



High serum serotonin in sudden infant death syndrome

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Sudden infant death syndrome (SIDS), the leading cause of postneonatal infant mortality, likely comprises heterogeneous disorders with the common phenotype of sudden death without explanation upon postmortem investigation. Previously, we reported that ~40% of SIDS deaths are associated with abnormalities in serotonin (5-hydroxytryptamine, 5-HT) in regions of the brainstem critical in homeostatic regulation. Here we tested the hypothesis that SIDS is associated with an alteration in serum 5-HT levels. Serum 5-HT, adjusted for postconceptional age, was significantly elevated (95%) in SIDS infants ($n = 61$) compared with autopsied controls ($n = 15$) [SIDS, 177.2 ± 15.1 (mean \pm SE) ng/mL versus controls, 91.1 ± 30.6 ng/mL] ($P = 0.014$), as determined by ELISA. This increase was validated using high-performance liquid chromatography. Thirty-one percent (19/61) of SIDS cases had 5-HT levels greater than 2 SDs above the mean of the controls, thus defining a subset of SIDS cases with elevated 5-HT. There was no association between genotypes of the serotonin transporter promoter region polymorphism and serum 5-HT level. This study demonstrates that SIDS is associated with peripheral abnormalities in the 5-HT pathway. High serum 5-HT may serve as a potential forensic biomarker in autopsied infants with SIDS with serotonergic defects.

asphyxia | brainstem | pulmonary neuroepithelial bodies | platelets | high-performance liquid chromatography

Sudden infant death syndrome (SIDS) is defined as the sudden death of an infant less than 1 y of age that remains unexplained after a complete autopsy and death scene investigation (1). Typically, SIDS is associated with a sleep period and with risk factors in the sleep environment, e.g., prone/face-down sleep, bed-sharing, soft bedding, and overbundling (2–4). Despite the national safe sleep campaign, SIDS remains the leading cause of postneonatal infant mortality, with an overall rate of 0.40 SIDS deaths per 1,000 live births (5). Our research in SIDS is based upon the premise of the triple-risk model that posits that SIDS results from three interacting factors impinging upon the infant simultaneously: (i) an underlying vulnerability (disease process); (ii) a critical developmental period (infancy); and (iii) an exogenous stressor that precipitates sudden death, e.g., asphyxia secondary to sleeping in the prone or face-down position (6). SIDS is likely due to a heterogeneous group of disorders that present as sudden, unexplained, sleep-related death, with subsets of vulnerabilities to be discovered with the same clinical presentation. Although death is sudden, the putative underlying vulnerability may be present subclinically for days or months before death, even possibly originating during gestation (7), remaining latent until the vulnerable infant is sufficiently stressed in the critical postnatal period. Reports of apnea, cardiac rate abnormalities, and arousal deficits in infants who subsequently die of SIDS support this possibility (8–12) and suggest that as yet undiscovered biomarkers of the disease process may be detectable in readily accessible tissues as a predictor in living infants at risk for sudden death. However, at the present time, there is no clinically available biochemical or physiological biomarker of SIDS.

We have previously reported an important subset of SIDS (~40%) associated with abnormalities in the neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) in regions of the caudal brainstem (medulla oblongata) that play major roles in homeostatic regulation of hypercarbia and hypoxia during sleep (13–16). The medullary 5-HT abnormalities in SIDS cases are notable for decreased levels of 5-HT and its key biosynthetic enzyme, tryptophan hydroxylase 2 (TPH2), thereby characterizing the brainstem disorder in a subset of SIDS as potentially due to 5-HT deficiency (14). Other abnormalities in the medullary 5-HT network in SIDS include aberrations in 5-HT_{1A} receptor binding and 5-HT cell density (13, 14). There are no biomarkers of these 5-HT-related defects in autopsied infants, and their demonstration at autopsy requires special research techniques in frozen, and not fixed, tissues. Although 5-HT produced centrally is critical to homeostasis, greater than 90% of total body 5-HT is produced peripherally in the gastrointestinal tract and other organs (17). Approximately 95% of the 5-HT in blood is carried in platelet dense granules (18), and serum contains the secretion products of activated platelets, including their dense granules. Despite this predominance of peripherally produced 5-HT, nothing is known about peripheral levels of 5-HT in SIDS infants. In the current study, we address this issue by analyzing serum 5-HT levels, a peripheral marker of 5-HT metabolism, in SIDS cases compared with autopsy controls. This information may provide insight into the extent of defective 5-HT pathology in SIDS: whether it

