Sensorineural hearing loss in children with sickle cell anemia and its association with endothelial dysfunction

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ABSTRACT

Objectives: To investigate the prevalence of sensorineural hearing loss (SNHL) in children and adolescents with sickle cell anemia (SCA) and its association with endothelial dysfunction (ED).

Methods: Fifty-two participants with stable SCA and 44 apparently healthy (AA genotype) participants aged 6–18 years were evaluated for pure tone audiometry and endothelial function using ultrasonographic imaging of the brachial artery to assess flow-mediated dilation (FMD). Laboratory analysis of the lipid profile and C-reactive protein levels was performed.

Results: In the SCA group, 15 (28.8%) patients presented with SNHL. The FMD values were reduced in the SCA with SNHL group compared with the SCA without SNHL and healthy groups. Logistic regression analysis showed that FMD was associated with SNHL independent of the lipid profile and SCA characteristics (odds ratio [95% confidence interval] = 0.614 [0.440–0.858]; p = 0.004).

Discussion: SNHL is a common complication in SCA; furthermore, this study identified a significant association between ED and SNHL. Damage to the vascular endothelium because of inflammation in SCA reduced blood flow in the inner ear. Thus, this circulatory disorder culminates in vaso-occlusive process and induces auditory disorders, such as SNHL.

Introduction

Sickle cell anemia (SCA) is the most common hemoglobinopathy, resulting from the homozygous presence of altered hemoglobin (HbS) [1]. Among the clinical manifestations of SCA, vasoconstriction is the most frequent, occurring in multisystemic vessels including the auditory system [2–5]. The hearing loss typically found in SCA is sensorineural, with great variability in the characteristics and prevalence described in the literature [6–13].

The most accepted etiopathogenesis of sensorineural hearing loss (SNHL) involves sickle-shaped blood cells that lead to decreased blood flow in the cochlear venous system with a consequent chronic deficiency in oxygen balance leading to Corti’s Organ hypoxia and death of the outer hair cell (OHC) [14].

Studies have reported the influence of the vascular endothelium in the formation of cell clusters that interact with the vessel wall, culminating in vaso-occlusive processes [15,16]. Reduced flow-mediated vasodilatation (FMD) is considered an early marker of vascular alterations in sickle cell disease (SCD) patients [17]. The relationship between endothelial dysfunction (ED) and SNHL is documented in sudden SNHL and idiopathic SNHL, supporting the presence of vascular damage [18–20]. Our exploratory study in children and adolescents with SCA showed an association between otoneurological symptoms (vertigo) and decreased FMD of the brachial artery, indicating the presence of ED [21].

Taking into account the chronic inflammatory state of SCA and the presence of ED in SCA, the objective of this study was to investigate the prevalence of SNHL in children and adolescents with SCA and its association with ED.

Methods

Selection and description of participants

This cross-sectional study assessed children and adolescents ≥6 and ≤18 years of age, of both sexes. The sample included 52 participants with SS hemoglobinopathy (SCA group) diagnosed using hemoglobin electrophoresis, and 44 apparently healthy participants with standard AA hemoglobin (healthy control (HC) group). There were no differences in age and sex between the groups. Patients in the SCA group were from the University Hospital Complex Professor Edgar
Santos of the Federal University of Bahia (UFBA), and HC group participants were from the outpatient clinic of the School of Medicine of Bahia of the UFBA. Part of this sample had participated in our earlier study [21].

None of the participants had associated comorbidities, such as genetic syndromes, obesity, diabetes, hypercholesterolemia, stroke, neurological disease, heart disease, or lung disease. Participants with a history of meningitis, traumatic brain injury, or family history of hearing loss were also excluded. In addition, the criteria for non-inclusion in the SCA group included blood transfusion during the previous 90 days and history of an acute event in the previous 30 days.

All participants had normal otoscopy and middle ear findings, defined as the presence of tympanometric curve type ‘A’ [22] and a contralateral acoustic reflex threshold in at least one of the analyzed frequencies.

