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INTRODUCTION

Erythrocytes containing haemoglobin S (HbsS) experience chronic redox imbalance from increased production of hemichromes and therefore reactive oxygen species (ROS). The associated haemolysis contributes to many of the pathophysiological pathways in sickle cell anaemia (SCA), potentially mediated by oxidant stress, decreased nitric oxide bioavailability, inflammation and hypoxia. The compromise of endothelial function may be exacerbated by intermittent nocturnal hypoxia (1) associated with obstructive sleep apnoea (OSA), common in SCA (2). The delta 12 s index (delta12 s), the absolute difference in haemoglobin oxygen saturation (SpO2) between successive 12-s intervals, measures baseline SpO2 variability. In adults in the general population, delta12 s values of >0.4 predict an apnoea/hypopnoea index (AHI) of >15 with 88% specificity and 70% sensitivity (3). In 71 children with sickle cell disease enrolled in the Sleep Asthma cohort (4), using the same cut-offs for delta12 s and AHI, specificity and sensitivity were 100% and 89%, respectively (Gavlak et al., unpublished).

OSA is associated with oxidative stress and endothelial dysfunction (5). Supplemental vitamin C improves endothelial function in OSA in adults in the general population (6). Dysfunctional endothelium in OSA in adults in the general population, delta12 s values of >0.4 predict an apnoea/hypopnoea index (AHI) of >15 with 88% specificity and 70% sensitivity (3). In 71 children with sickle cell disease enrolled in the Sleep Asthma cohort (4), using the same cut-offs for delta12 s and AHI, specificity and sensitivity were 100% and 89%, respectively (Gavlak et al., unpublished).

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cohort and sibling controls; those with SCA also had steady-state vitamin C levels measured.

**METHODS**

Ethical permission was granted by the Muhimibili University of Health & Allied Sciences ethics committee (Ref: MU/RP/AECNoI.XII/77). Children were recruited from confirmed HbSS patients enrolled in a cohort study at Muhimibili National Hospital, Dar-es-Salaam, from April to July 2009 and their siblings. They were not selected as having sleep or breathing problems. Informed consent was obtained from parents of the children; where appropriate, assent was obtained from children themselves. Pulse oximetry was sampled in the day at rest and over a single night using a 2-s averaging time and 1 Hz sampling rate (Masimo Radical, Irvine, CA, USA). Data analysis was performed with Download 2001 software (Stowood Scientific, Oxford, UK). Poor perfusion, low signal IQ and movement artefact data were rejected. Analysis software yielded standard measures including mean and minimum SpO2, delta12 s and desaturation index of 3% or greater from baseline. Analyses of artefact-free recordings were conducted and data were compared between children with SCA and their siblings using the independent t-test for normally distributed data or the non-parametric Mann–Whitney U-test. Steady-state vitamin C concentrations were measured using a fluorometric method by Human Nutrition Research, Cambridge, UK, in plasma samples separated and stabilized within 2 h of collection with metaphosphoric acid.

In the children with SCA, associations between logarithmically transformed vitamin C concentrations and oximetry variables were assessed using linear regression and by logistic regression of VCD and binary oximetry data. All oximetry variables were tested for associations with the potential covariates: age, sex, body mass index (BMI-z-score) or steady-state haemoglobin. Vitamin C concentration also decreased with higher numbers of episodes of SpO2 desaturations >3%/h (6.2% decrease [95% CI –11.8%/~2.5%], p = 0.042). There were no associations with vitamin C and the other oximetry variables. A high delta12 s (>0.4) was significantly associated with an odds ratio for VCD of nearly nine times greater (Table 2).

**RESULTS**

Eighteen control siblings, six boys, median age 7 (range 2–12) years, underwent overnight pulse oximetry, as did 23 children with SCA, 15 boys, median age 7.8 (range 2.9–15.1) years, who had had steady-state vitamin C concentrations measured prior to the sleep study. Ethics was not granted for venepuncture in the controls.

Descriptive statistics for haemoglobin and pulse oximetry data in controls and children with SCA and for steady-state vitamin C in those with SCA are given in Table 1. Daytime haemoglobin oxygen saturation was lower in the children with SCA than in the controls and there was a trend for lower mean nocturnal haemoglobin oxygen saturation but there was no difference between children with SCA and controls in sleep duration, minimum overnight SpO2, number of overnight SpO2 dips >3%/hr and the delta 12 s index (Table 1).

Forty-eight per cent (11/23) of children with SCA had VCD (<11.4 μmol/L, a similar proportion to all patients with SCA with data available (58%; 463/799) but higher than the proportion in a historical group of Tanzanian control children (32%, 24/74) (Cox et al., unpublished data). There was no association between vitamin C and age, sex, nutritional status (BMI-z-score) or steady-state haemoglobin in the children with SCA.

