Morphometric investigation of carotid body in Sudden Infant Death Syndrome

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Abstract

Background. The pathomechanism of sudden infant death syndrome (SIDS) has not been clarified yet. The high rate of early progenitor cells in carotid body has been reported as a pathognomic feature for SIDS. *Aim and Study design*. The morphometric analysis was done by NIKON Eclipse microscope with a morphometric program Lucia G. *Subjects*. This study was designed to investigate the structure and developmental state of carotid body in SIDS and non-SIDS cases. A comparison was made between the rates of dark and early progenitor cells. *Outcome measures*. The Kruskal-Wallis test showed a significantly higher number of progenitor cells in the SIDS group than in controls (p=0.0003). *Results and conclusion*. In this study on Hungarian SIDS cases we confirmed the observation that infants who died suddenly have an underdeveloped carotid body.

Keywords: sudden death, morphometry, carotid body, progenitor cell.

Introduction

Sudden Infant Death Syndrome (SIDS) is defined as sudden death of any infant or young child which is unexpected by history, and lacking explanation after post-mortem investigation (Beckwith, 1970). Risk factors include altered sleep patterns, prone sleeping position and infections. The upper respiratory tract infections would be insufficient to cause SIDS; however, malfunctioning respiratory system can produce fatal upper airway obstruction and death.

Developmental delay of peripheral chemoreceptors and hypoxic-ischemic injury may have important links to respiratory instability (Becker, 1996).

Ventilatory dysfunction correlates to structural abnormalities of carotid body and respiratory nuclei of brainstem (Gillan *et al.*, 1989). Hypoxia has been assumed to have an effect on the development of SIDS. The carotid body as peripheral chemoreceptor precipitates hyper-ventilation or waking reaction at decreased oxygen level.

The aim of this study was to investigate the structure and developmental state of carotid body and to compare the density of dark and early progenitor cells in SIDS and in control groups.

Material and methods

1. Selection of cases

In this study we examined infant death cases between 1 week and 1 year of age autopsied in a four-year period (1996-1999).

SIDS was defined according to the original definition by Beckwith, as "… the sudden death of any infant or young child which is unexpected by history, and in which a thorough post mortem examination fails to demonstrate an adequate cause of death" (Beckwith, 1970).

Data were collected from 9 (4 males, 5 females) SIDS and 18 (10 males, 8 females) non-SIDS cases.

The non-SIDS group involved infants died of well-defined natural or violent causes. Altogether four violent (aspiration: n = 1, infanticide by strangulation: n = 2, battered child: n = 1) cases were included.

The autopsy was done within 24-48 hours after death. The bodies were stored at 4°C before the autopsy. Following a preliminary fixation in 10% (V/V) buffered formalin for standard period of time (3-6 days) the carotid bifurcations removed at autopsy were dissected under stereomicroscope NIKON SMZ II.

Serial sections (5 mm thick) were cut from each block of paraffin-embedded tissues. HE staining was used for the identification of different chief cell types, and Székely-Goldner trichrome was used to limit cell clusters and lobules.

2. Immunohistochemical method

Labelled Streptavidin Biotin (LSAB) immunohistochemical method was applied for the identification of chief cells (Type I) by chromogranin A, (DAKO DAK-A3, 1:100) and sustentacular cells (Type II) by S100 protein (DAKO Polyclonal Rabbit anti-Cow, 1:600) with overnight incubation with primary antibody at room temperature. S100 protein was used in the heat induced epitope retrieval (HIER) method (5 min boiling after maximal pressure in pressure cooker, with citrate buffer, pH 8.0). For visualization we used diamino-benzidin (DAB) and hematoxylin for counterstaining.

3. Morphometric analyses

The measurement was done by NIKON Eclipse microscope with a morphometric program Lucia G 4.1. (Laboratory Imaging LTD cz). The measurement of the area on the monitor screen was 112.57 μm vertically and 154.84 μm horizontally.

The cells displayed on one monitor screen were counted by an objective 40, including the measurement of the diameter of nuclei. There were five sections analysed from different levels of series.

Depending on the size of carotid body on the section 2-3 areas without interlobular connective tissue were investigated.

