

Laryngeal Inflammation in the Sudden Infant Death Syndrome

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Abstract: *Objective:* Sudden infant death syndrome (SIDS) is marked by 'the sudden death of an infant that is unexpected by history and remains unexplained after a thorough forensic autopsy and a detailed death scene investigation'. The cause is unknown.

Excessive subglottic submucosal glandular tissue and excessive sulphated mucus glycoprotein in the larynges of SIDS babies have been previously reported from our institution. We now report on laryngeal immunohistology.

Methods: Larynges from 7 children who died from Sudden Infant Death Syndrome (SIDS) at under 16 weeks of age were examined immunohistologically and compared to those from 8 age- matched control infants who died from other causes.

Results: The SIDS babies had increased inflammatory changes in the laryngeal epithelium and sub- epithelium with raised numbers of cells staining for elastase ($p < 0.01$), EG2 (a marker for activated eosinophils) ($p < 0.01$) and CD4 ($p < 0.05$) suggesting that some SIDS deaths involve preceding inflammation.

Conclusions: Although death may be sudden and unexpected it appears that, at least in some SIDS victims, there is a preceding inflammatory process in the larynx which may allow hyper-reactivity of laryngeal reflexes and consequent apnoea. This observation concurs with others in the SIDS literature and offers a field for further research and possible prevention.

Keywords: Eosinophils, hyper- reactivity, inflammation, larynx, neutrophils, sudden infant death syndrome (SIDS).

INTRODUCTION

The cause of SIDS is unknown but it is regarded as multifactorial in origin [1]. The lack of definitive, easily identifiable postmortem marker(s) for SIDS complicates investigation of its aetiology [2]. Risk factors, such as infants lying prone to sleep, have been identified, however the reason(s) behind them are unknown. The incidence of SIDS correlates with the sex and age of the infant, and also with race, and with parental education and socio-economic status.

SIDS cases peak between two to four months after birth, when infant antibody levels are low since maternal immunoglobulins are waning and their own production is not yet fully established.

Inflammatory changes in the respiratory and digestive tracts, nervous system, and blood have been reported in SIDS [1]. Frothy, mucoid, sometimes blood- stained or nasal secretions are more common in SIDS cases [3].

At the Royal National Throat Nose and Ear Hospital a series of post-mortem larynges were obtained in the 1990s by the late Professor DN Harrison from infant fatalities: those due to SIDS and also from age -matched children dying from other causes, predominantly cardiac defects. He showed that the available airway had reduced by more than half in 35 per cent of the SIDS larynges within the two to four month age group due to excessive subglottic, submucosal glandular tissue [4] In a third of this group

the airway was reduced by over 60 per cent. Hyperplasia of subglottic mucous glands was proposed as a cause of fatal hypoxia [4].

Larynges from 24 of these SIDS victims, aged from two to 4 months, and 10 controls, aged from two days to 24 weeks, were available for further study of mucus glycoproteins: acid, neutral and mixed [5]. The results suggested that excess sulphated mucus glycoprotein was secreted in some SIDS victims [5]. The significance of this is unknown, but in rat noses similar changes follow stimulation with lipopolysaccharide (a bacterial component) [6]. In the gut also mucus composition and the microbiome are related [7]. Muco-ciliary clearance, vital for airways health, may be adversely affected by alterations in mucus [8].

Other investigators have found laryngeal abnormalities in SIDS. An increase in laryngeal mucosal glands was found [9]. Basement membrane thickening of the vocal cords was noted by Shatz [10], although not by others [11, 12]. More recently SIDS infants with high IL-6 levels in CSF (suggestive of infection) had higher laryngeal IgA immunocytes and HLA-DR expression (also suggesting a response to infective stimuli) than SIDS infants with low/ normal IL-6 CSF levels [13].

The advance in immunohistological methods has allowed us to re-visit the remaining larynges from the Harrison collection for further information on laryngeal changes in the 2-4 month age group. We have examined inflammatory cells using standard techniques in sections from a series of 7 larynges from SIDS fatalities and have compared them with those from 8 babies of a similar age who died of cardiac conditions.

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METHODS

Subjects

Larynges from infants dying with a diagnosis of SIDS and from those dying at similar ages from other causes, predominantly cardiac, were obtained by Professor Harrison as previously described [4]. The subjects in this paper represent the remaining specimens from that series which were in the 2-4 month age group. The Royal Free Hospital Ethics Committee approved their use.

