Postmortem blood ferritin concentrations in sudden infant death syndrome

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Abstract

Aims—To confirm the observation of extremely high concentrations of ferritin in postmortem serum samples in sudden infant death syndrome (SIDS); to examine the factors influencing blood ferritin concentrations postmortem; to determine whether or not these high blood ferritin concentrations are characteristic of SIDS.

Methods—Postmortem samples of cardiac blood were obtained from 58 full term infants who died of SIDS and 14 full-term infants who died of a variety of other causes. Whole blood and serum ferritin concentrations were determined and compared with age at death, liver iron concentration, serum iron concentration, and serum lactate dehydrogenase activity.

Results—The median postmortem blood ferritin concentration for all infants was 18 600 µg/l, which is about 200 times the concentration found in the serum of normal, live infants. Serum iron concentrations were high and there was a highly significant correlation between serum ferritin and iron concentrations suggesting that much of the serum iron was contributed by ferritin. There was no significant difference between serum and whole blood ferritin concentrations. H to L type ferritin ratios were higher in blood from the left than the right ventricle of the heart but the ferritin was always predominantly L type. Blood ferritin concentrations rose rapidly after death but in samples collected at postmortem examination there was a significant correlation with liver iron concentration and an inverse correlation with age. Median values for blood ferritin were higher in SIDS (22 500; n = 58) than in control cases (6900; n = 7) dying under one year of age; however, in both groups ferritin concentrations decreased with age.

Conclusions—Release of ferritin into the blood postmortem seems to be characteristic of infants dying before the age of one year rather than characteristic of SIDS. Two factors may cause such ferritin release postmortem: tissue breakdown and the high level of storage iron in cells of the reticuloendothelial system (including endothelial cells lining vessel walls). SIDS occurs when tissue iron concentrations are higher than at any other time of life. It is possible that the ready availability of iron

enhances free radical damage which might be implicated in SIDS.

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The peak incidence of the sudden infant death syndrome (SIDS) is between two and four months of age¹ when mean blood haemoglobin concentrations are lower² than at any other time of life.

We have recently reported that postmortem liver iron concentrations are higher in SIDS than for deaths from other causes, and that there is an inverse correlation with age.³ As the normal range for liver iron concentrations in infancy has not yet been established, it is not possible to say that liver iron concentrations in SIDS differ from those in normal healthy infants.

We have also noted extremely high ferritin concentrations in postmortem cardiac blood in SIDS.⁴ This paper reports on postmortem blood ferritin concentrations infants who died of SIDS and who died of other causes, and describes some of the factors which influence postmortem blood ferritin concentrations. Iron is an important factor in oxidative cell damage and may be involved in the final metabolic events in SIDS.

Methods

Postmortem samples of cardiac blood were obtained from 58 full-term infants (36 weeks gestation or more) who died of SIDS and 14 full-term controls who had died from a variety of causes when aged less than 2½ years. The cause of death was described as SIDS after full examination by the pathologist at each of the four centres and in accordance with the definition given by Beckwith.⁵ Details of the controls are given in table 1. For the 23 cases of SIDS and four controls collected in the West Midlands, blood was obtained from both left and right ventricles and serum separated before storage at -20°C. In other cases whole blood was collected in EDTA and frozen. In every case a sample of liver was collected and stored frozen.³ In 12 cases blood samples were obtained as soon as the infant reached the accident and emergency clinic or even before death (table 2). The maximum time interval between death and this sample was estimated from information provided by parents or hospital staff.

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Table 1 Control subjects (dying when aged between one month and two years)

Patient No.	Sex (F/M)	PCA (weeks)	Cause of death	Liver iron ($\mu\text{g/g wet wt}$)	Blood ferritin ($\mu\text{g/l}$)
1	M	40	Streptococcal septicaemia	1016	91 000
2	M	41	Asphyxia at birth	261	11 000
3	F	50	RSV pneumonitis	284	20 800
4	M	59	Intussusception	220	1600
5	M	65	RSV pneumonitis and bronchopneumonia	105	6900
6	M	69	Endocardial fibroelastosis (sudden death)	258	1470
7	M	74	Congenital heart disease, right ventricular failure	66	120
8	—	105	Freshwater drowning	46	2200
9	F	109	Viral myocarditis	57	700*
10	F	122	No satisfactory cause of death	155	3900
11	—	126	Fractured skull	26	2450
12	M	138	Gastroenteritis, gut perforation	168	240*
13	F	140	Ventricular septal defect, influenza A	54	1200
14	M	144	Freshwater drowning	53	200

* Serum ferritin. RSV = respiratory syncytial virus.