Significance

Sudden infant death syndrome (SIDS), the leading cause of postneonatal infant mortality, is defined as the sudden death of an infant less than 1 y of age that remains unexplained after a complete autopsy and death scene investigation. Although SIDS has been associated with deficiencies in central (brainstem) serotonin (5-hydroxytryptamine, 5-HT), there are no known peripheral biomarkers for SIDS. Here we demonstrate increased serum serotonin levels in a subset (31%) of SIDS infants compared with control infants. These findings suggest the potential of a high serum serotonin level as a forensic biomarker at autopsy to differentiate SIDS deaths with serotonergic defects from other causes of sudden death and, importantly, as evidence of a peripheral 5-HT abnormality in SIDS.

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The authors declare no conflict of interest.

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is a generalized disorder of 5-HT metabolism, and if measures of serum 5-HT could ultimately be used as a biomarker of 5-HT peripheral and central abnormalities in autopsied infants, as well as living infants at risk for sudden death due to central 5-HT defects. We specifically hypothesized that serum 5-HT levels are altered in SIDS cases compared with infants dying of known causes.

Methods

Serum Cohorts.

Postmortem cohort. A cohort of SIDS cases ($n = 61$) and autopsied control infants ($n = 15$) was accrued from 2007 to 2013 in collaboration with the San Diego Office of the Medical Examiner in San Diego County, CA (Table 1). Serum was available for research under the auspices of California Law Chapter 955, Statutes of 1989 (SB 1069). The case and control adjudications were based upon autopsy reports, clinical information, and death scene investigations, and were performed blinded to serum 5-HT values. In this study, all SIDS cases, classified according to the National Institute of Child Health and Human Development classification of SIDS (1), were sudden, unexpected deaths of infants under 1 y of age that remained unexplained after a complete autopsy and death scene investigation (1). The controls consisted of seemingly healthy infants who died of definable acute disorders with no or only minor signs of clinical illness within 1 wk of death. Clinical variables, e.g., gestational age at birth, postnatal age at death, gender, race, perinatal and/or postnatal illnesses, and time of death were recorded. Variables related to the sleep environment, i.e., sleep position at discovery or history of bed-sharing the night of death, were also recorded, as well as variables related to acute illness 48 h and 1 wk before death.

Living infant cohort. Serum samples were obtained from 40 living infants (20 males and 20 females, ranging from 4.6 postnatal weeks to 1 y) through the Canadian Laboratory Initiative on Pediatric Reference Intervals Project, a multicenter, nationwide initiative aimed at developing complete and accurate pediatric reference intervals. These serum samples were collected from metabolically healthy infants seen in outpatient dentistry, dermatology, and eye clinics. No specific clinical information was available on these cases. Serum was collected and transferred to Boston Children's Hospital under the auspices of a Research Ethics Board-approved protocol from The Hospital for Sick Children, including informed consent from the parent/guardian.

Living adult cohort. To determine the potential effect on serum 5-HT levels of time between blood collection and serum separation, serum was collected at Boston Children's Hospital from five healthy adult donors. These subjects gave written informed consent in accordance with the Declaration of Helsinki before participation in this Institutional Review Board-approved study.

Serum 5-HT and 5-HIAA Measurements. At autopsy, blood was collected from large blood vessels, placed into BD Vacutainer SST serum separator tubes (containing a silica clot activator and polymer gel for serum separation; BD Biosciences), and centrifuged. Serum was frozen at -80°C until analysis. Serum samples (25 μL) were analyzed in duplicate with an ELISA specific for 5-HT (catalog no. BAE 8900; Rocky Mountain Diagnostics, Inc.). All serum samples were run on the same 96-well ELISA plate, and duplicates were run in an identical fashion on a separate ELISA plate. A subset of SIDS ($n = 45$) and control ($n = 12$) serum samples were selected for confirmation of 5-HT levels by the independent method of high-performance liquid chromatography (HPLC) performed by the Vanderbilt Neurochemistry Core, Vanderbilt University School of Medicine. Selection of this subset was based on sample availability. Because brainstem tissues were not available in every case, correlative analysis of serum and brainstem 5-HT parameters could not be performed in this cohort. Serum 5-hydroxyindoleacetic acid (5-HIAA, a stable

breakdown product of 5-HT) was also measured by HPLC by the Vanderbilt Neurochemistry Core. Serum samples from living infants were collected into the same type of BD Vacutainer serum separator tubes used for the postmortem samples and analyzed by HPLC at the Vanderbilt Neurochemistry Core, Vanderbilt University School of Medicine.