Written informed consent was obtained from the legal guardian and from each participant old enough to understand the study goals. This study was approved by the Research Ethics Committee of the Bahiana School of Medicine and Public Health (No.33705714.3.0000.5544).

The sample size was calculated to compare the proportions the SNHL prevalence between SCA group (27.32%) and controls (3.6%) [7,8,11–13]. Study power of 80% and an alpha error of 0.05. Sample size for each group was 34 participants. (WinPep*, statistical software. Abramson, licensee BioMed Central, London, UK).

Technical information

Demographic and clinical data were entered into a questionnaire conducted by the researchers. Clinical data were obtained from patient records, including hemoglobin and fetal hemoglobin levels, the presence and number of painful vaso-occlusive crises (VOC) in the last 12 months, the use and number of transfusions in the last 12 months, and the use and duration of hydroxyurea (HU) therapy. The physical examination included measurement of weight and height to calculate body mass index (BMI) (kg/m²).

The biochemical analyses and evaluation of endothelial function were performed at the cardiovascular research laboratory at the Bahiana School of Medicine and Public Health in Salvador, Brazil.

Endothelial function was evaluated using a protocol established under the guideline for the ultrasonographic evaluation of FMD with reactive hyperemia of the brachial artery [23–25] and already used in previous studies carried out by our research group [21,26]. Briefly, the moving images were scanned, beginning 30 seconds before the cuff was deflated until 2 minutes later, allowing for subsequent analysis. The FMD was calculated using the following formula: ((maximal arterial diameters-basal arterial diameters) × 100). An experienced vascular ultrasonographer, blinded to the patients’ diagnoses, obtained, and analyzed all images using a high-quality computer. ED was determined to be present if the FMD value was below the 10th percentile cutoff of the HC group [27,28].

Following a fast of at least 8 hours, blood samples were collected for biochemical analysis and complete blood count in untreated tubes and in ethylenediaminetetraacetic acid, respectively. The levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) were analyzed using enzymatic methods. High-sensitive C-reactive protein (hs-CRP) levels were analyzed using turbidimetry. Participants with TC > 170 mg/dL [29] and/or hs-CRP > 10 mg/L [30] were excluded from the analysis.

The tympanometry and analysis of the contralateral reflex threshold at 0.5, 1, 2, and 4 kHz were performed using a MADSEN® Ototest 100 (Otometrics*, Taastrup, Denmark) to investigate the normal middle ear.

Pure-tone audiometry test (PTA) was performed using an AD 229 Clinical audiometer (Interacoustic*, Middlefart, Denmark). The hearing thresholds (HT) were evaluated at frequencies of 250 Hz; 500 Hz; and 1, 2, 3, 4, 6, and 8 kHz, using a TDH-39 supra-aural earphone (Thelephonics*, New York, EUA). Bone thresholds were measured using a bone vibrator transducer at frequencies of 500 Hz and 1, 2, and 4 kHz when the air thresholds were ≥20 dB.

The criteria used to determine hearing loss were HT >20 dB in two or more frequencies in one or both ears, as described in previous studies [10,11,31]. The audiological tests were performed at the Speech Therapy Clinic of the State University of Bahia, Salvador, Brazil.

Statistical analysis

Quantitative variables were represented as means and standard deviations (SD) or as medians and interquartile ranges (IQR). Categorical variables were described using frequencies and proportions. Bivariate analysis was performed using Student’s t-test or Mann–Whitney U-test. Differences between proportions were calculated using Fisher’s exact or chi-square tests. Kruskal–Wallis correction for ranking comparison was used to compare variables with more than two categorized groups. Logistic regression analysis was conducted to evaluate the relationship between endothelial function and SNHL after adjusting for potential confounding factors. Data analyses were conducted using IBM SPSS Statistics for Windows, version 21.0 (IBM Corp., Armonk, NY, USA).

