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ORIGINAL ARTICLE

Nicotine and cotinine in infants dying from sudden infant death syndrome

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Abstract The aim of this component of the *German Study on Sudden Infant Death* was to determine (1) nicotine concentrations in hair (NCH), as a marker of long standing exposure to tobacco, (2) cotinine concentrations in pericardial fluid (CCP) and (3) cotinine concentrations in liquor cerebrospinalis (CCL), the latter measures being markers of recent exposure to tobacco in the last few hours of life. The results obtained were compared with data on parental smoking revealed from interviews. In 100 cases of sudden infant death syndrome, material was taken at autopsy to determine NCH. In 41 cases, NCH and CCP, and in 70 cases, NCH and CCL were determined. Infants of mothers who stated having smoked during pregnancy had higher NCH than infants of non-smoking mothers (p=0.008). Furthermore, there was a weak but statistically significant relationship between NCH's and the daily cigarette consumption of the mother during pregnancy (n=64, r=0.24, p=0.05). In 43% of infants, nicotine could be detected in their hair, although the mothers had said at the interview that they did not smoke during pregnancy. On the other hand, in 33% of infants whose mother stated they had smoked during pregnancy nicotine was not detectable in the infant's hair. CCP's were strongly correlated with CCL's (r=0.62, p=0.0027). For this reason, both parameters were treated as equivalent for the detection of tobacco smoke exposure in the last hours before death. The influence of breast-feeding was evaluated by comparison of the nicotine concentrations in breast fed and non-breast-fed infants from smokers and non-smokers. Fivefold higher nicotine concentrations were determined in non-breast-fed infants of parents who smoked as compared to all other groups. It can be concluded that nicotine intake by passive smoking is much more important than by breast-feeding. We conclude that both interview data and biochemical measures should be sought to understand the true exposure to tobacco smoke.

Keywords Sudden infant death syndrome (SIDS) · Smoking · Nicotine · Cotinine · Breast feeding

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Introduction

In Germany, sudden infant death syndrome (SIDS) is still the leading cause of death in infancy after the neonatal period. The incidence was 0.4/1,000 live births in the year 2004 [1]. In various studies as well as in the Westphalian cot death study [2, 3], smoking by the mother during pregnancy has been identified as a main risk factor for sudden infant death [4–8]. Furthermore, some authors have shown that the risk increased with increasing cigarette consumption [2, 9]. Information about smoking habits is usually obtained during the parent's interview performed days or weeks after the death or is obtained from obstetric records. As parents might be aware of the detrimental health effects of smoking, the answers given may not always have been correct.

In the German SIDS study (GeSID) [10], objective information about long standing exposure and recent exposure to nicotine were to be obtained by the determination of (1) nicotine concentrations in hair (NCH), as a marker of exposure over weeks, (2) cotinine concentrations in pericardial fluid (CCP) and (3) cotinine concentrations in liquor cerebrospinalis (CCL), the latter ones being markers of exposure over a few hours [11–15]. The data obtained were compared to the smoking habits of the parents as reported by interview. The validity of the interview data has to be evaluated.

Furthermore, we examined whether there was an association between tobacco smoke exposure and the frequency and severity of infectious diseases of the respiratory tract in SIDS cases found at the autopsy.

To study the possible significance of breast feeding as an alternative source of nicotine intake after birth, interview data concerning the infants feeding were evaluated.

Materials and methods

Cases

In 100 cases of SIDS from the GeSID study, samples were taken during autopsy to determine the NCH, CCP and CCL (Table 1). The primary selection criterion of these cases was the presence of head hair of sufficient quantity. A post-mortem investigation was performed using a standardised autopsy protocol including extended histology, toxicology, neuropathology, virology, microbiology and clinical chemistry [10, 16, 17].

Table 1 Characterisation of the SIDS cases and the number of samples analysed

Characterisation	Number of samples
Total number of subjects hair nicotine (NCH) analysed	100
Category 1 (without pathological findings)	10
Category 2 (with minor diseases/inflammatory changes)	72
Category 3 (more severe changes/ inflammation)	18
NCH and pericardial cotinine (CCP) analysed	41
NCH and cerebrospinal fluid cotinine (CCL) analysed	70
NCH and CCP and CCL analysed	12

Parents' interview

The questionnaire for the interview consisted of 106 questions dealing with all major topics of infant development including five questions regarding the cigarette consumption of the parents during pregnancy and after birth as well as five questions regarding the duration and intensity of breast feeding. From these data a *breast feeding index* (BFI) was defined (calculated by dividing the number of months the infants were breast-fed at least 3× per day by the age of the infants). The 100 SIDS cases for which NCH could be measured were subdivided into three groups depending on the BFI: infants who were not breast-fed (BFI=0), had little breast feeding ($0<BFI\leq0.5$) and were predominantly or exclusively breast-fed ($0.5<BFI\leq1$).