Analysis of Adult Donors. Freshly drawn venous blood from the healthy subjects, who had not taken any antiplatelet medications within 7 d before the study, was collected into 10-mL glass serum Vacutainers and 5-mL clot activator and serum separation Vacutainers (BD Biosciences). After 0, 12, 24, and 36 h, content from the serum Vacutainers was transferred into a serum separation Vacutainer. To isolate serum from blood clots at these specific time points, serum separation Vacutainers, including the one that was used to directly draw blood into, were centrifuged ($1,850 \times g$, 10 min). Supernatant was taken off, and serum was frozen at -80°C until analysis for 5-HT by ELISA.

The 5-HTTLPR Genotyping. We sought to determine if the putative abnormal serum 5-HT levels were associated with a genotype of the serotonin transporter (5-HTT) promoter polymorphisms (5-HTTLPR). DNA from brain, kidney, or liver tissue was extracted using the Puregene DNA Extraction Kit and DNA purification from Tissue protocol (Gentra Systems). Acquired DNA was then amplified via PCR using published primer sequences and methods (19). To determine the 5-HTTLPR genotype, PCR products were separated by gel electrophoresis in a 1.5% agarose gel with ethidium bromide. The short [S; 486 base pairs (bp), 14 repeats] and long [L; 529 bp, 16 repeats] allele variants of 5-HTTLPR were determined from the PCR fragment sizes, and cases were assigned as S/S, S/L, or L/L genotypes.

Statistical Analysis. The t tests compared the following factors between SIDS and controls: gestational age, postnatal age, postconceptional age (PCA), postmortem interval (length of time between death and autopsy), and storage length interval (length of time from serum collection to 5-HT measurement by ELISA). The χ^2 and Fisher exact tests were used to determine potential differences in sex, race, and premature birth (<37 gestational weeks). Analysis of covariance (ANCOVA) tested for differences in serum 5-HT levels between SIDS cases and controls while controlling for potential effects of age. Differences in the frequency of risk factors within SIDS subgroups, as defined by serum 5-HT levels, were determined with t tests. ANCOVA tested for differences in serum 5-HT levels between 5-HTTLPR genotypes, controlling for diagnosis and age. Differences in the frequency of SIDS cases with high versus normative levels of serum 5-HT within 5-HTTLPR genotypes were assessed by Fisher exact test. To examine the effect of time of death on serum levels of 5-HT, Loess curves were fit by diagnosis. The curves did not assume a shape to the relationship, but rather fit the best curve through the data with a 95% confidence interval around the curve. All analyses were conducted using SAS v9.3 (SAS Institute Inc.), and statistical significance was tested at level 0.05.

Results

Clinicopathologic Database. A final cohort of SIDS cases ($n = 61$) and autopsied control infants ($n = 15$) was examined for serum 5-HT levels (Table 1). The causes of death of the controls were crib accidents and mechanical suffocation, $n = 5$; acquired lung disease, $n = 3$; clinically unsuspected congenital heart disease, $n = 2$; accidental head trauma, $n = 1$; infection, $n = 1$; inborn metabolic disorder, $n = 1$; histiocytoid cardiomyopathy, $n = 1$; and congenital myopathy, $n = 1$. There were no significant differences between SIDS and controls in the frequency of different

Table 1. Comparison of clinicopathologic data between SIDS and control infants

| Characteristic | SIDS | Controls | <i>P</i> value |
|---|------------|-------------|----------------|
| Number of cases | 61 | 15 | |
| Age in weeks, mean (SD) | | | |
| Postconceptional age at death | 52.9 (8.3) | 50.0 (14.1) | ns |
| Gestational age at birth | 38.9 (2.4) | 38.1 (2.9) | ns |
| Postnatal age at death | 14.0 (7.8) | 11.9 (13.0) | ns |
| Postmortem interval in hours, mean (SD) | 21.5 (5.7) | 18.8 (7.6) | ns |
| Storage interval at -80°C in years, mean (SD) | 5.6 (1.4) | 4.8 (1.6) | 0.04 |
| Male gender, % | 46 | 53 | ns |

ns, not significant.

