examination of these organs in combination with other history and laboratory data can clarify the causes of death and to avoid forensic medical errors. In the verification of the diagnosis with sudden unexpected infants death (SUID) leading role played by microscopic examination of the lungs, which in 43% of cases contributed to clarify the causes of death.

Our previous studies have shown the importance diagnostic value of comprehensive studies of the lung tissue to verify the cause of death in different types of unexpected death in infants (Shodiev, Giyasov and Tukhtaev, 2011; Tukhtaev, Giyasov and Shodiev, 2012). The results of these studies to some extent avoid the expert errors in terms of hypo- or overdiagnosis in establishing the causes of death associated with the violation of respiratory function.

The aim of this work was a comprehensive study of the morphological and immunohistochemical features of the lungs in SIDS, comparing them with other cases of sudden infant death with a verified diagnosis. Based on these data, we have also sought to clarify the role of pathological changes in the lungs and the state of pulmonary surfactant in the development of sudden infant death syndrome. In addition, we wanted to find out to what extent the comprehensive studies of the lungs may help to identify additional risk factors and more accurate determination of the cause of death in cases of sudden infant death.
Materials and methods

The material for the study was collected from 50 corpses of children aged 1 month to 1 year. Of these, in 20 cases there was SIDS (I group). 18 children patients died of pneumonia and acute respiratory infection (II group). In 12 cases was a category of violent death (mechanical asphyxia, mechanical injuries and thermal burns - III group). The last two (II and III) groups served as controls for SIDS. For morphological studies of lung tissue taken within 2-12 hours after death were fixed in 12% formalin solution in phosphate buffer (pH - 7.3) and after the respective wires embedded in paraffin. Paraffin sections 5-7 microns thick were stained with hematoxylin-eosin and Schiff's reagent. To identify of pneumocytes type II (P2) sections were stained by Sudan III. Counting the number of P2 in each case carried out by 1000 all pneumocytes and expressed in absolute numbers and percentages.

Immunohistochemical detection of mature surfactant protein B-type was performed on paraffin sections of lungs using a set UltraVision (Thermo Scientific, USA). These studies were conducted in 10 samples of lung SIDS, 5 - in cases of pneumonia and acute respiratory infections, and 5 - in the categories of violent death. Evaluation of the results of immunohistochemical studies were conducted on a scale of intensity and localization of the reaction product. All digital data is processed by the method of variation statistics using the software package Microsoft Excel 2010. Differences satisfying P <0.05 were considered significant.

Results

Macroscopic examination of the lung tissue with SIDS in some cases revealed the presence of foci of punctate hemorrhages under the visceral pleura, segmental pulmonary edema with emphysematous areas. A microscopic examination revealed a slight swelling and minor bleeding lesions of lung parenchyma, and pulmonary vascular engorgement in violation of the permeability of the walls and diapedesis of red blood cells in the surrounding tissue. In the lumen of the large bronchi determined locally homogeneous mass of reddish color, and in some cases showed a small peribronchial infiltrates consisting mainly of lymphocytes (Figure 1). Small pockets of advanced alveolar atelectasis and disatelectasis have been found frequently. Often in the alveoli and bronchi increased epithelial desquamation, and sometimes the presence in the lumen of the alveoli small hemorrhagic exudates were revealed (Figure 2). Approximately one-third of infant deaths from SIDS in the respiratory passages and the alveoli found signs of moderate catarrh. Along with them in about one-third of deaths in SIDS morphological manifestations of lung immaturity have been found.

Microscopic changes in the lungs of the control groups of infants (II and III) are fully consistent with the diagnosis and cause of death, revealed by the expert investigation. Thus, in some cases of SIDS in the lung tissue are revealed slight morphological changes with characteristics of moderate serous-desquamative or serous-catarrhal pneumonia. These changes are not enough to qualify as a major cause of death. However, it is possible that they may play a role as provoking factor (trigger) that causes sudden respiratory failure during sleep with outcome to death.

Immunohistochemical studies have shown that surfactant protein is localized in the surface of the alveoli and terminal bronchioles and bronchi of various calibers. Typically, the reaction product was localized in the form of aggregates, its diffuse distribution on the surface of the airways is determined very rarely. In the lung tissue of infants of the II and III groups showed rather intense and widespread deposition of surfactant protein B (Figure 3). At the same time, in the lung tissue in 6 of the 10 infants with SIDS expression of surfactant protein B was negative or very low compared to the control groups (Figure 4). This is indicative of the qualitative changes of surfactant, which generally confirmed
Discussion

SIDS remains a third leading cause of all infant mortality and is the leading cause of death in infants from 1 month to 1 year (Moon, 2011). Despite the huge number of studies, many problems associated with the etiology, pathology, epidemiology, genetics and risk factors for SIDS remain undecided. In fact, SIDS is a diagnosis of "exceptions", where the use of all possible methods of investigation did not establish the true cause of death. Proposed many theories, however, there is still no unified approach to the mechanisms of SIDS and how to prevent the development of this syndrome. Critical review of existing hypotheses and provide an outlook for future research on SIDS recently has been conducted by P.N. Goldwater (2011). The author points out that some of the hypotheses can be crossed, such as respiratory failure may include apnea, wrong position during sleep, which in turn may be closely associated with sleep disorders. Only obviously one that death in SIDS is caused by sudden respiratory and/or cardiac failure. Although sudden death in infants resulting from cardiac arrhythmias are well documented these appear to account for no more than 5-10% of SIDS cases. Sudden respiratory failure currently is being seen as the most likely cause of death in the other cases of SIDS (Thach, 2005).