Immunohistochemistry

Serial sections of larynges from 7 SIDS victims were stained for elastase (a neutrophil constituent), EG2 (a marker for activated eosinophils), CD68 (a macrophage marker) and CD4 (a marker for helper T lymphocytes). They were compared with sections of 8 larynges from age-matched control infants dying from causes other than SIDS.

Sections were deparaffinated in xylene, dehydrated in ethanol, and washed in PBS. Antigen Retrieval was performed using Citrate Buffer pH6 (for CD68 and CD4 only). Successive permeabilization steps of 30 minutes each (with Triton 0.2% in PBS) and hydrogen peroxide (5% in PBS) were performed. The sections were then washed in PBS three times for 5 minutes before incubation for 30 minutes with a universal blocking solution (Dako Cytomation, MN). Tissue sections were incubated overnight at 4°C with mouse monoclonal IgG antibodies against Elastase, EG2, CD4 or

CD68. The secondary antibody, biotinylated rabbit anti mouse, was applied at a concentration of 1/100, followed by incubation with SA-HRP (Vector Laboratories, Burlington, ON). The immunostainings were developed using the Liquid DAB+ Substrate Chromogen System (Dako) according to the manufacturer's instructions and counterstained with hematoxylin and lithium carbonate. The number of immune cells positive for Elastase, EG2, CD68 or CD4 were quantified and expressed as the number of positive immune cells per square millimetre of subepithelium by an observer blind to the coding of the specimen.

RESULTS (FIGURE 1)

The SIDS larynges, compared to the controls, showed increased numbers of immunoreactive cells when stained for elastase ($p < 0.01$), EG2 ($p < 0.01$) and CD4 ($p < 0.05$), (Mann Whitney U test). These represent neutrophils, eosinophils and helper T lymphocytes respectively.

There was no significant difference in CD68 +ve cells (macrophages).

The inflammatory cells were found in the epithelial and sub-epithelial layers of the larynx.

INTERPRETATION

The larynx has previously been suggested as the shock organ in SIDS [4, 13].

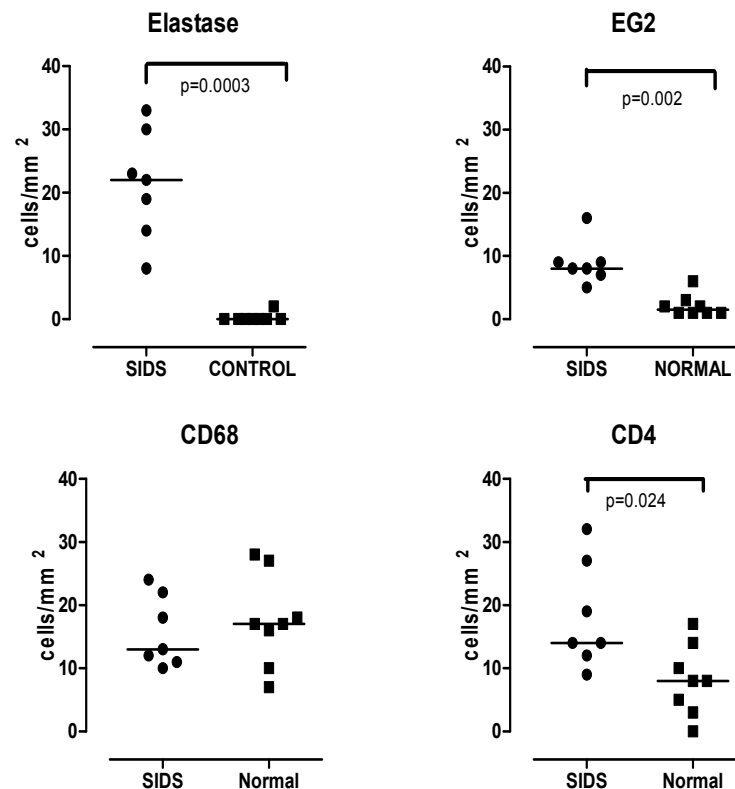


Fig. (1). Serial sections of larynges from 7 SIDS victims were stained for elastase, EG2, CD68 and CD4 to identify neutrophils, eosinophils, granulocytes and helper T cells respectively. They were compared with sections of 8 larynges from age-matched control infants dying from causes other than SIDS. The SIDS babies had increased inflammatory changes in the laryngeal epithelium and sub-epithelium with raised numbers of cells staining for elastase ($p < 0.01$), EG2 ($p < 0.01$) and CD4 ($p < 0.05$); there was no difference in CD68 cells (Mann Whitney U test).