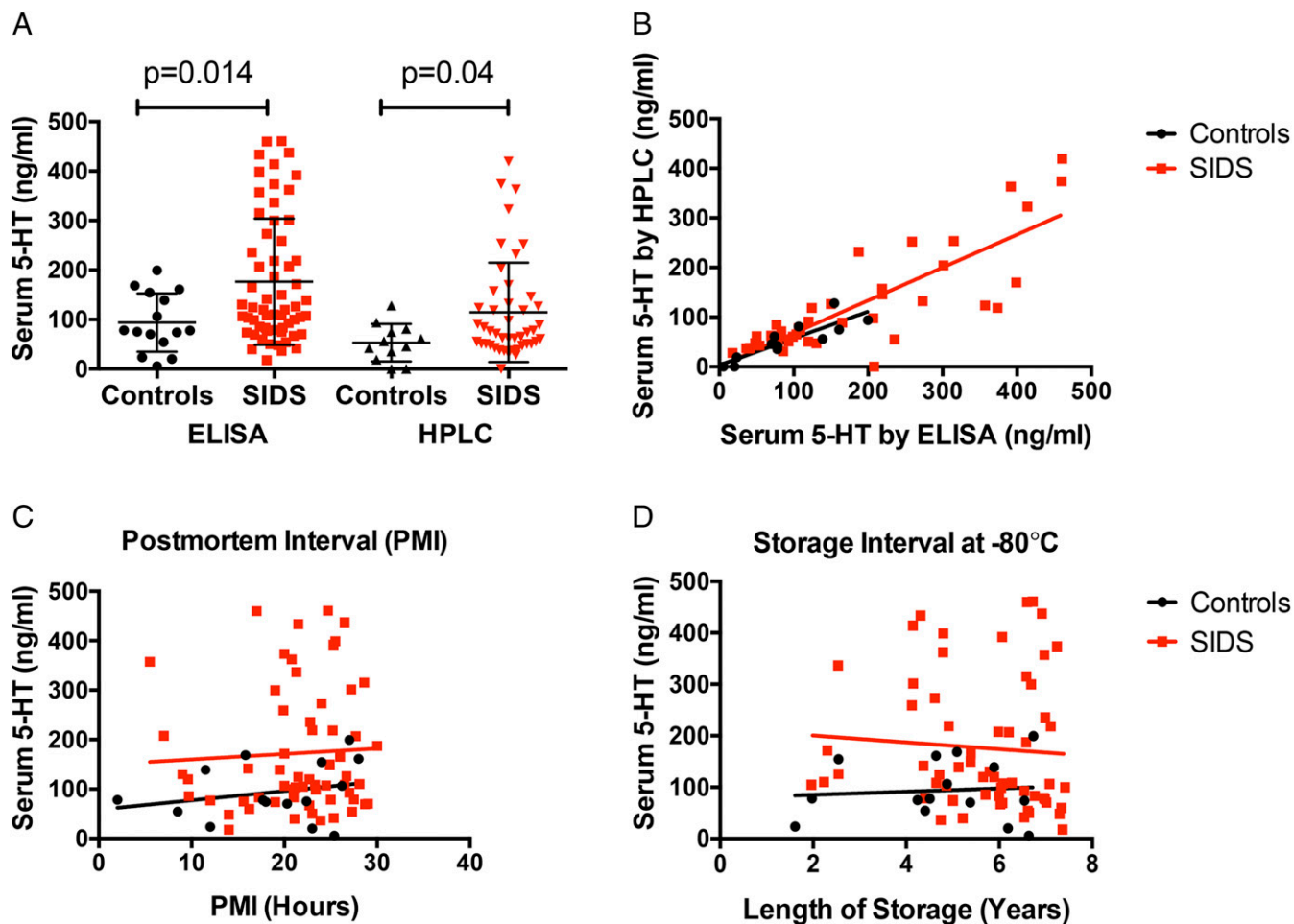


Fig. 1. Serum 5-HT values determined by ELISA and HPLC. (A) There was a significant elevation (average of 95%) in SIDS infants [177.2 ± 15.1 (SE) ng/mL] compared with controls [91.1 ± 30.6 (SE) ng/mL], $P = 0.014$, by ELISA. HPLC confirmed this finding in a subset of cases. The bars in the graph represent SD. (B) Serum 5-HT levels obtained with two different methods, ELISA and HPLC, showed significant correlation in SIDS cases [Spearman correlation of 0.84 ($P < 0.001$)] and controls [Spearman correlation of 0.87 ($P < 0.001$)]. There is no significant effect of (C) postmortem interval or (D) storage length at -80°C on the levels of serum 5-HT in control or SIDS infants.

ances (data not shown), male gender, gestational age, postnatal age, PCA, and postmortem interval (Table 1). There was a significant difference in storage interval at -80°C with SIDS being stored for 5.6 ± 1.4 y and controls being stored for 4.8 ± 1.6 y ($P = 0.04$) (Table 1). Potential effects of postmortem interval, sex, storage interval, and interactions between diagnosis and age were included in initial statistical modeling, but no associations were found, and these variables were eliminated in final models.