Results

Data from 96 children and adolescents (age range, 6–18 years) were analyzed. Of these, 52 had SCA and 44 were
Apparently healthy (12.3 ± 3.27 vs. 11.1 ± 3.17 years; \( p = 0.065 \)); male sex (27 [51.9%] vs. 17 [38.6%]; \( p = 0.193 \), respectively). There was a predominance of participants of non-white ethnicity in both groups (100% in the SCA group vs. 81.8% in the HC group) and white ethnicity was observed only in the HC group (8 participants [18.2%], \( p = 0.001 \)). The BMI values were similar between groups (16.9 ± 3.27 vs. 18.0 ± 3.07 kg/m² in SCA and HC, respectively; \( p = 0.055 \)).

The SCA group had lower mean hemoglobin, TC, HDL-C, and LDL-C levels and higher TG levels, TG/HDL-C, and hs-PCR. The FMD value was significantly decreased in the SCA group compared with the HC group. The cutoff point for ED classification was 9.05% (see Methods section). The SCA group had a higher occurrence of ED (Table 1).

The PTA tests showed that 15 (28.8%) participants in the SCA group presented with SNHL, compared with none of the HC group (0%, \( p < 0.001 \)). Of these 15 participants, 7 (46.66%) were 6–11 years of age and 8 (53.33%) were 12–17 years. SNHL occurred in both sexes, and affected 9 (52.9%) female participants. Regarding the severity of SNHL, 100% presented with a mild degree of SNHL (≤40 dB). Unilateral SNHL occurred in 13 (86.66%) participants and these total 9 (69.23%) in the right ear.

Among the 17 ears affected, high frequencies (4–8 kHz) were more often affected (7 ears, 41.7%), followed by both low and high frequencies (250–500 Hz and 4–8 kHz; 5 ears, 29.4%), low frequencies (250–500 Hz; 4 ears, 23.5%), and all frequencies (1 ear, 5.89%).

The median HT in PTA tests in the left ear was significantly higher in the SCA group than in the HC group in the following frequencies: 250 Hz (15, median IQR [10–18.7] vs. 10, median IQR [5–15]; \( p = 0.008 \), 500 Hz (15 [5–15] vs. 10 [5–10]; \( p = 0.024 \), 2 kHz (7.5 [5–15] vs. 5 [5–10]; \( p = 0.015 \), 3 kHz (5 [5–10] vs. 5 [0–5]; \( p = 0.001 \), and 4 kHz (5 [5–13.75] vs. 5 [5–10]; \( p = 0.013 \). There were no differences at 1 kHz (5 [5–13.7] vs. 5 [5–10]; \( p = 0.561 \), 6 kHz (10 [5–15] vs. 10 [5–10]; \( p = 0.304 \), and 8 kHz (10 [5–15] vs. 10 [5–13.7]; \( p = 0.167 \) (Figure 1(A)).

In the right ear, the HT exhibited differences at 250 Hz (15, median IQR [10–20] vs. 10, median IQR [5–15]; \( p = 0.003 \), 500 Hz (10 [10–15] vs. 10 [5–10]; \( p = 0.013 \), 2 kHz (7.5 [5–15] vs. 5 [5–10]; \( p = 0.034 \), 4 kHz (10 [5–15] vs. 5 [1.2–10]; \( p = 0.004 \), 6 kHz (10 [5–15] vs. 5 [5–10]; \( p = 0.022 \), and 8 kHz (10 [5–18.7] vs. 5 [0–10]; \( p = 0.006 \). There were no differences at 1 kHz (10 [5–15] vs. 10 [5–10]; \( p = 0.229 \) and 3 kHz (5 [5–13.7] vs. 5 [5–10]; \( p = 0.131 \) (Figure 1(B)).

In the comparison between the SCA with SNHL and SCA without SNHL groups, the SCA with SNHL group included a greater number of participants with painful VOC. In addition, the FMD was significantly lower in the SCA with SNHL group. Moreover, this group had a higher occurrence of ED (Table 2).