Although the role of sudden respiratory failure in the development of SIDS is recognized by many authors, the mechanisms of its occurrence are not fully understood. It is known that in infants in the rapid phase of sleep is defined by a set of changes of respiratory characteristics, such as irregular breathing, episodes of hypo- and apnea, which could predispose to pathological disorders of respiratory function and the development of severe hypoxia (Kelmanson, 2010). There are in infants associated with brain stem reflex mechanisms that are responsible for recovery from severe hypoxia (autoresuscitation) and are important for the survival of hypoxic infants (Thach, 2005). Disorder of these mechanisms may play an important role in SIDS, because brain stem-mediated hypoxic gasping is essential for successful autoresuscitation (Thach, 2008).

In recent years again has become popular plausible neurocentric hypothesis that the risk factors matter only in the context of some pre-existing brainstem neurological deficit (Kinney, 2009). Specifically, sudden respiratory and cardiac disorders in SIDS and death would result from prenatally defects of brainstem mechanisms that have evolved to protect against stressors and that occur while asleep during a critical period of development. Study of neurons of different nuclei of the brain stem at the SIDS identified deficiency of serotonin and its key enzyme tryptophan hydroxylase (5-HT and TPH2). Later, it was shown that these changes are due to lack of protein isoforms that regulate the exchange of 5-HT and TPH2 (Duncan, Paterson, Hoffman, Mokler et al., 2010; Broadbelt, Rivera, Paterson, Duncan et al., 2012). The authors believe that the disorders of brain response in victims of SIDS potentially associated with the molecular defect of regulation of the TRN2 and isoforms of signaling proteins are critical in this process. It is assumed that the lack of these factors leads to the insolvency of the response of protective mechanisms in hypoxia (autoresuscitation) and results sudden death in SIDS. Besides, there are experimental studies in rats and mice, which attempts to prove the lead role of serotonergic neurons in the violations of autoresuscitation mechanism, leading to death in SIDS (Cummings, Commons, Hewitt, Daubenspeck, Li, Kinney and Nattie, 2011a; Cummings, Hewitt, Li, Daubenspeck and Nattie, 2011b). However, despite this, neurocentric hypothesis raises a number of objections. First, many researchers are not agree that the observed changes in the brain stem are prenatal, resulting in violations of the embryonic development of the nervous system. Such changes in the serotonergic neurons of the infants could well develop after birth as a result of hypoxia (Guntheroth and Spiers, 2002). On the other hand, the deficit in 5-HT receptors can be seen as the result of excessive serotonin release, but not its depression. Since the results of neurological studies clearly show the absence of mass destruction serotonergic neurons in
the brain stem in SIDS, it was suggested that more subtle defects in serotonergic transmission may be only a predisposing factor rather than a major cause of SIDS. Categorical assertion that the origin of SIDS already predetermined prenatally, does not have sufficient evidence. Available in some cases of SIDS morphological findings are more likely explained by a non-specific effect of hypoxia on neurons (Guntheroth, 2011).

In mammals that are born at an early stage of brain development such as rodents and man, the brainstem respiratory network undergoes substantial postnatal maturation, breathing is more vulnerable and unstable during sleep and apneas are common. As a result of this instability, the tipping point between breathing resumption and irreversible hypoxic depression of the brain stem may be reached more easily in early infancy than later in life (Guyenet, 2011). Therefore, at this age it is important to condition of the lung tissue, which should quickly eliminate hypoxic depression of the brain and restore mechanism of self recovery.

Our histological studies in approximately one-third of infant deaths from SIDS showed signs of moderate catarrhal inflammation in the respiratory passages and alveoli. Along with them, in about one-third of deaths in SIDS morphological manifestations of lung immaturity has been revealed. However, these changes were not enough to qualify as a major cause of death. In respiratory function, especially in the age of 2 to 4 months, plays a special role of pulmonary surfactant. Structural and functional impairments of the pulmonary surfactant lead to aggravation of hypoxia, which is the basis of many respiratory diseases in children and adults (Enhorning, 2008). Study of surfactant system of the lungs in SIDS devoted few works. Some researchers in the broncho-alveolar lavage (BAL) of SIDS infants found various changes in the chemical composition of the surfactant in the form of reducing the concentration of phosphatidylcholine, violation ratio of phospholipids/proteolipids, reducing the physical properties of surfactant (James, Berry, Fleming and Hathaway, 1990; Hills, Masters, Vance and Hills, 1997). In contrast, other researchers did not find any significant changes in surfactant, which could explain the cause of sudden infant death (Funayama, Kageyama and Ohtani, 1994).