Serum 5-HT and 5-HIAA Levels in SIDS Postmortem Cases Versus Postmortem Controls. Serum 5-HT, measured by ELISA, was 177.2 ± 15.1 (SE) ng/mL for the SIDS cases ($n = 61$) compared

with 91.1 ± 30.6 ng/mL for the controls ($n = 15$) ($P = 0.014$) (Fig. 1A and Table 2), an average increase of $\sim 95\%$ in the SIDS infants compared with controls. In a subset of 57 cases (45 SIDS and 12 controls), serum levels of 5-HT were also measured by HPLC. Results confirmed the increase in serum 5-HT levels in SIDS infants (114.6 ± 13.7 ng/mL) compared with controls (52.4 ± 26.5) ($P = 0.04$) (Fig. 1A and Table 2). Serum 5-HT levels determined by ELISA and by HPLC correlated positively with each other [Spearman correlation of 0.84 in SIDS cases ($P < 0.001$) and 0.87 in controls ($P < 0.001$)] (Fig. 1B). There was no significant effect of postmortem interval (Fig. 1C) or storage interval at -80°C (SIDS, mean of 5.6 ± 1.4 y; controls, 4.8 ± 1.6 y) (Fig. 1D) upon the serum

Table 2. Serum data for 5-HT and 5-HIAA (ng/mL) in SIDS cases compared with controls, adjusted for postconceptional age

| Variable | Method | SIDS mean \pm SE, ng/mL; <i>n</i> | Postmortem controls mean \pm SE, ng/mL; <i>n</i> | <i>P</i> value SIDS vs. postmortem controls |
|--------------|--------|--|---|--|
| 5-HT | ELISA | 177.2 ± 15.1 ; 61 | 91.1 ± 30.6 ; 15 | 0.014 |
| 5-HT | HPLC | 114.6 ± 13.7 ; 45 | 52.4 ± 26.5 ; 12 | 0.04 |
| 5-HIAA | HPLC | 70.1 ± 9.1 ; 44 | 36.3 ± 17.5 ; 12 | 0.09 |
| 5-HT/ 5-HIAA | HPLC | 3.45 ± 0.57 ; 44 | 1.7 ± 1.1 ; 12 | 0.17 |

All postmortem serum data are adjusted for postconceptional age at death.

Table 3. Frequency of cases with known SIDS risk factors within SIDS groups defined by normative and high levels of 5-HT

| SIDS risk factor | Normative serum 5-HT, % (numbers) | High serum 5-HT, % (numbers) |
|-----------------------|--------------------------------------|---------------------------------|
| Male sex | 45 (19/42) | 47 (9/19) |
| Black race | 7 (3/41) | 5 (1/19) |
| Prematurity | 7 (3/42) | 21 (4/19) |
| Prone sleep | 62 (18/29) | 38 (5/13) |
| Prone or side sleep | 68 (23/34) | 50 (8/16) |
| Face-down or covered | 38 (12/32) | 29 (5/17) |
| Sleeping in adult bed | 66 (21/32) | 59 (10/17) |
| Bed-sharing | 33 (13/39) | 44 (8/18) |
| Illness within 48 h | 26 (10/38) | 29 (5/17) |
| Illness within 1 wk | 36 (14/39) | 41 (7/17) |

High serum 5-HT levels were defined as greater than 2 SDs above the mean of the control group. Risk factor information was not available on every case.

5-HT measurements. In our analysis examining time of death and serum 5-HT, the Loess curves suggested that serum 5-HT was slightly higher in the morning (data not shown). The confidence intervals, however, reflected too much variation to permit conclusions of an effect. In the HPLC analysis of serum, levels of the 5-HT breakdown product 5-HIAA were also obtained. Although there was a numerical increase in 5-HIAA levels in SIDS cases compared with control cases, this increase did not reach statistical significance ($P = 0.09$) (Table 2). There was also no statistically significant difference in the ratio of 5-HT/5-HIAA between the two groups (Table 2).