FMD values were different between the SCA with SNHL, SCA without SNHL, and HC groups. The SCA with SNHL group presented with the lowest FMD value (Figure 2).

The results of binary logistic regression analysis to evaluate the relationship between FMD and SNHL showed that FMD was associated with SNHL, even after adjustment for lipid profile (HDL-C and TG/HDL-C), number of episodes of painful VOC, and use of HU (odds ratio [OR] [95% CI] = 0.614 [0.440–0.858]; \( p = 0.004 \). This finding also demonstrated that each percentage increase in the FMD value decreases the chance that the SCA patient has SNHL by 39%. The use of HU was not associated with SNHL (OR [95% CI] = 0.153 [0.022–1.053]; \( p = 0.056 \).

Discussion

In the present study, 28.8% of the SCA group exhibited SNHL. This finding supports the hypothesis that this alteration is more prevalent in SCA than in the healthy population, which is similar to data reported in the literature showing that the incidence of SCA ranges from 3.8 to 66% [7,8,11–13,32]. Few studies have only considered the age group used in the present study. The SCA prevalence in the United States (US) (12%) [33] was lower than that of our study, and in Nigeria the prevalence ranges from 3.8 to 21% [9,12,34]. Furthermore, studies with a broad age range, including adults, showed a greater SCA prevalence, such as studies in Nigeria (66%) [32] and the US (36%) [35]. It might be expected that as patients with SCA grow older they will experience repeated crises and an increased incidence of SNHL would be found.

Table 1. Laboratory data and endothelial function parameters of the study groups.

|                      | Sickle cell anemia (n = 52) | Healthy control (n = 44) | \( p \)-value |
|----------------------|-----------------------------|--------------------------|--------------|
| Hemoglobin (g/dL), mean (SD) | 8.03 (0.82)                 | 13.4 (0.96)              | <0.001*      |
| Total cholesterol (mg/dL), mean (SD) | 122.2 (24.95)             | 157.1 (26.76)            | <0.001*      |
| High-density lipoprotein-cholesterol (mg/dL), mean (SD) | 30.62 (7.17)               | 47.21 (11.04)            | <0.001*      |
| Low-density lipoprotein-cholesterol (mg/dL), mean (SD) | 70.76 (22.15)             | 94.26 (23.37)            | <0.001*      |
| Triglycerides (mg/dL), mean (SD) | 103.5 (40.19)             | 72.9 (26.35)             | <0.001*      |
| TG/HDL-C (mg/dL), median/IQR | 3.02 (2.41–4.71)          | 1.45 (1.05–2.11)         | <0.001*      |
| High-sensitive C-reactive protein (mg/dL), median/IQR | 2.81 (0.99–4.73)         | 0.55 (0.20–1.46)         | <0.001*      |
| Flow-mediated dilation – %, median/IQR | 10 (6.65–14.29)         | 15 (11.6–22.4)           | <0.001*      |
| Endothelial dysfunction, n (%) | 20 (38.5%)                | 4 (9.1%)                 | <0.001*      |

Note: SD: standard deviation; IQR: interquartile range; TG/HDL-C: triglyceride to high-density lipoprotein cholesterol ratio. *Statistically significant values at the level of \( p < 0.05 \).
In addition, the haplotypes of the β-globin gene region, which play an important role in determining the severity of the disease [36], may influence SNHL; moreover, the prevalence of SNHL in SCA for each geographical region is variable beyond this factor. Besides the prevalence in the countries mentioned above, the SCA prevalence has also been reported in Guadeloupe (44%), (France) [11], Saudi Arabia (36%) [7], Italy (31.8%) [13], and the United Kingdom (13.5%) [31]. Several reasons, including socio-economic class, regular follow-up, and a balanced diet, have been proposed to explain the varying prevalence rates of SNHL in SCA [10,12]. Nevertheless, the lack of criteria used to define SNHL can contribute to this variability.