In the children with SCA, geometric mean vitamin C decreased by 66% per 0.1 unit increase in delta12 s ([95% CI –86%/–15%] p = 0.023) but delta12 s was not associated with duration of sleep, age, sex, BMI-z-score or steady-state haemoglobin. Vitamin C concentration also decreased with higher numbers of episodes of SpO2 desaturations >3%/h (6.2% decrease [95% CI –11.8%/~2.5%], p = 0.042). There were no associations with vitamin C and the other oximetry variables. A high delta12 s (>0.4) was significantly associated with an odds ratio for VCD of nearly nine times greater (Table 2).

**DISCUSSION**

In line with previous data (1), our study reports lower daytime and mean nocturnal haemoglobin oxygen saturation in children with SCA than in controls, although the latter did not reach statistical significance. OSA is commoner in black children, but the limits acceptable as within the normal range have not been defined in this population (8). There are few data comparing measures of desaturation and OSA

### Table 1: Vitamin C, steady-state haemoglobin and pulse oximetry data in 23 children with SCA and 18 control siblings

<table>
<thead>
<tr>
<th>Variable</th>
<th>Children with SCA</th>
<th>Control siblings</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median steady-state plasma vitamin C μmol/L</td>
<td>12.5 (IQ range 3.1–24.1)</td>
<td>98.6 (SD 1.95) (range 93–100)</td>
<td>0.005</td>
</tr>
<tr>
<td>Mean steady-state haemoglobin* (g/dL)</td>
<td>7.7 (SD 1.16) (range 5.8–10.9)</td>
<td>9.37 (IQ range 5.98–11.06)</td>
<td>0.8</td>
</tr>
<tr>
<td>Mean daytime SpO2 (%)</td>
<td>96.0 (SD 3.68) (range 85–100)</td>
<td>97.9 (SD 2.0) (range 91.4–99.9)</td>
<td>0.1</td>
</tr>
<tr>
<td>Median recorded study duration (h)</td>
<td>9.47 (IQ range 7.78–10.82)</td>
<td>85.9 (SD 9.1) (range 63–95)</td>
<td>0.8</td>
</tr>
<tr>
<td>Mean overnight SpO2 (%)</td>
<td>96.3 (SD 4.06) (range 77.9–100)</td>
<td>97.9 (SD 2.0) (range 91.4–99.9)</td>
<td>0.1</td>
</tr>
<tr>
<td>Mean minimum overnight SpO2 (%)</td>
<td>85.3 (SD 9.3) (range 65–95)</td>
<td>85.9 (SD 9.1) (range 63–95)</td>
<td>0.8</td>
</tr>
<tr>
<td>Median delta 12 s Index</td>
<td>0.38 (IQ range 0.28–0.51)</td>
<td>0.35 (IQ range 0.23–0.48)</td>
<td>0.25</td>
</tr>
<tr>
<td>Median number of overnight SpO2 dips &gt;3%/h</td>
<td>5.19 (IQ range 1.09–7.64)</td>
<td>2.44 (IQ range 1.11–6.16)</td>
<td>0.4</td>
</tr>
</tbody>
</table>

*Mean of multiple measurements made at between 3 and 15 routine steady-state (no fever, reported pain, malaria parasites or antigens, or admission within 90-day period) routine clinic visits preceding sleep study.

SpO2 = haemoglobin oxygen saturation; IQ range = interquartile range.
between children with SCA and ethnically matched controls, although in one study, mean and minimum overnight SpO2 of <95.8 and <80%, respectively, were not seen in 50 controls, half of whom were siblings (2). Interestingly, in our data, 2 and 3 controls, respectively, had values below the mean and minimum overnight SpO2 in Samuels’ study of children living in England (2). In addition, there was no difference between children with SCA and sibling controls in study duration, minimum overnight SpO2, the number of SpO2 dips >3% and delta12 s, a measure of the variability of SpO2 predictive of OSA in adults in the general population (300 mg)

Table 2 Logistic regression between abnormal oximetry measures and steady-state vitamin C deficiency, N=23

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vitamin C sufficient (%)</th>
<th>Vitamin C deficient (%)</th>
<th>OR [95% CI], p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean daytime SpO2 &lt;96%</td>
<td>3/12 (25)</td>
<td>6/11 (54.6)</td>
<td>3.60 [0.62 – 21.03], p = 0.155</td>
</tr>
<tr>
<td>Mean overnight SpO2 &lt;94%</td>
<td>2/12 (16.7)</td>
<td>2/11 (18.2)</td>
<td>1.11 [0.13 – 9.61], p = 0.92</td>
</tr>
<tr>
<td>Delta 12 s Index&gt;0.4</td>
<td>2/12 (16.7)</td>
<td>7/11 (63.7)</td>
<td>8.75 [1.24 – 61.7], p = 0.029</td>
</tr>
<tr>
<td>Overnight SpO2 dips (&gt; 3%) &gt;4/h</td>
<td>5/12 (41.7)</td>
<td>8/11 (72.7)</td>
<td>3.77 [0.65 – 21.6], p = 0.14</td>
</tr>
</tbody>
</table>

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References


