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Morphology, immunohistochemistry and morphometry of pancreatic islets in cases of sudden infant death syndrome (SIDS)

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Abstract The pancreatic islets from 112 infants (66 males and 46 females) who died of SIDS during the years 1990–1992 have been studied. The control group consisted of endocrine pancreas tissue from 19 infants who died of a clear cause of death (pneumonia, drowning, sepsis, etc.). The mean age of the SIDS group was 5.1 months. We found histologically normally developed organs in all the SIDS cases. By evaluating the relative endocrine cell area of the pancreas by immunohistochemical investigations, A-cells were found to make up 10–30%, B-cells 30–60%, D-cells 10–30% and pancreatic polypeptide cells less than 10% in the SIDS group and in the controls with a small increase in glucagon and insulin cells among SIDS cases. The morphometric evaluation revealed that cell enlargement and cytoplasm shrinking occurred slightly more often in the SIDS group than in the control group. The diameter of the islets was normal and the maximal volume was not enlarged. The results did not show significant differences so that a relationship between alterations of the endocrine pancreas and sudden infant death syndrome could not be demonstrated.

Key words SIDS · Endocrine pancreas · Morphology · Immunohistochemistry · Morphometry

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Introduction

The sudden infant death syndrome (SIDS) is defined as the unexpected death of an infant child from the previous history and where a thorough postmortem examination did not reveal the cause of death [4, 10]. Despite many efforts during the last decades SIDS is still the most frequent cause of death in infants between the age of 1 week and 1 year [6]. The frequency of SIDS cases ranges between 0.3 and 3 per 1000 live births [18] with a decrease during the previous 5 years [35]. Numerous hypotheses for SIDS have been proposed including infections [1, 2, 5, 17, 31], hypoxia [20, 28, 30, 34] and primary cerebral impairment [3, 32, 36]. From the literature only very few studies have considered the involvement of the endocrine system e.g. the adrenal gland [21, 22], the pituitary [19, 25] or the thyroid gland [26, 27, 29]. Nevertheless an investigation of the pancreas is always included in a protocol of a standardized autopsy in suspected SIDS cases as proposed previously [16, 38]. The aims of this study were to investigate whether the pancreas islets are responsible for alterations leading to SIDS or if they show secondary lesions with regard to morphological, immunohistochemical and morphometrical alterations of the pancreatic islets.

Materials and methods

During the years 1990–1992 autopsies were carried out in the Institutes of Legal Medicine in Münster and Essen, Germany, in 112 SIDS cases and 19 age-matched infants with a clear cause of death (Table 1). All of the SIDS cases fulfilled the criteria for SIDS. The causes of death in the control group were drowning (n = 4), hyperthermia (n = 1), pneumonia (n = 6), prematurity (n = 1), central death by hypoxia (n = 1), myocarditis (n = 1), pulmonary oedema (n = 1), peribronchitis with beginning sepsis (n = 1), reflex death (n = 1), severe upper airway infection (n = 1) and severe sepsis (n = 1). The pancreas samples were fixed in formalin and embedded in paraffin and the slides were stained with hematoxylin-eosin (HE) and periodic acid Schiff (PAS).

For morphometric evaluations the average frequency of the islets per visual field was counted by light microscope at a magni-

Table 1 Details of infant death cases in this study

Sex	SIDS cases			Control cases		
	Number	Age (months)	Peak death age	Number	Age (months)	Peak[1] death age
M	66	0.7–13.6	2nd and 4th months	14	0.6–20.4	–
F	46	1.4–16.7	2nd and 3rd months	5	3.1–9.8	–

[1] Evenly distributed

fication of × 25. By using the point-counting method [15] and a micrometer ocular (Leitz, Germany) the samples were projected on to a transparent lattice with a net of 12 interval sections of 0.5 mm and up to 100 fields were evaluated. The maximal and minimal diameters of the islets were measured and the volume of the islet with the maximal diameter was calculated using the formula $V = 4/3\pi R^3$. The majority of the islets were elliptic, however the formula could still be used to obtain an approximate result and the range of errors could be neglected [7].