Laryngeal inflammation - both neutrophilic and eosinophilic - appears to be involved in SIDS pathogenesis in the under 16 week deaths. A chronic process may manifest in the larynx prior to a sudden fatal outcome. The histology is similar to that of chronic severe asthma.

The epithelial and sub-epithelial location suggest a luminal origin of inflammation which could be related to infection, pollution, reflux, or a combination of these. Laryngeal involvement fits with many known aspects of the syndrome.

1. Position

The switch to lying on the back to sleep has reduced SIDS deaths, particularly in the 2-4 month age group with infections [14]. In the prone position the oesophageal inlet is above the larynx and reflux of gastric contents into it is more likely.

2. Pollution

Exposure to second hand smoke is connected to SIDS [15]. Higher concentrations of nicotine and cotinine (a biological marker for second hand smoke exposure) are found in body fluids from infants who die from SIDS compared to those who die from other causes [16].

Levels of gaseous air pollutants, e.g. carbon monoxide, sulphur dioxide, nitrogen dioxide, and hydrocarbons and peak in the winter, as do SIDS deaths. NO₂, which is a product of automobile exhaust and tobacco smoke, is related to SIDS: acute high NO₂ exposure in the last day of life showed an OR = 2.43(95% CI 1.13 to 4.87), after adjusting for tobacco smoke exposure [17].

3. Chronic Hypoxia

Brain stem astrogliosis found in half of SIDS infants probably relates to previous episodes of hypoxia [18], which is also suggested by 20% more pulmonary artery muscle and, increased haemoglobin and erythropoietin [19]. This suggests a chronic process rather than a sudden acute death. In another study [20] higher levels of vascular endothelial growth factor (VEGF) in cerebrospinal fluid were found in 51 SIDS infants compared to 33 control infants who died from known causes, again suggesting that hypoxia frequently precedes death from SIDS.

4. Immunity and Infection

Most SIDS deaths occur between 2 - 4 months of age when maternally-acquired immunoglobulin G is low, as is the infant's own immunoglobulin G production. Breast feeding reduces the risk of SIDS by approximately 50% [21], possibly via protective cytokines and immunoglobulins. Minor symptoms of infection, often in the respiratory tract, are present in half of SIDS cases in the days preceding death, although probably insufficient alone to have caused death [22, 23].

Hyperstimulation of the immune system has been reported in SIDS victims with HLA-DR and secretory component upregulated in salivary glands [24] and changes in immunoglobulins in palatine tonsils [25]. Increased macro-

phages, eosinophils, and T and B lymphocytes have been reported in lungs and in tracheal infiltrates [26].

Associations with polymorphisms of IL-10, a regulatory cytokine, which could reduce control of the inflammatory response, have been noted [27, 28]. HLA-DR up-regulation in the laryngeal mucosal glands and surface epithelium was related to IL-6 elevation in cerebrospinal fluid, which is a sign of infection (12) and may correlate with genetic polymorphisms of IL-6 and IL-8 relevant to immunity [29].

Blackwell and colleagues postulated an uncontrolled inflammatory reaction to infectious agents (especially pyrogenic toxins of *Staphylococcus aureus* which is isolated from the respiratory tract of 56% of healthy infants, but 86% of SIDS infants) as a cause of some SIDS deaths, possibly augmented by cigarette smoke [30, 31].

Our data would support this and suggest that SIDS in some babies is not a single acute event, but involves chronic airway-compromising inflammation in the larynx which may be multi-factorial in its induction. The final event may be acid reflux stimulating a laryngeal reflex resulting in apnoea [32]. It is known that in some conditions the contact between refluxate and the larynx can trigger several reflexes leading to cardio-respiratory inhibition [33].