Definition of the SIDS Subset with Elevated Serum 5-HT. After finding the significant elevation in serum 5-HT in this SIDS cohort, we defined post hoc “high” serum 5-HT as greater than 2 SDs above the mean of the controls as determined by ELISA (≥ 211.8 ng/mL) and “normative” serum 5-HT as below this cutoff (< 211.8 ng/mL). The percent of SIDS cases with high serum 5-HT was 31% (19/61) (Fig. 1A). There were no controls above this cutoff level of serum 5-HT (Fig. 1A). The percent of SIDS cases with known SIDS risk factors was compared between SIDS cases with high serum 5-HT and those SIDS cases with normative levels. There were no significant differences in risk factors for SIDS between the SIDS subset with high serum 5-HT levels ($n = 19$) and the SIDS subset with normative 5-HT levels ($n = 42$) (Table 3). The frequencies of male sex, prematurity, and discovery in the prone position were not significantly different between the two groups (Table 3). There was no significant difference in postmortem interval ($P = 0.58$) or length of storage interval ($P = 0.91$) between SIDS cases with high and normative levels of 5-HT.

The 5-HTTLPR Genotype Association with Serum 5-HT Levels. In a second post hoc analysis, we tested the hypothesis that the high serum 5-HT subset was associated with the LL genotype of 5-HTTLPR. Of the SIDS cases, 58 out of 61 cases were genotyped; all control cases were genotyped. Among SIDS cases and controls, there was no significant difference in the serum 5-HT values across the different genotypes (Table 4). In the SIDS cases, there was no significant difference in the frequency of

cases with high versus normative serum 5-HT levels within the different 5HTTLPR genotypes (Table 5).

Effect of Breastfeeding on Serum 5-HT Levels. There were no significant differences in serum 5-HT concentrations between (i) control cases who were breastfed vs. control cases who were not breastfed or (ii) SIDS cases who were breastfed vs. SIDS cases who were not breastfed (Table S1 and Fig. S1).

Serum 5-HT and 5-HIAA Levels in Living Control Infants. As measured by HPLC, living infant controls had higher 5-HT concentrations (300.8 ± 25.0 ng/mL, mean \pm SE, $n = 40$) than postmortem SIDS (114.6 ± 13.7 ng/mL, $n = 45$) and postmortem control infants (52.4 ± 26.5 , $n = 12$). As measured by HPLC, living infant controls had lower 5-HIAA concentrations (9.9 ± 1.1 ng/mL, $n = 40$) than postmortem SIDS (70.1 ± 9.1 ng/mL, $n = 44$) and postmortem control infants (36.3 ± 17.5 , $n = 12$). There were no significant differences in serum values of 5-HT, 5-HIAA, and 5HT/5HIAA between living male infants ($n = 20$) and living female infants ($n = 20$).

Effect of Delay in Serum Separation on Serum 5-HT Concentrations. To assess the potential impact of a postmortem delay in serum separation on serum 5-HT values, normal adult blood was collected using serum separator tubes identical to those used for the postmortem cohort. Of note, it was logistically impossible to obtain IRB-approved, fresh serum samples from normal, living infants. There was no significant difference in serum 5-HT concentration as determined by ELISA between serum separated immediately (346.3 ± 56.9 ng/mL), at 12 h (319.7 ± 98.7 ng/mL), 24 h (312.1 ± 102.1 ng/mL), or 36 h (297.6 ± 54.8 ng/mL).

Discussion

This study provides evidence of a blood biomarker for peripheral 5-HT abnormalities in SIDS. We identified an elevation in serum 5-HT levels in SIDS cases compared with postmortem controls, with a subset of 31% of the SIDS cases with levels greater than 2 SDs above the mean of the controls. This study expands upon the brainstem evidence suggesting that SIDS is a 5-HT disorder related to an underlying vulnerability posited in the triple-risk

Table 4. Serum 5-HT values across different 5-HTTLPR genotypes in SIDS and controls

| 5HTTLPR genotype | SIDS | | | Controls | | |
|----------------------------|-------------------|-------------------|-------------------|-----------------|------------------|-------------------|
| | SS | SL | LL | SS | SL | LL |
| <i>n</i> | 11 | 27 | 20 | 2 | 4 | 9 |
| Serum 5-HT \pm SD, ng/mL | 169.5 \pm 134.4 | 157.4 \pm 105.9 | 189.6 \pm 146.1 | 96.6 \pm 59.8 | 78.23 \pm 60.9 | 100.2 \pm 63.86 |

ANCOVA controlling for diagnosis and postconceptional age; $P = 0.62$ (not significant).

