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Morphological and immunohistochemical studies of the pituitary in Sudden Infant Death Syndrome (SIDS)*

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Summary. The morphological structure and immunohistochemical reactions of 100 pituitaries from cases of SIDS children (58 males and 42 females, average age 5.34 ± 3.12 months) were studied. Controls consisted of 19 pituitaries from children (14 males and 5 females, average age 5.63 ± 2.52 months) with a clearly identifiable cause of death e.g. drowning or strangulation. The microscopical and immunohistochemical studies for identifying pituitary cell types revealed normally developed organs. Unspecific necroses and haemorrhages were observed in 2 cases of SIDS but in none of the controls. Hyperaemia was detected in 51 (30 male/21 female) cases of SIDS. No significant differences were found in the distribution of microfollicles (54%), cysts of the intermediate zone (14%), persistency of the Rathke's pouch (44%), Erdheim's squamous epithelium (8%) or heterotopic salivary glands (3%). The semiquantitative immunohistochemical evaluations of the different cell types showed no significant variations from the control group. The pattern of distribution of the intracytoplasmic vacuolisations of the ACTH and gonadotropic cells showed no significant differences. Folliculo-stellate cells were either not demonstrable – commensurate with age – or showed a normal distribution. The results for both study groups may be defined as consequences of terminal agony, but failed to reveal the cause of the sudden infant death.

Key words: SIDS – Pituitary morphology – Newborn – Immunohistochemistry

Zusammenfassung. 100 Hypophysen von SIDS-Fällen (58 männlichen und 42 weiblichen Geschlechtes mit einem Durchschnittsalter von 5,34 ± 3,12 Monaten) wurden untersucht. Die Kontrollgruppe bestand aus 19 Hypophysen (14 männlichen und 5 weiblichen Geschlechtes mit einem Durchschnittsalter von 5,63 ± 2,52 Monaten) mit jeweils eindeutig geklärter (z.T. nicht-natürlicher) Todesursache. Die lichtmikroskopischen und immunhistologischen Untersuchungen zur Typisierung der einzelnen Zellgruppen zeigten regelrecht entwickelte Hypophysen. Unspezifische Nekrosen und Blutungen fanden wir in zwei SIDS Fällen und keinem Fall der Kontrollgruppe. Hyperämien bestanden in 51 (30 M/21 W) SIDS-Fällen. Mikrofollikel (54%), Zysten der Intermediärzone (14%), Reste der Rathke'schen Tasche (44%), Erdheim'sches Plattenepithel (8%) oder Speicheldrüsenheterotopien (3%) bildeten keine als signifikant zu wertenden Befunde. Bezüglich der immunhistologischen Verteilungsmuster der dargestellten Zellen fanden sich für die Quantitäten keine Auffälligkeiten. Die intrazellulären Vacuolen bei ACTH- und gonadotropen Zellen zeigten keine signifikanten Unterschiede. Die S-100 Protein-positiven Zellen ließen sich altersentsprechend stellenweise gar nicht und sonst nur regelhaft darstellen. Die Ergebnisse können als Folgen der terminalen Agonie, nicht aber als Ursache des plötzlichen Kindstodes interpretiert werden.

Schlüsselwörter: SIDS – Hypophysen-Morphologie – Säugling – Immunhistochemie

Introduction

The sudden infant death syndrome (SIDS) is defined as the unexpected death of an infant or young child in whom a thorough examination fails to reveal the cause of death [2, 6]. It is thought to be the most common cause of death in infants during the first year of life beyond the first week after birth.

The first reports on systematic and scientific research were published in 1953 by Garrow and Werne [4]. As a consequence of more cases being investigated, specific pathological findings and physiological reactions have been connected with SIDS [1, 11–13]. The multifactorial aspects of SIDS are nowadays widely accepted.

* Dedicated to Prof. Dr. H. D. Herrmann (Hamburg) on the occasion of his 60th birthday