To estimate the proportion of the islet tissue in the pancreatic parenchyma, immunohistochemistry was performed by using avidin-biotin complex method and the polyclonal antisera anti-insulin (guinea pig anti-porcine insulin, DAKO Hamburg, dilution 1:300), anti-glucagon (rabbit anti-glucagon, DAKO Hamburg, dilution 1:300), anti-somatostatin (rabbit anti-human somatostatin, DAKO Hamburg, dilution 1:250) and anti-pancreatic polypeptide (rabbit anti-human pancreatic polypeptide, DAKO Hamburg, dilution 1:600). Positive staining was graded into four categories: group 1 < 10%, group 2 > 10% – < 30%, group 3 > 30% – < 60% and group 4 > 60%.

The data were analysed by a computerized questionnaire EPI-INFO version 6.03 and the results were evaluated using a χ^2-test.

Results

Morphology

No significant differences were found between the histological aspects of the cytostructure of the study group and the control group (Table 2). In one case from the study group enlargement of the nuclei could be observed and one other case showed shrinking of the nuclei, another SIDS case showed both phenomena. In three cases of the SIDS group a cell enlargement was found but not in the controls and in ten cases a cytoplasm shrinking was found with only one in the control group.

Immunohistochemistry

The immunostaining for insulin (Fig. 1) did not show any significant differences (p = 0.884) (Table 3). In one case from the SIDS group immunostaining was not possible. Insulin cells represented the most abundant cell type with 30–60% insulin-positive islets in 106 SIDS cases (85.5%) and 18 control cases (94.7%), in 6 cases (5 of the SIDS group and 1 of the control group) more than 60% of cells stained positive.

The islets stained for glucagon (Fig. 2) did not reveal any significant differences (p = 0.340) (Table 4). In three

Table 2 Morphology of histological findings

Grading	Number of cases	Control group	SIDS group
Monomorph inconspicuous cytostructure			
30–60%	3	1	2
> 60%	125	18	10
	128	19 (15%)	109 (85%)
		$p = 0.362$	
Nucleus enlargement			
< 10%	116	18	98
10–30%	2	0	2
	118	18 (15.2%)	100 (84.7%)
		$p = 0.545$	
Cell enlargement			
< 10%	2	0	2
10–30%	1	0	1
	3	0	3
Nucleus shrinking			
< 10%	123	19	104
10–30%	2	0	2
	125	19 (15.2%)	106 (84.8%)
		$p = 0.546$	
Cytoplasm shrinking			
< 10%	10	1	9

cases immunohistochemistry could not be performed. In 104 SIDS cases (95.2%) and 18 cases from the control group (94.7%) 10–30% of cells were positively stained. Positivity for glucagon for less than 10% of cells could be seen in 1 case of the control group and positivity of 30–60% in 5 cases from the SIDS group.

No statistically significant differences were detected in the immunostaining for somatostatin (Fig. 3) (p = 0.051) (Table 5). In three cases immunostaining was not possible. In 126 cases, 108 of the SIDS group (99.1%) and 18 of the control group (94.7%), 10–30% of the cells were stained positive, one case of the control group less than 10% and one case of the SIDS group 30–60% positive. The calculation of the χ^2-test was not carried out as the differences in the results were too small.

Immunostaining for pancreatic polypeptide (Fig. 4) did not reveal any significant difference (p = 0.51) (Table 6). Only two samples showed no usable results. In 123 stained tissues, 105 SIDS cases (95.4%) and 18 control cases (94.7%) less than 10% positively stained cells were found. Only three samples showed 10–30% and another three showed 30–60% positive cells.

Morphometry

Using the point-counting method for determining the maximal (p = 0.409) and minimal diameter (p = 0.778) of the islets, no significant difference was found (Table 7).