Chemical analyses

For the determination of NCH from hair, a minimum of 25–30 mg of hair from the head (back of the scalp) was carefully washed twice using dichloromethane to eliminate all external contaminants. After NaOH-lysis (1 ml of 0.5 M NaOH) and HCl-neutralisation (1 ml of 0.5 M HCl), an amount of 50 ng deuterated nicotine as internal standard (ISTD) and 3 ml of 0.01 M $(NH_4)_2CO_3$ buffer pH 9.3 were added. The mixture was transferred to preconditioned Chromabond C_{18} cartridges (Macherey and Nagel, Düren, Germany) for solid phase extraction. After several washing steps, nicotine was eluted with two aliquots of 0.5 ml methanol into vials, which contained 50 µl of 0.1 M HCl in 2-propanol. The combined eluates were lyophilised, reconstituted in 2-propanol and analysed by gas chromatography–mass spectrometry (GC–MS) in the selected ion monitoring (SIM) mode [18].

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For the determination of CCP and CCL, 0.5 ml of the samples were mixed with 50 ng of deuterated cotinine as ISTD and 3 ml of 0.01 M $(NH_4)_2CO_3$ buffer pH 9.3. Solid-phase extraction and GC–MS SIM were the same as described for nicotine [18]. The precision of the method was tested by repeated measurements using aliquots of the same sample. In particular, retesting was done in cases showing high concentrations of nicotine and cotinine and in those cases where these concentrations were near the detection limit (0.2 ng/mg for nicotine and 5 ng/g for cotinine).

Statistical method

Agreement between smoking during and after pregnancy was estimated using a kappa statistic. Distributions of NCH, CCL and CCP were compared between smokers and non-smokers using the non-parametric Wilcoxon test. Correlations of NCH, CCL and CCP were calculated using Spearman's rank correlation co-efficient.

Results and discussion

The GeSID study is a large population based study, which covered half of the population in Germany. Case ascertainment was extensive, as during the whole study period, a standardized protocol was used and extensive examinations were done in each case to define SIDS cases [10]. To our knowledge, the GeSID study is the first study to examine in such a high number interview data on maternal smoking and compare them to NCH, CCP and CCL.

Smoking behaviour

Mothers who reported smoking during pregnancy continued smoking after birth. The level of agreement was excellent (kappa=0.86).

CCP and CCL

The levels of CCP and CCL were strongly correlated (r=0.62, p=0.0027). Therefore, they were handled as equivalent (Fig. 1).

Nicotine concentrations in the hair

After intake, nicotine is rapidly oxidised into its main metabolite cotinine. Whereas the plasma half life of nicotine is in a range of 24 to 84 min with an average of 40 min, the half life of cotinine is in the range of 18 to 24 h [19, 20]. Nicotine is incorporated into the hair fibres by diffusion from the systemic circulation through the hair bulb, but the incorporation of cotinine is poor [21, 22]. For this reason, the determination of NCH from scalp hair is a suitable measure of long standing exposure to tobacco smoke [18, 23–26]. Growing hair reaches the scalp surface after approximately 3 weeks [27]. Unfortunately, no precise data are available on hair growth in babies.

There were 61 SIDS cases that were positive for NCH and 39 that were negative. In the 61 cases of NCH positive infants, the NCH ranged from 0.5 to 258.4 ng/mg (median, 5.5 ng/mg; mean, 16.3 ng/mg). The infants of mothers who said that they had smoked during pregnancy had higher NCH than those of non-smoking mothers. In addition, there was a weak but statistically significant relationship between NCH and the number of cigarettes smoked during pregnancy (Fig. 2, p=0.05).

However, we found inconsistencies that require further analysis and discussion. Firstly, there is an enormous inter-individual variation of the NCH, CCL and CCP for each reported amount of cigarettes smoked. Secondly, there are cases where reported heavy tobacco consumption was associated with zero concentrations (Fig. 2). Furthermore, there were cases in which reported exposure to tobacco was negative but showed high NCH.