In Brazil, only one study was found in literature [8], showing a prevalence of 21.4% (range age, 6–55years). Farther standardized epidemiological studies are required to elucidate the prevalence in each region. It is important to note that all SCA participants in our study were of Afro-descendants, as well as the majority of the HC group (>80%).

Unilateral SNHL of mild severity was found in both sexes. Although a number of audiograms exhibited both low- and high-frequency losses (250–500 Hz and 4–8 kHz), several of them demonstrated atypical configurations with isolated frequency regions at high or low frequencies, demonstrating impairment. These characteristics are comparable to those described in other studies [6,7,10,11,13].

In SCA, most investigators attribute SNHL to impaired blood flow in the cochlea or to the nerve pathways from the inner ear to the brain. The blood supply of the inner ear is provided by the labyrinthic artery, which is a functional end artery. Even occasional limited ischemia culminates in Corti’s Organ hypoxia and lesion of the OHC in the stria vascularis [3–5]. The base of the cochlea

Figure 1. Comparison of hearing thresholds between the sickle cell anemia (SCA) and the healthy control (HC) groups. Box plots represent the medians and interquartile ranges for each frequency for the left ear (A) and right ear (B). *Statistically significant values for p < 0.05.
is typically involved because of its tonotopic organization; the hair cells of the basal turn of the cochlea are more sensitive to anoxia pathogena than the apex. This may explain why SNHL initially affects isolated frequency regions, and usually high frequencies. Changes in the other frequencies suggest diffuse damage to the cochlea, rather than damage limited to the basal turn [34].

Corroborating with the pathophysiological mechanism of SNHL in SCA, our data showed a significant association between SNHL and ED. To the best of our knowledge, this is the first study that has demonstrated this association in SCA.

The lower FMD value in the SCA group than that in the HC group suggests a diminished vasodilatation capacity. FMD is simple, noninvasive, and useful diagnostic tool for the measure of endothelial function, and has been widely used [23–25]. Other studies demonstrated an abnormal response in FMD after wall shear stress (WSS) for transient arterial occlusion in SCA adults [17,37] and children [38]. Studies by our research group showed similar results with decreased FMD in SCA [21,26].

In SCA, hemolysis and ED are observed in addition to inflammation and vaso-occlusion. Moreover, increased

Table 2. Characteristics of the groups sickle cell anemia with sensorineural hearing loss (SNHL) and without SNHL.

|                         | SNHL (yes) (n = 15) | SNHL (No) (n = 37) | p-value |
|-------------------------|---------------------|--------------------|---------|
| Age (years), mean (SD)  | 12.3 (3.75)         | 12.3 (3.11)        | 0.986   |
| Male sex, n (%)         | 7 (46.7)            | 20 (54.1)          | 0.629   |
| Body mass index (kg/m²), mean (SD) | 16.8 (2.89) | 16.9 (2.36) | 0.916   |
| Painful vaso-occlusive crisis (last 12 months), n (%) | 15 (100) | 23 (62.2) | 0.005*  |
| Number of painful vaso-occlusive crises (last 12 months) median (IQR) | 1 (1–4) | 2 (0–3) | 0.117   |
| Blood transfusions (last 12 months), n (%) | 2 (13.3%) | 9 (24.3%) | 0.614   |
| Number of blood transfusions (last 12 months) median (IQR) | 0 (0–1.5) | 0 (0–1) | 0.823   |
| Use of hydroxyurea therapy, n (%) | 6 (40.0) | 22 (59.5) | 0.202   |
| Duration of hydroxyurea therapy (>2 years), n (%) | 3 (50%) | 11 (30%) | 0.184   |
| Hemoglobin (g/dL), mean (SD) | 7.84 (0.87) | 8.10 (0.81) | 0.297   |
| Fetal hemoglobin (g/dL), median (IQR) | 7.65 (2.7–11.8) | 8.9 (3.8–12.1) | 0.775   |
| Total cholesterol (mg/dL), mean (SD) | 127.78 (24.93) | 119.96 (24.94) | 0.310   |
| High-density lipoprotein-cholesterol (mg/dL), mean (SD) | 32.88 (6.87) | 29.71 (7.18) | 0.150   |
| Low-density lipoprotein-cholesterol (mg/dL), mean (SD) | 75.33 (22.69) | 68.91 (21.97) | 0.348   |
| Triglycerides (mg/dL), mean (SD) | 97.76 (27.38) | 105.86 (49.60) | 0.516   |
| TG/HDL-C (mg/dL), median (IQR) | 3.04 (2.62–5.34) | 3.00 (2.02–3.89) | 0.437   |
| High-sensitive C-reactive protein, median (IQR) | 2.74 (0.8–3.74) | 4.53 (1.03–6.42) | 0.258   |
| Flow-mediated dilation – %, median (IQR) | 6.60 (4.20–9.90) | 11.50 (8.25–15.70) | <0.001* |
| Endothelial dysfunction, n (%) | 10 (66.7) | 10 (27) | 0.008*  |