Our previous studies showed that the vast majority of children with SIDS had marked impairment of the functional properties of surfactant. These changes are manifested in the form of disruption of the surface-active properties of surfactant, significant reduction in performance stability (Tukhtaev, Giyasov and Shodiev, 2012). Using immunohistochemistry method A. Stray-Pedersen, A.Vege, A.Stray-Pedersen et al. (2008), have found that children with SIDS in the first months observed a significant decrease in surfactant protein-A (SP-A). It is these periods of life coincide with the peak of the highest infant death. Based on this, the authors had been hypothesized that the reduction of SP-A is a one of risk factor for SIDS. We have previously found that SIDS in some cases accompanied by severe reduced number of pneumocytes type II (Shodiev, Giyasov and Tukhtaev, 2011). It is known that pneumocyte type II (P2) is the main source of phospholipid and protein components of pulmonary surfactant (Enhorning, 2008). Lack of maturity, reduced number and impaired functional activity of pneumocytes type II, of course, can lead to quantitative and qualitative changes in lung surfactant. By method of immunohistochemistry we also have found decreased expression of surfactant protein B in the majority of children with SIDS compared to infants who died of pneumonia or in accidents. However, in violation of the surfactant system can not exhaustively explain the cause of death in SIDS. As we are discussed above, many authors are deciding that the prenatal or perinatal defects of regulatory mechanisms of respiratory or/and cardiovascular centres of the brain stem in SIDS have no conclusive evidences (Guntheroth, 2011). In this regard, the concepts of multifactorial causation with interaction of risk factors with variable probabilities are less restrictive and more in keeping with the large number and varying prevalence of demonstrated risk factors (Guntheroth and Spiers, 2002). In our opinion, the SIDS has undoubtedly multi-etiologic character with the sophisticated multi-level pathogenesis. In the development of the syndrome very important role are playing multiple risk factors arising in the prenatal and
postnatal periods in the life of infants (Athanasakis, Karavasiliadou and Styliadis, 2011). Based on these data, we also believe that impaired lung surfactant system is just one of the risk factors, which creates a kind of unfavorable background. Against this background, under certain conditions (wrong position the baby during sleep, lack of oxygen in the room, parental smoking and other risk factors), it is possible worsening hypoxia neurons of the brain stem. This, in turn, leads to a negative reflex effect, contributing to infringement of self-repair mechanisms (autoresuscitation) with sudden death. It is not excluded that this same hypoxic depression has an adverse effect on the cardio-vascular center and causes impaired heart function up to sudden heart failure.

Conclusion

Integrated morphological and immunohistochemical studies of lung tissue in some cases allow to verify the diagnosis in sudden infant death syndrome (SIDS). Immunohistochemistry in a significant portion of babies with the syndrome observed decrease in expression of surfactant protein B, probably due to qualitative changes in pulmonary surfactant. The data obtained suggest that the comprehensive morphological and immunohistochemical studies of lung tissue of infants may contribute to clarifying the degree of risk and more accurately determining the causes of death in some cases of sudden infant death. This will help reduce the risk of diagnostic errors, i.e., hypo- or over-diagnosis SIDS and is of practical importance for forensic examination. This is especially true for those cases that require the need for differential diagnosis of SIDS with different types of pneumonia or mechanical asphyxia. Further investigations in this area should focus on the study of the molecular and genetic mechanisms of synthesis and transport of surfactant proteins, and to develop simple inexpensive methods for detection of the lungs surfactant deficiency in infants.

References


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Appendix

Figure 1. Lung tissue of infant 3.5 month old (expert opinion N 334), diagnosis: SIDS. Dilatation of blood vessels, diapeesis of red blood cells in the interstitial tissue. Hematoxylin and eosin. Magnification 100X

Figure 2. Lung tissue of infant 3 month old (expert opinion N 273), diagnosis: acute pneumonia. In the lumen of the bronchus destructive and necrotic masses, bleeding in the surrounding tissue. Hematoxylin and eosin. Magnification 100X
FigurE 3. Lung tissue of infant 2.5 month old (expert opinion N 811), diagnosis: acute brain injury. Immunohistochemical reaction to the surfactant protein B. Intense deposition of the reaction product in the wall and lumen of the alveoli. Magnification 400x

FigurE 4. Lung tissue of infant 3 month old (expert opinion N 375), diagnosis: SIDS. Immunohistochemical reaction to the surfactant protein B. The product of the reaction in the wall and lumen of the alveoli virtually undetectable. Magnification 400x