Table 5. Frequency of high or normal serum 5-HT values within each 5-HTTLPR genotype in the SIDS cases

| 5-HTTLPR genotype | High 5-HT, % (numbers) | Normal 5-HT, % (numbers) |
|---------------------|------------------------|--------------------------|
| SS (<i>n</i> = 11) | 27 (3/11) | 73 (8/11) |
| SL (<i>n</i> = 27) | 22 (6/27) | 78 (21/27) |
| LL (<i>n</i> = 20) | 40 (8/20) | 60 (12/20) |

High serum 5-HT levels were defined as greater than 2 SDs above the mean of the control group.

model (6). High serum 5-HT levels at autopsy may help to distinguish the 5-HT-related vulnerability in infants with sudden, unexpected death from those infants with similar presentation mimicking SIDS, but due to unsuspected infection, inborn errors of metabolism, trauma, and accidental asphyxia. The evidence of brainstem (13–16), and now peripheral, abnormalities in 5-HT pathway markers suggests a key role for 5-HT metabolism in SIDS.

Platelets avidly take up 5-HT from the bloodstream via the 5-HT transporter and store it in dense granules, where it is protected from the action of mitochondrial monoamine oxidases. Indeed, ~95% of the 5-HT in blood is carried in platelet dense granules (18), and serum contains the secretion products of activated platelets, including the contents of their dense granules. An extensive body of investigation has used platelets as a peripheral model of neuronal function in the central nervous system (20). Multiple neurological disorders demonstrate functional abnormalities in platelets, including Alzheimer disease, Parkinson disease, various psychiatric disorders, and autism (21–27). The major forces driving these studies are the inherent difficulty of directly accessing brain function *in vivo* and the critical molecular similarities between platelets and central neurons. The presently reported data suggest that measurement of the platelet stores of 5-HT, via measurement of serum 5-HT, may be an accessible means of premortem identification of infants at risk for SIDS.

We did not find any associations of the high serum 5-HT levels in the SIDS cases with any previously known risk factors definable by autopsy and investigative reports in the cohort, including prematurity, sleep position when found at discovery, history of minor illness around the time of death, or with LL genotype of the 5-HT transporter (5-HTT) polymorphism. Known polymorphisms of the 5-HTT gene (SLC6A4) have been extensively studied in various disorders, including SIDS (28–30). The 5-HTTLPR is an insertion–deletion polymorphism that consists of a repeated sequence of 22 to 23 base pairs that is present in 14 copies producing a short or “S” allele or in 16 copies producing a long or “L” allele. The L allele and the LL genotype are associated with increased gene transcription and protein expression compared with the SS and SL genotypes (31, 32). Although the association of the LL genotype on 5-HT uptake rate in platelets has been documented (33), the effect of this genotype on serum content of 5-HT is less well understood (33, 34). In the present study, the lack of association between the LL genotype and high values of serum 5-HT suggests that specific 5-HTTLPR genotypes, shown to influence 5-HTT expression levels (31, 32), do not contribute to the increased serum 5-HT in SIDS.

The cause(s) of the elevated serum 5-HT levels in the SIDS subset in this study remains unknown. One potential cause is overproduction of peripheral 5-HT in SIDS without a proportional increase in breakdown. In the HPLC analysis, we found a nonsignificant trend toward an increase in the levels of 5-HIAA in SIDS cases compared with control cases, consistent with an increase in 5-HT peripheral breakdown; the lack of difference in the ratio of 5-HT/5-HIAA further suggests that the breakdown of 5-HT into 5-HIAA is proportionate with the increased 5-HT, and that there is no lack of breakdown of 5-HT to account for the increased serum 5-HT. Other potential sources of elevated serum 5-HT include enterochromaffin (EC) cells of the gut and pulmonary

neuroendocrine cells (PNEC) and neuroepithelial bodies (NEBs) of the lung. Greater than 90% of the peripheral 5-HT is produced in the gut EC cells, and these cells are known to increase their secretion of 5-HT under various conditions, including hypoxia (35) and infection (36). PNEC and NEBs cells of the lung function as airway oxygen/carbon dioxide sensors and release 5-HT in a dose-dependent manner in response to hypoxia (37, 38). Although there have been no reports in SIDS of increased 5-HT production from EC cells of the gut, hyperplasia and hypertrophy of PNEC/NEB within the lungs of infants dying of SIDS have been reported, and secretory products of these PNEC/NEB cells have been proposed previously as a potential biological marker of SIDS (38). The relationship in SIDS between increased serum 5-HT and gut/lung synthesis and release of 5-HT requires further research.