Cells were classified according to the morphology and size of nuclei.

4. Statistical method

Averages of cell numbers were calculated significances between groups were tested by Kruskal-Wallis test (SPSS 93).

The permission number of the Hungarian Scientific Ethics Committee was 134/1999.

Results

The average age was 6.3 months in the SIDS group and 5.5 months in non-SIDS group. The average birth weight was 3050 g for the SIDS and 3200 g for the non-SIDS group. Prior lower or upper respiratory tract infections were found in 6 cases. However, every infant was symptom-free within two weeks before death.

The main epidemiological and pathomorphological data are shown in Tables 1 and 2. The most frequently observed changes in SIDS cases were the subepicardial and subpleural petechial haemorrhages on thymus or mild inflammation of the respiratory tract.

In non-SIDS group disease of natural disease (pneumonia, meningitis) was detected in 15 cases as the cause of death.

Table 1 – ***Number of progenitor cells of glomus caroticum in SIDS cases***

No. of patients	Age (months)	Sex	Pathomorphological changes	Average number of progenitor cells/screen
1.	2	male	subcapsular petechiae of thymus, subpleural petechial haemorrhages, infection of upper respiratory tract	5.2
2.	3	female	subcapsular petechiae of thymus, subpleural petechial haemorrhages	22.8
3.	3	female	subepicardial petechial haemorrhages, infection of upper respiratory tract	25.2
4.	3	male	subcapsular petechiae of thymus, infection of upper respiratory tract	9.6
5.	6	female	subcapsular petechiae of thymus	9.4
6.	6	female	subpleural petechial haemorrhages	11.6
7.	11	male	subpleural petechial haemorrhages subcapsular petechiae of thymus, subepicardial petechial haemorrhages	30.4
8.	11	male	subpleural petechial haemorrhages	13.0
9.	12	male	subepicardial petechial haemorrhages	21.3

Table 2 – ***Number of progenitor cells of glomus caroticum in non-SIDS cases***

No.	Age (months)	Sex	Cause of death	Average number of progenitor cells / screen
1.	3	female	pneumonia	3.3
2.	1	female	hydrocephalus internal	2.6
3.	2	male	pneumonia	6.4
4.	4	male	pneumonia	5.4
5.	10	female	meningitis	1.6
6.	1	female	cardiac malformation	4.0
7.	1	male	subdural haemorrhage	6.2
8.	1	female	cardiac malformation	12.3
9.	3	male	pneumonia	5.2
10.	3	female	pneumonia	12.0
11.	4	female	pneumonia	4.4
12.	5	male	infanticide by strangulation	4.8
13.	6	male	sepsis	3.0
14.	9	male	infanticide by strangulation	3.8
15.	10	male	pneumonia	4.2
16.	12	male	pneumonia	4.2
17.	12	female	cardiac malformation	1.4
18.	12	male	aspiration	3.4

In the investigated groups the cases were divided by the number of progenitor cells in carotid body. The low and moderate number (<15) of progenitor cells could be seen both in SIDS and non-SIDS groups.

However, high number (>15) of progenitor cells was found only in the SIDS group.

The Kruskal-Wallis test showed a significantly higher number of progenitor cells in the SIDS group than in controls ($p = 0.0003$) (Table 3).

Table 3 – ***Average number of progenitor cells in SIDS and non-SIDS cases***

	No.	Average	Standard deviation
All cases	27	8.67	7.8
SIDS	9	16.36	8.7
non-SIDS	18	4.82	2.7

High number of progenitor cells in SIDS cases is displayed by Figures 1 and 2.

There was a difference in the number of dark and light cells in SIDS and non-SIDS groups. However, there was no statistically significant difference.

In the non-SIDS group the number of dark and light cells was comparable to the numbers in normal adults (Figures 3 and 4).

Discussions

The objective of the study was to determine the rate of progenitor cells, dark and light cells of carotid body in Hungarian SIDS and control cases.

The difference of the proportion of progenitor cells in SIDS and controls was significant; and, as in the previous studies we confirmed the observation [2, 4, 5] that infants died suddenly have underdeveloped carotid body.