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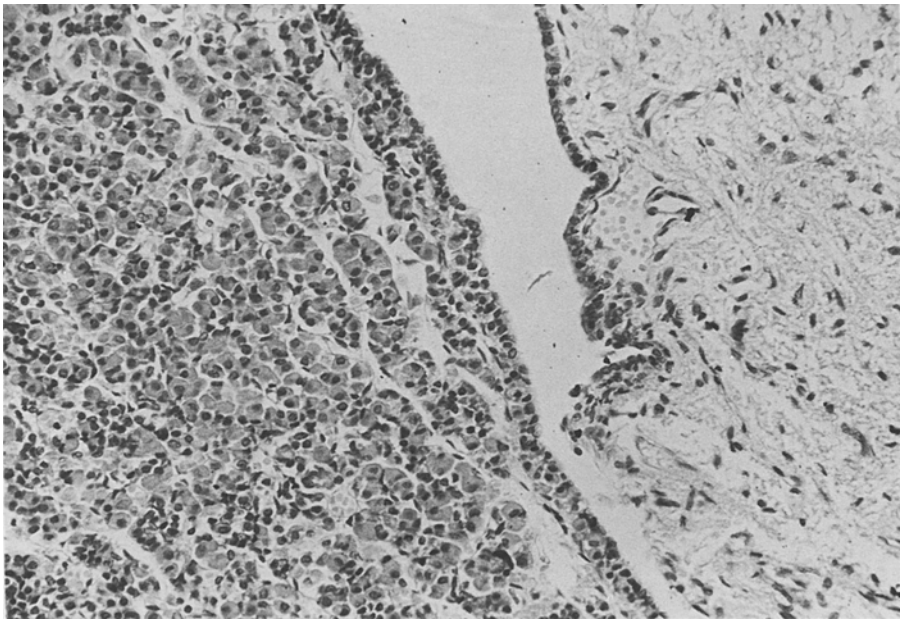

Fig. 1. (3.5-months-old male). Persistence of Rathke's pouch *(centre)* surrounded by cuboid epithelia. Regular adenohypophysis *(left)* and neurohypophysis *(right)*. Hematoxylin-eosin, magnification × 225

The pathological findings usually involve alterations to the non-endocrine organs excluding the chromaffin tissue in the adrenal medulla and gliosis of the brain stem [11–13], but these findings are usually not acceptable as a cause of death. Detailed investigations of endocrine organs and their regulatory systems have not yet been performed.

Endocrine malfunction and insufficiency of the pituitary may have a slow progression and long term inapparent clinical processes are described. Sudden deaths from these diseases are very rare, but should be excluded in any case of SIDS. Defects caused by dyscirculation, inflammation and necrosis are not described in the literature as a cause of SIDS but have to be considered.

The aim of our study, as part of a multicenter study, was to investigate pituitaries from SIDS cases for possible light-microscopical or immunhistological alterations.

Material and methods

A total of 100 cases of SIDS from Münster and Essen consisting of 58 males (average age 5.14 ± 2.88 months) and 42 females (average age 5.62 ± 3.45 months) have been investigated. The control group consisting of 19 cases included 14 males (aged 4.79 ± 1.81 months) and 5 females (aged 8.00 ± 2.92 months) where the cause of death was known.

The pituitaries were fixed in formalin and embedded in paraffin. Slides were stained with hematoxylin-eosin and PAS reagent.

Immunohistochemistry was performed with 8 polyclonal antibodies (Anti-GH, Dako Hamburg, dilution 1:500; Anti-PRL, Dako, 1:300; Anti-ACTH, Dako, 1:300; Anti-TSH, Dako, 1:1000; Anti-FSH, Dako, 1:200; Anti-LH, Dako, 1:800; Anti-alpha-subunit Dr. Gräßlin/Dr. Saeger, 1:800; Anti-S100-Protein, Dako). Positive staining was graded as < 10%, > 10% – < 30%, > 30 % – < 60 % and > 60 % positively stained cells. Negative or unclear results were repeated to exclude technical failures.

Data were processed on a modified questionnaire of EPI INFO version 5.0, a public domain software for epidemiology and disease surveillance. Results were tested using Chi-square tables with extended test procedures for verifying significant findings.

Results

Microscopical findings

All pituitaries showed a corresponding age-related level of development. Focal necroses in 2 cases and inflammation (as part of one case of the necroses) were observed. Significant decreases or increases in cell types and malformations were not observed.

Hyperaemia was detected in 51 (30 males/21 females) cases of SIDS. The distribution of microfollicles (54%), cysts of the intermediate zone (14%), persistency of the Rathke's pouch (44%) (Fig. 1), Erdheim's squamous epithelium (8%) or heterotopic salivary glands (3%) was not significantly different comparing the study group and the control group.

Immunhistological evaluation

The *GH-cell population* (normal value 40%–60%) showed a normal distribution with 94 cases (52 male/42 female) containing between 30%–70% immunohistologically positive cells for the SIDS group. In 6 cases of SIDS (all males) less than > 10% and < 30% of the cells were positive (Table 1). In the control group 17 cases had > 30% and < 60% positive cells (14 male/3 female) and in 2 cases > 10% and < 30% (1 male/1 female). A homogeneous distribution was found in all areas of the pituitary.

The *PRL positive cells* (normal rating 10%–40%) showed in 2 cases < 10% (2 males), in 46 cases > 10% – < 30% (23 male/23 female) and in 52 cases (33 male/19 female) > 30 % – < 60% immunohistologically positive cells for the SIDS group (Table 1). In the control group immunohistologically positive cells were observed as follows: 2 cases < 10% (2 male), 15 cases > 10%– < 30 % (11 male/4 female) and 2 cases > 30% – < 60% (1 male/1 female). A homogeneous distribution was found in all areas of the pituitary for both groups.