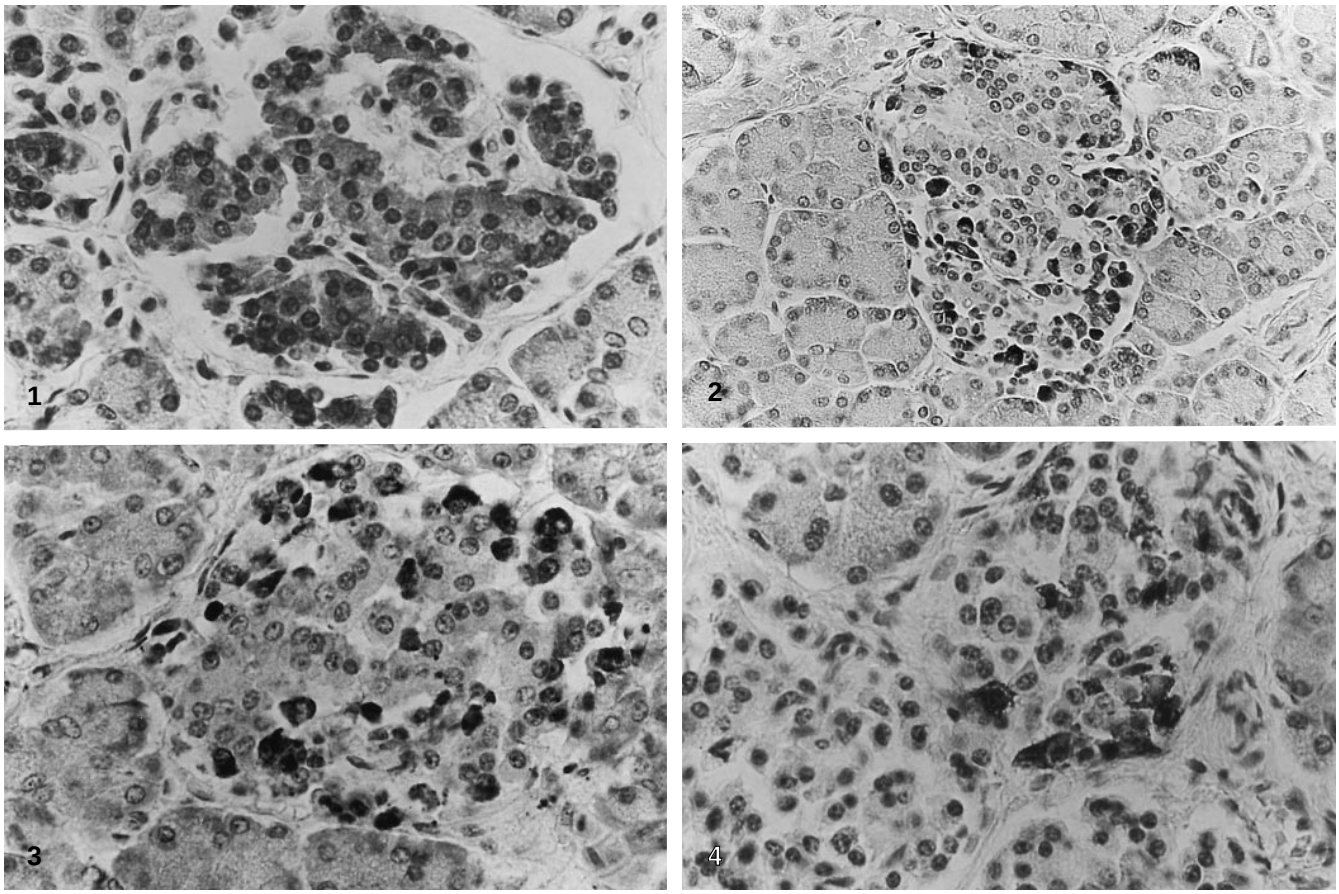

Fig. 1 Pancreatic islet in SIDS (case No. M66/90) with a regular number of insulin-positive cells (black). Anti-insulin, diaminobenzidine, magnification × 440

Fig. 2 Pancreatic islet in SIDS (case No. M88/91) with some glucagon-positive cells, mainly in the periphery (dark grey). Anti-glucagon, diaminobenzidine, magnification × 270

Fig. 3 Pancreatic islet in SIDS (case No. M 148/90 with sparse somatostatin-positive cells (black). Anti-somatostatin, diaminobenzidine, magnification × 440

Fig. 4 Pancreatic islet in SIDS (case No. E594/90) with few pancreatic polypeptide-positive cells (black). Anti-pancreatic polypeptide, diaminobenzidine, magnification × 440

Table 3 Results of the staining for insulin

Positive cells	Number of cases $n = 130$	Control group $n = 19$	SIDS group $n = 111$
< 10%	0	0	0
10–30%	0	0	0
30–60%	124	18	106
> 60%	6	1	5
χ^2-test	$p = 0.884$		

This applies also to the frequency of the islets (p = 0.421) and the calculated volume of the cells with the maximal diameter (p = 0.217). A measurement of the cell diameter could not be done in nine cases due to severe autolysis of the tissues.