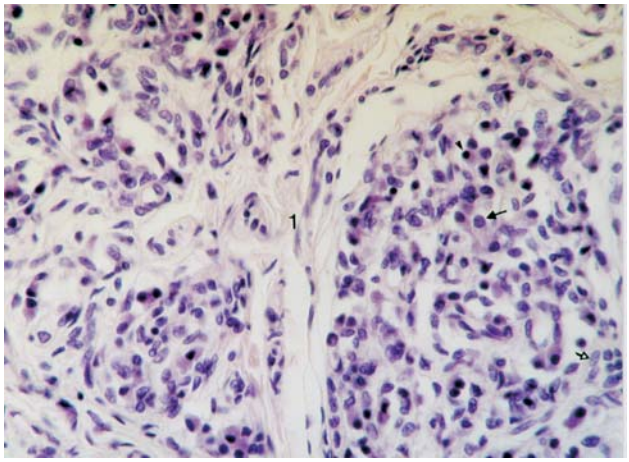

Figure 1 – ***Predominance of dark and progenitor cells in the cell clusters, 6 month old female SIDS (ob. ×20): (1) – interlobular septum, (arrow) – dark cell, (arrow-head) – progenitor cell, (light arrow) – sustentacular cell***

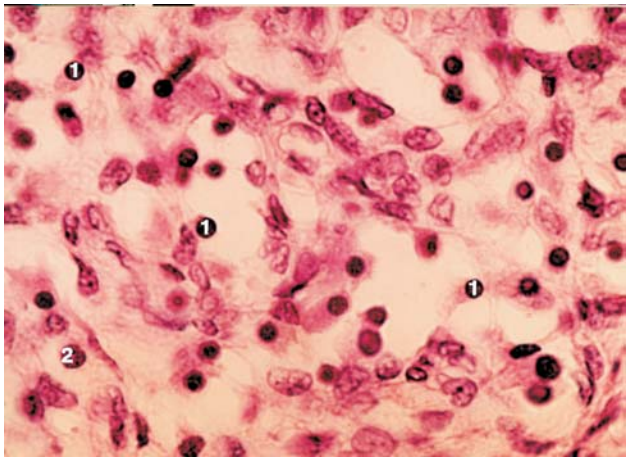

Figure 2 – ***Higher magnification of progenitor cells, 6 month old female SIDS (ob. ×40): (1) – progenitor cell, (2) – dark cell***

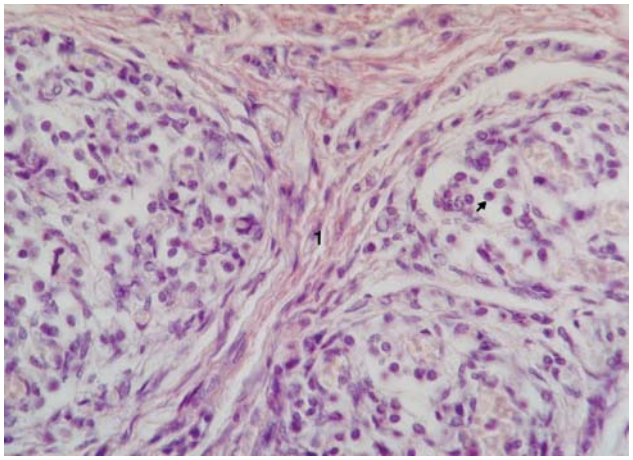

Figure 3 – ***High number of light cells in the cell cluster, 12 month old male non-SIDS (ob. ×20): (1) – interlobular septum, (arrow) – progenitor cell***