Table 1. Immunohistological evaluation of pituitary cell types

Cell type	Normal number		Increased number		Decreased number	
	SIDS	Control	SIDS	Control	SIDS	Control
GH+ Normal range 40%–60%	94/100	17/19	–	–	6/100	2/19
PRL+ Normal range 10%–40%	98/100	17/19	–	–	2/100	2/19
ACTH+ Normal range 15%–40%	54/100	12/19	–	–	46/100	7/19
TSH+ Normal range 1%–10%	92/100	19/19	8/100	–	–	–
FSH+ Normal range 10%–30%	96/100	19/19	–	–	4/100	–
LH+ Normal range 10%–30%	99/100	19/19	–	–	1/100	–
Alpha-subunit+ Normal range 10%–30%	99/100	19/19	–	–	1/100	–
S-100-protein+	80/100	12/19	–	–	20/100*	7/19*

* = No S100 protein-positive cells (folliculo-stellate cells) (see table 2)
+ = Immunohistologically positive cell type
Normal range values according to references [5, 8]

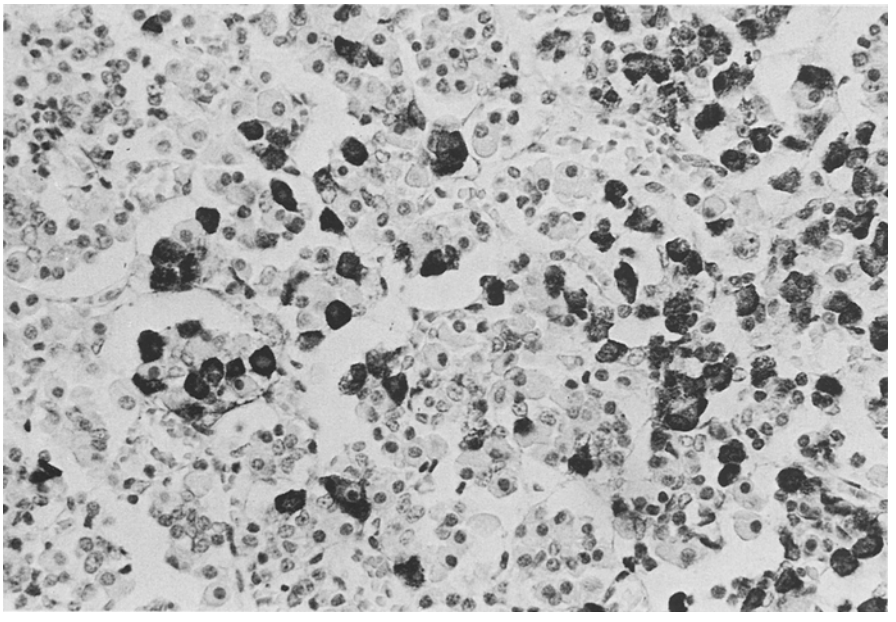

Fig. 2. (11-months-old male). Focal accumulation of ACTH cells with variable densities of granulation. Anti-ACTH-ABC-hematoxylin, magnification × 360

The *ACTH positive cells* (normal distribution 15%–40%) revealed 46 cases (28 male/18 female) < 10%, 54 cases (30 male/24 female) > 10% – < 30% immunohistologically positive cells for the SIDS case group (Fig. 2). The controls contained 7 cases (5 male/2 female) < 10% and 12 cases (9 male/3 female) > 10% – < 30%. An accumulation was observed near the intermediate zone. Two subtypes of ACTH positive cells could be differentiated, one with much small granular immunostaining in 78 cases (47 male/31 female) and a second group with fewer, but larger, immunostained cytoplasmic areas in 22 cases (11 male/11 female). In the control group, the first type was found in 13 cases (10 male/3 female) and the second type in 6 cases (4 male/2 female). Intracytoplasmic vacuoles were detected in 34 cases (25 male/9 female) of the SIDS group and in 11 cases of the controls (9 male/2 female) (Tables 1 and 3).

In the SIDS group total of 92 cases (57 male/35 female) showed < 10% immunohistologically positive cells for *TSH* (normal distribution 1%–10%) and 8 (1 male/7 female) cases in SIDS revealed > 10% – < 30% immunohistologically positive cells. The controls included 19 cases (14 male/5 female) < 10% positive cells (Table 1). Stained cells were usually located near the capsule.