Note: SCA: sickle cell anemia; SNHL: sensorineural hearing loss; SD: standard deviation; IQR: interquartile range; TG/HDL-C: triglyceride to high-density lipoprotein cholesterol ratio. *Statistically significant values at the level of p < 0.05.
inflammation-driven reactive oxygen species and inflammatory mediator production occurs, as well as decreased bioavailability of nitric oxide (NO) [39]. NO plays a key role in the regulation of vasomotor tone in response to WSS. Thus, reduced bioavailability of NO causes variation in vessel diameter adjustment, which potentially exacerbates the vaso-occlusive process [37].

Hadeed et al. [40] did not find ED in children with SCD, however, FMD value tended to be lower in SCD than in controls. The authors suggesting that these manifestations may be related to disease severity and duration. The difference in results may be explained by the fact that the population may have a less severe form of the disease, as only 16.6% presented with VOC.

In our study, all patients in the SCA with SNHL group (100%) presented with painful VOC in the last 12 months, and the median FMD value in the SCA group with SNHL was below the cutoff for ED (10th percentile of the FMD of the HC group). Furthermore, the SCA with SNHL group exhibited a higher frequency of ED in comparison with the SCA without SNHL group.

Considering the chronic inflammatory state of SCA, these findings lead us to hypothesize that damage to the vascular endothelial due to SSW in the labyrinthic artery reduces blood flow in the ear; this results in vaso-occlusive processes in the spiral ganglion in the basal part of the cochlea or to the auditory nerve, culminating in SNHL. Similar studies in other types of SNHL show the presence of ED in the auditory system, as evidenced by a lower FMD value [18–20]. In addition, there is evidence that high levels of adhesion molecules and unbalanced oxidative status are involved in idiopathic sudden SNHL, which supports the hypothesis that SNHL should be considered as a microcirculation disorder caused by ED [18].

An additional favorable effect on the microcirculation can be exerted by anti-inflammatory mediators, improving the vascularization of small ear vessels that usually cause inner ear injury. In SCA HU is considered an effective pharmacological therapy with several beneficial effects, such as increased HbF, improved red globule hydration, increased NO production, and decreased adhesion molecules, with consequent reduction of painful VOC [41,42]. It is possible that HU improved endothelial function. In our study, the implementation of HU no showed independent effect on SNHL. In the literature, we did not find studies on this approach. However, despite the similar use of HU in the SCA with SNHL and SCA without SNHL groups, the duration of HU therapy was variable, which may be a limitation of the study.

There are others methodological limitations that should be considered. PTA is a behavioral measure and may have variations in the HT. The endothelial function was assessed using an image-based methodology dependent on the ability of the person performing the procedure. The small number of participants with SNHL may also represent a limitation. However, considering the consistency of our data, these minor limitations do not invalidate our results.

In conclusion, the present study demonstrated that SNHL is a common complication in children and adolescents with SCA and is associated with ED. This findings suggests that disruption of the vascular endothelium generates circulatory disorders, culminating in auditory disorders such as SNHL.

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