Table 4 Results of the staining for glucagon

Positive cells	Number of cases $n = 128$	Control group $n = 19$	SIDS group $n = 109$
< 10%	1	1	0
10–30%	122	18	104
30–60%	5	0	5
> 60%	0	0	0
χ^2-test	$p = 0.340$		

Table 5 Results of the staining for somatostatin

Positive cells	Number of cases $n = 128$	Control group $n = 19$	SIDS group $n = 109$
< 10%	1	1	0
10–30%	126	18	108
30–60%	1	0	1
> 60%	0	0	0
χ^2-test	impossible for statistical reasons		

Table 6 Results of the staining for pancreatic polypeptide

Positive cells	Number of cases $n = 129$	Control group $n = 19$	SIDS group $n = 110$
< 10%	123	18	105
10–30%	3	0	3
30–60%	3	1	2
> 60%	0	0	0
χ^2-test	$p = 0.510$		

Table 7 Morphometric data of pancreatic islets

Maximum diameter (μm) $n = 121$		Minimum diameter (μm) $n = 121$		Maximum volume (cm^3) $n = 121$		Frequency of islets per visual field (× 25) $n = 125$	
SIDS group $n = 103$	Control group $n = 18$	SIDS group $n = 103$	Conrol group $n = 18$	SIDS group $n = 103$	Control group $n = 18$	SIDS group $n = 107$	Control group $n = 18$
142	143	41.8	42.3	1.661	1.802	5.056	4.833
$p = 0.409$		$p = 0.778$		$p = 0.217$		$p = 0.421$	

Discussion

The age and sex distribution of the cases investigated is typical for SIDS cases and similar to those reported by others [17, 18]. The few results of cases with severe autolysis of the pancreas parenchyma were not taken into account in this study but did not alter the main conclusions.

The present study shows that the relative proportion of the different cell types seems to be stable within each group. These results correspond quite well with earlier data published by Rahier et al. [24] and Hisoaka et al. [12]. The quantitative analysis in this study revealed a small increase in the number of insulin and glucagon cells in SIDS cases. These findings might give an indication for endocrine cell proliferation. Persistant hypoglycemia caused by inappropriate insulin secretion is reported to be the result of functional B-cell disorders without histological abnormalities or different forms of B-cell proliferation [11]. A cause for proliferation of endocrine cells is unknown [14]. In hyperinsulinemic hypoglycemia an absence of A-cells [13] and decreased numbers of D-cells [9] have been reported. An increase in A-cells in SIDS cases has not previously been detected and the reason for the small increase in A-cells in our study could merely be due to a slightly hypoglycemic condition of the fetus which leads to a predominance of A-cells [7].

Hyperplasia of islet tissue with islet hypertrophy has long been known to occur in infants of diabetic mothers [13] or rarely in cases of erythroblastosis fetalis [33]. Fetal cells from diabetic mothers react to a diabetic metabolism with hypertrophy [7]. Islet hypertrophy has been defined as islets larger than 250 μm [23] or 400 μm [8]. The maximal diameter in this study was 208 μm. Different methods have been described for evaluating islet cell hyperplasia, the enlargement resulting from increase in cells. Some authors have measured the relative proportion of islet cell area to the total pancreatic tissue [12, 13], the volume density of the islets [7] or the weight of pancreas parenchyma [24]. Due to the rapid growth of the exocrine pancreas with a subsequent decrease of the islet density there is a wide range of islet cell area in neonates [37]. Hisoaka et al. [12] reported a relatively high proportion of islet cell area in SIDS cases with a mean of 8.46% and Jaffe et al. [13] found 6.1–12.9% of endocrine tissue proportional to the total pancreatic tissue. In this study the relative proportion of islet cell area was not measured, therefore the number and the volume of the pancreatic islets were counted, but the results of the SIDS group did not vary from the control group.

From our results we conclude that significant alterations of the pancreatic islets are not demonstrable in cases of SIDS. Therefore it is unlikely that the endocrine pancreas plays an important role in these cases.

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