In 96 cases a normal distribution of cells immunohistologically positive for *FSH* (regular frequence 10%–30%) was found, where 79 contained slightly > 10% positive cells (52 male/27 female) and 17 cases > 10% – < 30% positive cells. In 4 cases less than < 10% positive cells (3 male/1 female) were found. In the controls 16 cases (10

Table 2. Structural variations of folliculo-stellate cells

	SIDS cases	Control cases
Mostly enlarged	36/100	3/19
Normal	44/100	9/19
Not demonstrable	20/100	7/19

Table 3. Intracytoplasmic vacuoles of ACTH and gonadotropic cells

	SIDS cases	Control cases
ACTH cells	34/100	11/19
FSH cells	42/100	11/19
LH cells	54/100	14/19

male/6 female) contained slightly above 10% positive cells and 3 cases > 10% – < 30% (3 male) positive cell staining (Table 1). In all cases a diffuse arrangement was observed within the adenohypophysis. Intracytoplasmic vacuoles were seen in 42 out of 100 SIDS cases (30 male/12 female) and in 11 controls (8 male/3 female) (Table 3).

In 53 cases (27 male/26 female) slightly > 10% positive cells immunohistologically positive for *LH* (normal distribution between 10%–30%) were found and 46 cases (31 male/15 female) up to < 30%. In one case (male) < 10% cells were positive. In the control group 7 cases (5 male/2 female) showed slightly above 10% and 12 cases (9 male/3 female) between > 10% – <30% (Table 1). In 54 cases of SIDS (32 male/22 female) and 14 control cases (12 male/2 female) intracytoplasmic vacuoles were observed (Table 3). The population of cells was arranged diffusely over the pituitary.

Concerning the *Alpha-subunit* distribution (normal range 10%–30%) 37 cases (20 male/17 female) were observed slightly above 10% and 62 cases (38 male/24 female) > 10% – < 30% immunohistologically positive cells in the SIDS group. The distribution was homogeneous in all areas of the pituitary. In 1 case (female) in the SIDS group less than 10% of cells were positive. In the control group 3 cases (males) were slightly above 10% positive and 16 cases (11 male/5 female) > 10% – < 30% positive (Table 1).

In 54 out of 100 cases of SIDS > 10% – < 30% of the cells were *folliculo-stellate cells* positively staining for S100-Protein (26 male/28 female) and in 12 out of 19 controls (10 male/2 female). In 26 cases of SIDS (18 male/8 female) < 10% of cells were positively stained for S100-Protein. No immunohistologically S100-Protein-positive cells were found in 20 out of 100 cases of SIDS (14 male/6 female) and 7 out of 19 controls (4 male/3 female). An enlargement of folliculo-stellate cells were recognizable in 36 out of 100 cases of SIDS (21 male/15 female) and 3 out of 19 (male) in controls (Table 2).

Discussion

The age and sex distribution of the SIDS cases studied were comparable to those stated in the literature [1, 7]. An accumulation of cases was observed between the 2nd and 5th month of life (52%), with a slight majority of the male sex. Differences in collectives have also been described by the literature but the reasons are not known [1].

Great efforts have been made to define possible epidemiological features or predispositions for the detection and identification of infants at risk. Morphological studies of the pituitary reported in the literature are very rare and incomplete [10]. Understanding of the maturation of the human brain is a complex field in which hormonal regulation plays a major role [8]. From this point of view the development of the pituitary in SIDS linking the central nervous system with endocrine organs and it's possible role in the unclear cause of SIDS is of major interest.

In comparison to the controls no significant decreases or increases of cell types and malformations were observed in this study as reported in the literature [3, 5, 9].

The hyperaemia detected in 51 (30 male/21 female) cases of SIDS is not the cause of SIDS but more or less a consequence of it. The distribution of microfollicles, the cysts of the intermediate zone, the persistency of the Rathke's pouch, Erdheim's squamous epithelium or heterotopic salivary glands are individual morphological alterations and do not seem to be significant due to the similarity between SIDS cases and controls.

The immunohistochemistry revealed an intact and normally developed organ.

The variations of the immunohistochemical content of the cell populations are equivalent for both groups and not statistically significant. The vacuolization of ACTH cells and gonadotropic cells should be interpreted as an individual variation or as a cytological reaction in terminal agony but the possibility of artefacts has to be considered.

The reason for the variation in size of the secretory granules of the ACTH-positive cells remains unclear. In our view the absence or alterations of the folliculo-stellate cells are age-related or may be artefacts.

Conclusion

The results regarding the clinical data are in accordance with other reports in the literature [2, 6, 7]. The pituitary forms the connexion between the central nervous system and the endocrine organs and alterations can therefore be of importance to detect possible endocrine dysfunctions. The observed alterations of the folliculo-stellate cells may be interpreted as an immunoreactive answer to an as yet unknown agent but artefacts cannot be excluded with certainty. The vacuolization of the ACTH and gonadotropic cells may be caused by stress or the terminal agony but could also be considered as an artefact.

The similar findings of the infants in the SIDS group to those of the control group lead us to the conclusion that the findings are reactions due to the terminal stage of the underlying disease and cannot be linked with the still unknown cause of SIDS.

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