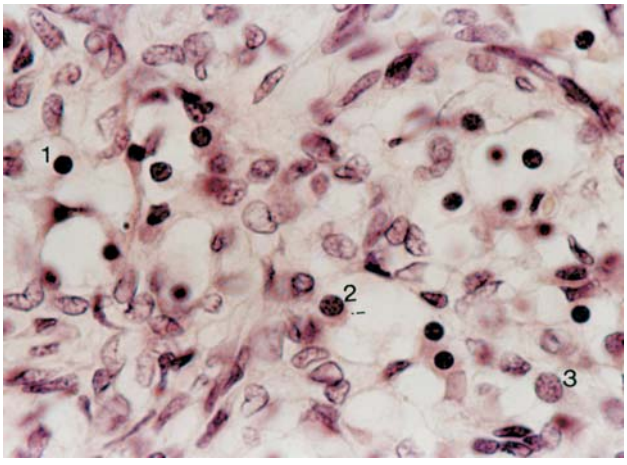

Figure 4 – ***Different cell types in 3 month old male SIDS case (ob. ×40): (1) – progenitor cell, (2) – dark cell, (3) – light cell***

The Hungarian infant mortality rate was among the highest of the Eastern European countries in the mid-1980s (22-24 / 1000 live births), but it decreased below the average in 1998 (9.7 / 1000 live births), and in 2002 it was 7.16 / 1000 live births. The decreasing trend in infant mortality and in SIDS rate was due to development of health policy and health care networks, and the early detection of developmental abnormalities.

The characteristics for the structure of carotid body are the lobes separated by connective tissue septum (Grimley and Glenner, 1968). In the lobes chief cells and surrounding supporting cells in cell-nest could be identified. Light cells, dark cells, and progenitor cells could be separated among chief cells. In light cells the pale/staining reaction of the nucleus with haematoxylin and eosin was detected.

The distinctive nucleus was pale, round or oval in profile. Their size was about 7μm in diameter. The cytoplasm of light cells is abundant and faintly eosinophilic and its outline is usually well defined. The light cells show by far the greatest degree of vacuolisation (Heath *et al.*, 1970).

In dark cells the nucleus has an average diameter of 6 μm. The nucleus was conspicuously more haematoxyphilic than in the light cells. The cytoplasm was also dark, purple with haematoxylin and eosin. Outlines of the cells are clearly seen. The cytoplasm commonly has an oval profile but may be elongated, with formation of streamers.

In the progenitor cells the nucleus was small, measuring 4 μm in diameter, situated on one side of purple-staining cytoplasm (Heath *et al.*, 1990). Seker *et al.* (1994) found that the percentage of the three varieties of type I (chief) cells depends on the time between death and fixation. The carotid bodies have been fixed for 3, 5 and 15 hours.

In this study the percentage of progenitor (pycnotic) cells increased by the post-mortem time. In our material the time difference between death and fixation was 24-48 hours. Because the autopsy times after death did not significantly differ between the groups we think that the changes noted are real.

The earliest histopathological response to hypoxia occurred in the carotid bodies in the form of an increase in the count of the dark variant of chief cells (Smith *et al.*, 1993). In SIDS cases overgrowth of sustentacular cells with predominant progenitor cells, and absence of dark cells were detected (Heath *et al.,* 1990).

Other studies have not found appreciable differences in architectural arrangement or cytological features of carotid body between SIDS and controls (Lack *et al.*, 1986; Perrin *et al.*, 1984; Cole *et al.*, 1979). However, carotid bodies of SIDS contained higher concentration of dopamine and noradrenaline than age-matched controls. Histopathological study reported that in neonates and infants the lobular pattern of the carotid body had been disorganised by an overgrowth of sustentacular cells, and the cellular constitution was abnormal with a predominance of progenitor cells (Heath *et al.,* 1990). Also there was disorganisation of the lobular structure by sustentacular cells which included an increased proportion of progenitor cells.

Using electron microscopic techniques marked reduction or absence of the dense cytoplasm granules of carotid chemoreceptor cells, as well as a reduction in cell number and size was detected. Carotid body chief cells from SIDS and control cases contained numerous electron-dense neurosecretory granules, and the distribution, frequency and size of neurosecretory granules did not show statistical difference (Perrin *et al.*, 1984; Cole *et al.*, 1979).

Carotid body situated at the bifurcation of carotid artery has an important role in the control of breathing. A defect in carotid body could block normal stimulation of respiration during hypoxaemia at episodes of sleep apnoea in infancy (Lack *et al.,* 1986). In case of functional abnormalities of carotid body the infant may not be able to restart breathing during a prolonged episode of apnoea (Naeye, 1980).