Therefore, we have carried out further analyses to try and identify the reasons for these inconsistencies:

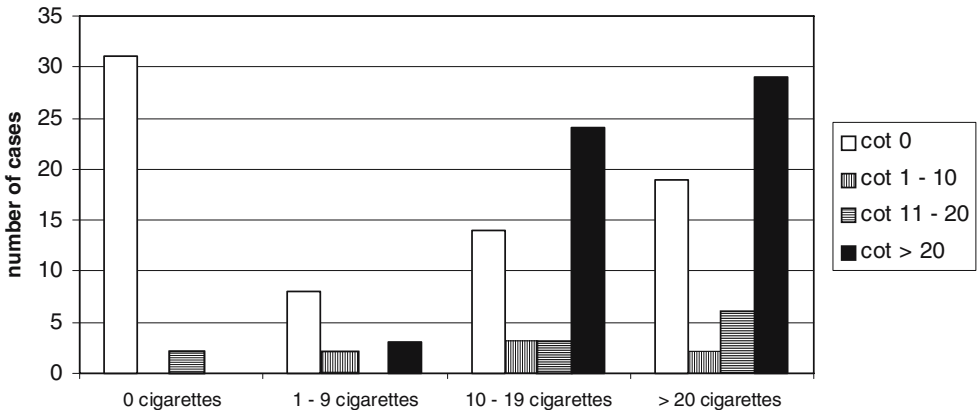

Fig. 1 Cotinine concentrations in pericardial fluid and/or cerebrospinal fluid and reported maternal smoking. *Cot* Range of cotinine concentration in ng/ml

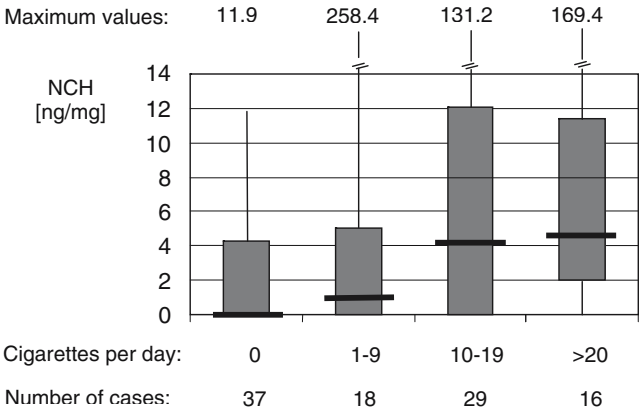

Fig. 2 Nicotine concentration in the hair (NCH) of SIDS cases (n=100) and reported cigarette consumption of the mother during pregnancy. The *boxes* give the 25–75 percentile. The medians are given by the *thick horizontal lines*

Reported smoking and chemical analysis

'Smoking' mothers Among the infants of 'smoking' mothers, one subgroup needs special attention: One third (33%, 22 of 67 cases) of the infants of mothers who reported smoking had no detectable NCH, whereas in 3 out of these cases, CCP and/or CCL were positive. The mothers of the latter babies were heavy smokers (more than ten cigarettes per day).

Thus, there are 19 cases where mothers reported that they smoked, but analytical data was negative. Possible explanations include (1) not smoking in the presence of the infants (in 9 out of the 19 cases, the mother reported that she had not smoked in the presence of the infant); (2) the fact that, in the remaining 10 cases, 6 were light smokers (<10 cigarettes); (3) environmental factors such as room size, ventilation, seasonal influences and opened windows; and (4) the nicotine content of cigarettes, which varies widely.

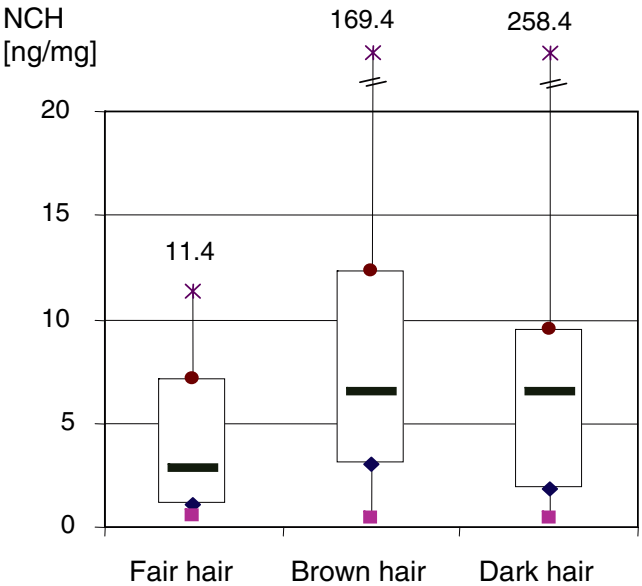

Fig. 3 Hair nicotine concentrations (NCH) and hair colour of NCH positive infants (n=61). The *boxes* give the 25–75 percentile. The medians are given by the *thick horizontal lines*