Samples were also obtained at the full post-mortem examination and the interval between the two samples was recorded. Samples were not included if the infant had received blood transfusions, except for one case in the study of changes in ferritin concentration after death (table 2).

Serum was obtained for ferritin estimation from 14 live infants age between one and six months undergoing venepuncture before surgery or for other investigations.

Samples were collected from four centres in the United Kingdom: the West Midlands (Wolverhampton, Birmingham, Stafford), Cardiff, Nottingham, and Edinburgh. All estimations were performed in Cardiff. Blood samples were stored at -20°C for up to two years before testing.

ASSAYS

Liver non-haem iron concentration

The iron content of liver was determined by the method of Torrance and Bothwell,⁶ using ferrozine as the chromogen. Results are expressed as $\mu\text{g iron/g wet weight}$.

Liver ferritin concentration

A small piece of liver weighing about 0.05 g was blotted dry and homogenised in 1 ml 0.9% NaCl. The homogenate was centrifuged at $12\,000 \times g$ for four minutes and diluted in assay buffer before being assayed at a range of dilutions from 1 in 10 to 1 in 10 000 by the enzyme linked immunosorbent assay (ELISA) for "L" type ferritin as described later. Results are expressed as $\mu\text{g ferritin/g wet weight}$.

Blood ferritin concentrations

Ferritin (L type) was determined by ELISA⁷ using a mouse monoclonal antibody to human spleen ferritin (kindly supplied by R Henley, Cardiff) as the solid phase antibody, rabbit antiliver ferritin coupled with horseradish peroxidase (Dako, High Wycombe, UK) as the second antibody, and spleen ferritin as the standard. The standard was calibrated against the second international standard for the assay of serum ferritin (reagent 80/578, National Institute for Biological Standards and Control, South Mimms, UK). H type ferritin was also determined in serum by ELISA with human heart ferritin as standard⁸ and monoclonal antibody to heart ferritin (kindly provided by Dr J Cook, Kansas City, Kansas, USA) for both the solid phase and labelled antibodies. When whole blood was assayed, the sample was diluted with an equal volume of deionised water, frozen (in dry ice) and thawed three times before centrifugation at $12\,000 \times g$ for three minutes. The supernatants were diluted in assay buffer and assayed at several dilutions.

Lactate dehydrogenase activity

Lactate dehydrogenase (LDH) activity was determined as described by Elliott and Wilkinson⁹ for the cases where serum was available.

Serum iron concentrations

These were determined as described by Worwood and Darke.¹⁰

Statistical analysis

This was carried out as indicated in the results section using Minitab Statistical Software.

Table 2 Changes in cardiac blood ferritin concentration after death

Patient No.	PCA (weeks)	Gestational age (weeks)	Cause of death	First sample		Second sample		Liver ferritin concentration ($\mu\text{g/g wet wt}$)
				Estimated time (h)†	Ferritin ($\mu\text{g/l}$)	Time (h)‡	Ferritin ($\mu\text{g/l}$)	
1	33	29	(Transfused)	a	2200	11	20 700	302
2	38	36	SIDS	<5	25 000	31	33 200	—
3	40	38	Streptococcal septicaemia	<2	11 400	30	91 000	1016
4	42	35	SIDS	<1	7300	32	106 000	421
5	45	40	SIDS	—	500*	24	11 000	—
6	46	36	SIDS	<2	1790	25	21 200	159
7	51	41	SIDS	<1	25 000	54	34 500	386
8	54	36	RSV pneumonitis, bronchopneumonia	b	1300	42	6900	98
9	54	T+3	SIDS	<1	4350	27	25 400	—
10	58	38	SIDS	<2	3650	6	8150	152
11	66	39	SIDS	<8	4930	24	23 400	147
12	112	35	Streptococcal septicaemia	<1	54	21	76	43

* Serum; † estimated time between death and first blood sample (see text); ‡ time between first blood sample (Accident and Emergency) and second blood sample (postmortem); a = alive on ward; no resuscitation; b = died in Accident and Emergency clinic.

Figure 1 Correlation between serum ferritin and serum iron concentrations in 21 postmortem samples (17 SIDS (+) and four controls (*)). Linear regression analysis for log ferritin against log serum iron concentration shows a highly significant correlation ($r=0.921$, $p<0.001$).