Sleep apnoeas have been documented in subsequent SIDS victims (McMurray and Holinger, 1997). Apnoea could be a terminal event in SIDS, however, the primary cause of obstructive apnoea is unknown. Distribution of petechial haemorrhages can be explained by negative intrathoracic pressure before death (Engelberts, 1995). In our material the SIDS cases showed subpleural and subepicardial petechial haemorrhages in a high number of cases.

The regulation of breathing relies upon chemical feedback concerning the levels of CO_2 and O_2. Carotid bodies provide tonic excitation to brainstem respiratory neurones and dramatic excitation in case O_2 levels fall (Nattie, 1999).

Stimulation of carotid body chemoreceptors by asphyxia during apnoea episode may contribute to sudden death (Daly *et al.*, 1979). Ventilatory responses to oxygen were ablated by carotid chemodenervation (Fagenholz *et al.*, 1979). Central chemoreceptors are critical for adequate breathing in sleep, but other aspects of control system can maintain breathing in wakefulness (Nattie, 1999).

Chemoreceptors with compensatory hypertrophy and hyperplasia occurred in most patients with chronic hypoxia (Lack *et al.*, 1985). Carotid bodies can undergo compensatory hypertrophy and hyperplasia in SIDS cases. Mean combined weights of carotid bodies from SIDS victims were detected greater than controls; however, both groups showed an equally intense degree of cytoplasm argyrophilia of chief cells (Lack *et al.*, 1986). In the mechanism of developmental arrest of the third bronchial arch and its pharyngeal part in the first trimester of pregnancy could lead to hypoplasia of the carotid body and to the underdevelopment of glossopharyngeal and superior laryngeal nerve.

Becker (1996) has made a series of observations implicating six links in the pathogenesis of SIDS: prenatal factors, immature cardiorespiratory sleep-arousal system, subclinical respiratory instability, hypoxemic / ischemic insults, neural maturation delay, and triggering or stressing factors. The neuron maturation delay manifested by delayed dendrite spine development and delayed myelinisation including the peripheral myelinisation of the vagus nerve is most probably important.

Sixty-three percent of SIDS victims had a subnormal volume and twenty-three percent had an enlarged volume of glomic cells in their carotid bodies (Neaye *et al.*, 1979).

Early study (Neaye, 1980) found that more than half of the victims of SIDS cases had been found with an underdeveloped carotid body. Associations between SIDS and brainstem astrogliosis, periventricular and subcortical leucomalacia, microglial activation and elevated neurotransmitters in carotid body were reported.

Many of the SIDS victims were found with apparent life-threatening event (ALTE) before death. ALTE is a term used to characterise an event of unknown cause after an infant was found limp, cyanotic, bradycardic with apnoea and/or requiring resuscitation (McMurray and Holinger, 1997).

ALTE infants would have died of SIDS (Engelberts, 1995). The relationship between ALTE and SIDS has not been clearly defined, although 7-15 percent of children with ALTE die of SIDS (McMurray and Holinger, 1997).

Triggering factors included altered sleep patterns, hyperthermia, prone sleeping position; infections of upper respiratory tract may produce fatal upper airway obstruction and SIDS (Becker, 1996).

Conclusions

In this study we confirmed the observation that infants died suddenly have underdeveloped carotid body. Among our cases there were differences in the number of progenitor and dark cells of carotid body in SIDS and control cases. Our findings, in line with earlier examination (Naye, 1980) emphasize the importance of chronic under-ventilation and hypoxemia in SIDS.

Our data suggested a significant increase in the numbers of progenitor cells in SIDS. The examination of structure and detection of morphological changes of carotid body can promote the pathological investigation of SIDS. The results showed that insufficient maturity of carotid body was found in SIDS cases.

The complexity of SIDS needs histological, toxicological and bacteriological test in addition to autopsy. The performance of these tests and the accurate processing of cases can assist to clear up the pathomechanism of SIDS. We associated high numbers of progenitor cells with inability of the carotid bodies to respond adequately to changes in arterial oxygen tension.

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