There are incomplete data on the maternal use of selective serotonin reuptake inhibitors (SSRIs) in our cohort. In any event, although gestational exposure to SSRIs appears to substantially reduce serum 5-HT in cord blood (39), physiologically meaningful SSRI exposure in infants through breastfeeding by mothers receiving SSRIs is probably infrequent (40–42). Thus, previous studies of maternal SSRI treatment in breastfeeding mother–infant pairs have demonstrated (*i*) elevated levels of SSRI and major metabolites in the maternal plasma, but SSRI and major metabolites at or below the lower limit of quantitation in paired breastfed infants; and (*ii*) maternal SSRI use significantly decreases serum 5-HT levels in the mother, but does not affect serum 5-HT levels in paired breastfed infants (41, 42). In this study, there were no significant differences in serum 5-HT concentrations between (*i*) control cases who were breastfed vs. control cases who were not breastfed or (*ii*) SIDS cases who were breastfed vs. SIDS cases who were not breastfed. Thus, 5-HT in breast milk does not account for the differences in serum 5-HT observed between SIDS cases and controls.

A potential limitation of this study concerns the effects of postmortem interval, postmortem delay in serum separation, and lengthy storage times on the stability of the serum 5-HT. We did not, however, observe an effect of either postmortem interval or the time of serum storage on the levels of serum 5-HT as measured by two independent assays (ELISA and HPLC) (Fig. 1 *C* and *D*), supporting the relative stability of the monoamine in postmortem samples stored under appropriate conditions. Likewise, we did not see an effect of delayed serum separation, albeit necessarily modeled using adult donor serum. A second limitation to this study is the relatively small number of control samples, a problem reflected in any postmortem infant study due to the difficulty in obtaining control tissue. Although all of our control infants died of definable acute disorders, the actual causes of death varied. Despite this variation among the controls, we were able to define a significant subset of SIDS cases with high serum 5-HT levels.

The presently reported 5-HT concentrations in healthy, living infants in the first year of life are data that are not readily available in the literature. Compared with postmortem infant SIDS and postmortem infant control samples, living infant controls had higher concentrations of serum 5-HT and lower concentrations of 5-HIAA. Likely explanations for the higher 5-HT and lower 5-HIAA in sera from living subjects compared with postmortem infants include that (*i*) the lower platelet counts in postmortem samples compared with living controls results in less 5-HT

available to be released from platelet dense granules into the serum, (ii) there is less catabolism of 5-HT to 5-HIAA in living subjects than occurs after death, and (iii) there are differences in plasma clearance rates of 5-HT and 5-HIAA. Because SIDS is, by definition, the sudden, unexplained death of an infant, we were unable to obtain serum samples from living infants who would subsequently die of SIDS, as there is currently no biomarker, biochemical or otherwise, to identify them. The present data do not therefore allow us to confirm that 5-HT levels are higher in living infants who will subsequently develop SIDS than in living infants who will not subsequently develop SIDS. To address this question, a large, long-term, prospective population study needs to be performed. Importantly, however, the present study does allow us to conclude that serum 5-HT is elevated in SIDS infants at postmortem, because we have the appropriate controls: serum from infants at postmortem who died of non-SIDS causes.

In summary, the available evidence of central and peripheral abnormalities in the serotonergic system in SIDS infants provides essential information on a biological vulnerability in a subset of infants who die of SIDS. Further research will be necessary to validate, in an independent cohort, the present finding of increased serum 5-HT in SIDS and to determine the relationship or correlation between central and peripheral 5-HT abnormalities. A high serum 5-HT value may ultimately prove to be a peripheral biomarker of central 5-HT abnormalities in SIDS

cases, and its determination will be useful in identifying such SIDS infants, i.e., as an autopsy biomarker. Postmortem serum analysis of 5-HT by HPLC or ELISA is easier, less expensive, and more efficient than analyses of brain 5-HT neurochemistry, e.g., Western blotting and HPLC on micropunches of discrete and tiny brainstem nuclei. These techniques require frozen brain samples and oftentimes lengthy experiments (months for exposure) such as receptor ligand autoradiography. HPLC on Guthrie cards may be an option for future population screening, given that 5-HT has been reliably measured from dried blood spots (43). Further research aimed at understanding the association between the increased serum 5-HT reported here and the decreased brainstem 5-HT reported previously (14) will be essential for a deeper understanding of 5-HT abnormalities as a pathological mechanism underlying SIDS death.

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