Results

In the 14 live infants age between one and six months serum ferritin concentrations ranged from 20 to 150 $\mu\text{g/l}$, falling within the expected range for this age group.¹¹ In postmortem samples the median blood ferritin concentration was 18 600 $\mu\text{g/l}$ (95% confidence interval (CI) 11 200–26 000 $\mu\text{g/l}$) about 200 times the concentration found in the serum of normal infants aged three months. In cases of SIDS the median blood ferritin concentration was 22 500 $\mu\text{g/l}$ (95% CI 15 600–32 000 $\mu\text{g/l}$) significantly higher than the median of 6900 $\mu\text{g/l}$ (95% CI 200–20 800 $\mu\text{g/l}$) in the non-SIDS cases under 12 months of age ($p=0.049$, two sample Wilcoxon rank sum test). In both groups, however, ferritin concentrations declined with age.

Figure 2 Correlation between blood ferritin and liver non-haem iron concentrations in 58 SIDS (+) and 14 control cases (*). For linear regression analysis the regression equation is $\log_{10}(\text{blood ferritin}) = 2.58 + 1.57 \log_{10}(\text{liver iron})$ ($r=0.698$, $p<0.001$).

Figure 3 Correlation between blood ferritin concentration and PCA in 58 SIDS (+) and 14 control cases (*). The regression equation is $\log_{10}(\text{blood ferritin}) = 2.45 - 0.0213 \text{ PCA}$ ($r=-0.680$, $p<0.001$).

In normal infants aged up to six months the total binding capacity of serum iron increases with age but does not exceed 80 $\mu\text{mol/l}$.¹² In 11 of the 21 postmortem samples examined the serum iron concentration was higher than this and there was a highly significant correlation between plasma ferritin and plasma iron concentrations (fig 1).

In the cases of SIDS there was no significant difference between serum and whole blood ferritin concentrations (Kruskal–Wallis test, $n=58$, $H=0.17$, $p=0.677$), and these concentrations have therefore been combined in the analysis of the data.

Cardiac blood ferritin concentrations correlated with liver iron concentration (fig 2) and inversely correlated with postconceptional age (PCA) (fig 3). Liver iron concentrations also decline with age.³ Blood ferritin concentrations were found to rise rapidly after death in a small group of infants for whom samples were available immediately after death as well as at the full postmortem examination (table 2). There was no significant correlation between the increase in ferritin concentration and the time lapse between the two samples or between the final ferritin concentration (either expressed in $\mu\text{g/l}$ or as a multiple of the first value) and estimated time since death in this group. However, in two cases the ferritin concentration was initially very high and in one older, non-SIDS case both concentrations were very low. The initial values were still considerably higher than the expected value of about 50 $\mu\text{g/l}$ in serum (about 250 $\mu\text{g/l}$ in blood) for a healthy infant aged six months.

Blood ferritin concentrations (transformed as $\log_{10}$) were compared with liver iron concentrations and the estimated time interval between death and postmortem examination for all cases, and for cases of SIDS, using multiple linear regression analysis. The time interval was not found to be a significant variable.

In the samples from Wolverhampton we were able to compare blood from the left and right ventricles of the heart in 16 cases of SIDS. Although the mean concentrations in the left (51 000 $\mu\text{g/l}$) and the right (52 000 $\mu\text{g/l}$) ventricles were almost identical, the concentration in the left was less than that in the right in 14 of 16 cases and the paired Student's t test showed that values were significantly lower in the left ventricle ($p<0.01$). Concentrations were also measured with assays specific for H and L type ferritin as described in the methods section. In cardiac muscle the ratio of H to L ferritin is in excess of 1 compared with a value of about 0.04 for liver ferritin.¹³ The mean value for the left ventricle samples was 0.097 and for the right 0.021. Using the paired t test this difference was highly significant ($p=0.003$). This suggests that a greater proportion of ferritin in the left ventricle may be derived from cardiac muscle than in the right ventricle.

Examination of serum from the left and right ventricles for the isoenzyme distribution of LDH¹⁴ revealed a predominantly "liver" pattern¹⁵ in one pair of samples from a case of SIDS and another pair from a control. There was a highly significant correlation between

liver iron and liver ferritin concentrations (linear regression analysis $r=0.63$, $p<0.001$) and also between blood and liver ferritin concentrations (linear regression analysis of data transformed to $\log_{10}$ $r=0.52$, $p<0.001$).