Brain centres coordinating breathing and swallowing were poorly understood, but research [34] has elucidated the two CNS mechanisms, demonstrating how they co-function in the presence of an irritant. When food or water enters the larynx by mistake a protective reflex brings the vocal cords together and initiates coughing and swallowing. This is vital to life, especially in babies, as they commonly regurgitate and saliva pools in their throats. In adults the temporary closing of the airway is a small compromise as breathing is only briefly stopped. In babies the response has more radical implications, especially if breathing stops for a long time, as they cannot take in oxygen or get rid of carbon dioxide. The exquisitely co-ordinated timing of breathing and swallowing may be awry in SIDS, where abnormal autonomic control and arousal responsiveness are implicated (1) especially if recurrent reflux is causing laryngeal inflammation and hyper-reactivity.

The concept of inflammation leading to hyper-activation of reflexes via mediator-induced stimulation of nerve endings is well known in the respiratory tract. Examples include nasal and bronchial hyper-reactivity caused by allergic rhinitis and reduced by topical nasal corticosteroid [35], and similar events in the asthmatic airway [36].

The strength of this study is the rare ability to compare infant larynges from SIDS victims with age-matched controls using contemporary immunological markers. The major weakness is the small numbers involved, however the changes shown are of sufficient magnitude to suggest clinical relevance as well as statistical significance.

This observation alters the concept of SIDS - which in some infants appears to be the end stage of a chronic process (Fig. 2). It opens a new field for further research and possible prevention. Future work could include non-invasive laryngeal assessment by vocal changes, MRI and measurement of eNO and other inflammatory markers in exhaled breath in infants with near death experiences, and

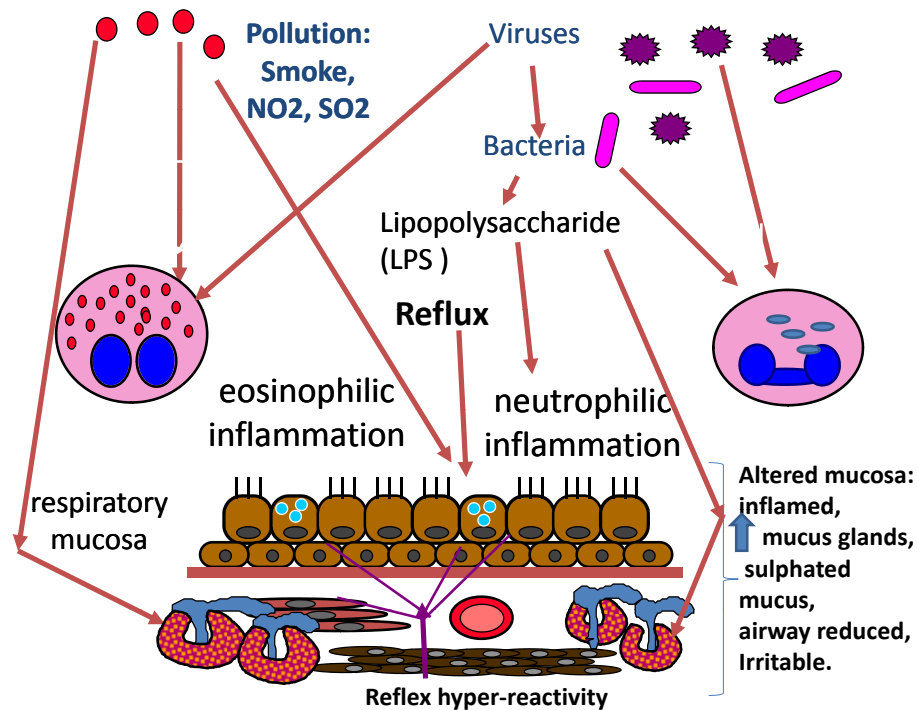


Fig. (2). Hypothesis of SIDS causation in 2-4 month old infants. The laryngeal epithelium responds to repeated stimuli such as infection, pollution, and reflux of gastric contents by inflammation allied to an increase in mucosal glands which reduce the airway. Additionally changes in mucus composition, with an increase of sulphated mucopolysaccharides, might reduce mucociliary clearance. Inflammation also leads to hyper stimulation of reflexes via irritation of nerve endings by mediators such as histamine. The final event could be reflux of gastric contents which elicits a disproportionately large and prolonged fatal response due to hyper-reflexia and airway compromise.

in those with a familial predisposition to SIDS. The use of topical inhaled corticosteroid could prove beneficial in at risk infants.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

Declared none.

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