Table 2 NCH and age distribution in breast-fed and not-breast-fed infants in the SIDS cases that were NCH positive

	NCH positive (n=61)	
	Breast fed (n=28)	Not breast fed (n=33)
NCH		
Range	0.6–11.9 ng/mg	0.5–258.4 ng/mg
Arithmetic mean	4.5 ng/mg	25.9 ng/mg
Geometric mean	3.4 ng/mg	7.5 ng/mg
Age (days)		
Range	20–347	15–307
Arithmetic mean	119	105
Geometric mean	97	95

'Non-smoking' mothers There were 37 mothers who reported that they did not smoke during pregnancy, and 33 of these mothers were not smoking after delivery. Amongst their infants, 16 were NCH positive. The nicotine concentration of these infants ranged between 0.5 and 11.9 ng/mg with an arithmetical mean of 5.1 ng/mg, which is similar to that of mothers who reported that they smoked. Of these 16 infants, 8 were exposed to environmental tobacco smoke by fathers who smoked. This left 8 infants with positive NCH in which both parents said they were non-smokers. One can only speculate about the possible reasons. Possibly parents are denying their smoking behaviour or the infant may be exposed to another source, e.g. grandparents.

Of the 100 SIDS cases, 64 infants were chemically exposed to nicotine and 36 infants were not exposed. At interview, mothers of 67 infants reported smoking during pregnancy, and 33 were nonsmokers. Although the proportions are similar, there are inconsistencies between the reported smoking behaviour and the biochemical data. We conclude that both interview and biochemical evidence

Table 3 NCH in infants without histological signs of air way infections and in infants with histological signs of air way infections

	No RTI n=22	RTI n=78
NCH negative cases	9 (41%)	30 (38%)
NCH positive cases	13 (59%)	58 (62%)
Range NCH positive cases	0.5–22.3 ng/mg	0.5–258.4 ng/mg
Arithmetic mean NCH positive cases	6.0 ng/mg	18.8 ng/mg
Geometric mean NCH positive cases	3.3 ng/mg	6.4 ng/mg

RTI Respiratory tract infection

should be sought to estimate the true exposure to tobacco smoke.

NCH positive infants and hair colour

Nicotine concentration has been shown to be higher in pigmented than in unpigmented hair. NCH in brown- and dark-haired infants were similar and were higher than those of fair-haired infants. However, these differences were not significant (Fig 3; $\chi^2=2.84$, $p=0.2423$).

Reported breast feeding and NCH

In addition to the nicotine intake by inhalation of tobacco smoke, an alternative route of intake is via breast-feeding [28, 29]. For this reason, the reported breast feeding behaviour was analysed. About one half of the infants were not breast-fed (BFI=0). The mean BFI, as well as the age distribution within the groups, did not differ significantly.

Amongst the 61 NCH positive infants, about one half was partially or totally breast-fed ($n=28$, BFI>0) and the other half was not breast-fed ($n=33$, BFI=0). The breast-fed children in this group had a lower mean NCH compared to the non-breast-fed children (Table 2). Looking at breast feeding and maternal smoking, we found 26 infants with a range of NCH from 0–9.5 ng/mg ($\bar{x} = 2.8$ ng/mg), whereas in the group of breast fed infants ($n=22$), whose mothers did not smoke after the delivery, we found a mean of 2.4 ng/mg (range 0–11.9 ng/mg). Breast-feeding is not a major source of nicotine intake in infants, which were exposed to tobacco smoke in our study. However, breast feeding and nicotine exposure are not necessarily independent variables [30, 31].

Respiratory tract infection and NCH

To study the influence of exposure to tobacco smoke on air way infections, we have subdivided the whole study sample into the following:

1. $n=22$ as a negative control composed out of SIDS cat. 1–3 without histological signs of air way infections and without preceding fever and
2. $n=78$ infants as test group composed out of SIDS cat. 2+3 infants, all of them with histological signs of airway infections not sufficient to explain the death [10].

The percentage of NCH negative infants is about the same in both groups (41 vs 38% respectively), and the mean levels did not differ statistically (Table 3, Wilcoxon two-sample test, $p=0.57$). Other investigators have reported that children of smoking parents have a higher rate of bronchitis, childhood asthma and otitis media [32, 33]. However, in this study among children dying from SIDS, maternal smoking was not associated with an increased frequency of respiratory infections.

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