LACTATE DEHYDROGENASE

LDH activity in blood was very high in both SIDS and control cases. The mean value in 23 cases of SIDS was 3509 $\mu\text{g/l}$ (range 722–7200 $\mu\text{g/l}$) and in five control cases (PCA 44–174 weeks) was 2735 $\mu\text{g/l}$ (559–4774 $\mu\text{g/l}$). This suggests a similar degree of cellular damage although haemolysis may also have contributed to high serum activities. There were no significant correlations between LDH activity and PCA, liver iron concentration, or blood ferritin concentration.

Discussion

This is the first reported study of postmortem blood ferritin concentrations in infants. In life the assay of serum ferritin has considerable clinical value as an indicator of the level of iron stored¹⁶ although this relationship is compromised by tissue damage particularly in the liver.¹⁷ In infants, postmortem, the remarkably high serum ferritin concentrations are also related to levels of stored iron (liver non-haem iron concentration). The high concentrations of serum ferritin found on immunoassay have been confirmed by the chemical determination of serum iron concentrations. In many cases serum iron concentrations were grossly in excess of the transferrin iron binding capacity of serum in live infants aged about six months, and the postmortem serum iron concentration was highly correlated with the serum ferritin concentration determined by immunoassay. Much of the iron is probably contributed by partial release of ferritin iron during the assay.¹⁸ In vivo plasma ferritin normally has a low iron content¹⁶ and provides only about 20% of blood ferritin, the remainder being supplied by blood cells.¹⁹ Even in completely lysed blood the ferritin concentration from the cellular elements will only increase the concentration found in normal serum by about four times. The grossly elevated plasma concentrations found postmortem must therefore be iron-rich ferritin derived from the tissues.

The extremely high serum ferritin concentrations described here appear to be characteristic of age rather than SIDS, but with the SIDS cases showing the highest concentrations. However, we were able to study only a few control cases up to one year of age and the 95% CIs overlap.

In life there are two conditions associated with ferritin concentrations in excess of 10 000 $\mu\text{g/l}$: necrosis of the liver¹⁷ and active erythrophagocytosis.²⁰ Autolysis occurs postmortem. Active erythrophagocytosis also occurs in infants aged from one to six months. Particularly high concentrations of stored iron may be found in cells of the reticuloendothelial system at this age²¹ because of the high rate of haemoglobin breakdown and the relatively low

rates of synthesis.² Iron concentrations in phagocytic cells in the first weeks of life are higher than at any other time and may reach concentrations usually associated with haemochromatosis in adults.²¹ It is these high concentrations of tissue iron (and ferritin) which cause the postmortem release of ferritin into the blood in infants, but endothelial cells lining vessel walls are also capable of producing high concentrations of ferritin²² and may contribute directly to ferritin release. Ferritin concentrations in serum, like LDH and some other enzyme activities,²³ increase rapidly after death so the lack of correlation between the blood ferritin concentration and the time of sampling after death was surprising. In some cases the ferritin concentrations were high at the time of death but in others the time of death could not always be established precisely, and the increase may have been completed already. Another cause of high ferritin concentrations may be the trauma caused by attempts at resuscitation. In the Nottingham series attempts to resuscitate in the ambulance or in hospital were recorded but these cases did not have higher concentrations than found in those where resuscitation was not attempted.

We have recently completed a study of ferritin concentrations in blood spots collected on "Guthrie cards" during the first week of life to determine whether infants who subsequently die of SIDS have higher tissue iron stores at birth (Raha-Chowdhury *et al*, submitted for publication). We have found no evidence of gross elevation of blood ferritin concentrations at birth in infants who subsequently died of SIDS. We have previously discussed ways in which excess iron may be acquired after birth³ but changes in tissue iron concentrations as a result of supplementation or administration of iron containing medicines are likely to be small.

By definition, there is no adequate explanation for SIDS at postmortem, suggesting an overwhelming toxic or metabolic disturbance as the cause of death. It is conceivable that oxidative cell damage may be the final biochemical event and the role of iron as an enhancer of free radical damage is well established.²⁴ If oxidative cell damage is found to be implicated in the aetiology of SIDS then high tissue iron concentrations may be an important factor. The extraordinary ferritin concentrations found in blood postmortem are a reflection of these high tissue iron concentrations.

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