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Original Article

Lower hair cortisol among patients with sickle cell disease may indicate decreased adrenal reserves

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Abstract: Introduction: Sickle cell disease (SCD) is a chronic illness that presents with a wide range of phenotypic variation. Stress may be a contributing factor to differences that are found in this population. Objectives: Our objective is to determine the relationship between hair cortisol content (HCC), a biomarker of stress, and other clinical measures in individuals with SCD. Methods: We collected hair samples and other clinical measures from 73 subjects with SCD (mean age: 39 ± 12 years, 63% female). Results: HCC was lower among individuals who had greater than 30% hemoglobin S, compared with those who had less than 30% hemoglobin S (W=272.5, P=0.01). Lower HCC was also associated with report of not being on a chronic transfusion program (β=48.34, SE=14.09, P=0.001) and higher ferritin levels (β=-0.006, SE=0.002, P=0.02). Furthermore, HCC was significantly correlated with serum cortisol (r_s=0.26, P=0.03) and corticosterone (r_s=0.29, P=0.01). We also observed a consistent pattern of low steroid values among our population. Conclusion: Our findings suggest that individuals with higher hemoglobin S and ferritin, both markers of severe SCD, may have decreased cortisol levels. This is consistent with the relationship we observed between higher HCC among individuals who are on a chronic blood transfusion program, which typically increases quality of life. Our results suggest that hair cortisol may be an indicator in patients with SCD who could be at risk for developing adrenal insufficiency. We recommend that clinicians treating patients with SCD follow the Endocrine Society guidelines for testing for adrenal insufficiency and treat accordingly.

Keywords: Sickle cell disease, sickle cell anemia, corticosteroids, steroids

Introduction

Sickle cell disease (SCD) is an inherited hemoglobinopathy that has significant variation in its presentation. It has been reported that every year approximately 300,000 infants are born with sickle cell anemia globally and that this number could rise to 400,000 by 2050 [1]. In the United States it is estimated that there are approximately 100,000 individuals living with the disease [2]. The disease phenotypic presentation includes chronic hemolytic anemia, and multiple acute and chronic complications, such as pain, infection, acute chest syndrome, retinopathy, leg ulcers, renal failure, cardiopulmonary dysfunction, and endocrine abnormalities, including impaired growth, delayed sexual development and hypoadrenalism [1, 3, 4].

Despite the understanding of the molecular basis of SCD, there is limited information regarding the mechanisms underlying variation in disease phenotype, as well as the interactions between non-genetic factors, such as biological mechanisms of stress and clinical complications, and disease severity [1].

To date, several studies have observed a high burden of psychological complications, such as

anxiety and depression, among individuals with SCD [5-8]. The chronicity of SCD, as well as the experiences of pain and other disease complications, are often associated with stress and poor psychological outcomes [9-12]. Although this consistent relationship between pain and stress has been observed, it is still unclear through which biological pathways this relationship is occurring in SCD. Therefore, we developed a study to evaluate chronic biological stress using hair cortisol as a biomarker since there has been limited research on cortisol and sickle cell disease.

Hair cortisol as a biomarker

Cortisol, a steroid hormone, is produced in the adrenal cortex, the outer part of the adrenal gland, and is released in response to stress. It aids the metabolism, mediates immune response, and the sleep/wake cycle. Cortisol is regulated by the hypothalamic-pituitary-adrenal (HPA) axis. Long-term stress has been associated with HPA dysfunction and poor health outcomes later in life, such as metabolic and cardiovascular morbidity [13]. For these reasons, cortisol levels have become an important biomarker of stress.

Dysregulation of cortisol has also been found to be associated with other diseases, such as cardiovascular disease, obesity, insulin resistance, type 2 diabetes, stroke, Cushing syndrome, adrenal insufficiency, depression, and cognitive impairment [14-19]. Chronically high levels of cortisol are associated with metabolic syndrome and mental illness, such as depression. Additionally, hypercortisolism in Cushing syndrome is associated with poor quality of life, morbidity, and mortality [20]. Adrenal insufficiency can present as chronic symptoms, such as weight loss, nausea, vomiting, fatigue, and anorexia, or acutely, as shock with disorientation, known as adrenal crisis [19].

Cortisol is commonly measured in the saliva, blood, and urine [21]. However, these methods are short-term measurements and influenced by the time of day of collection and often require multiple collections [13, 22, 23]. In contrast, a novel analysis of cortisol content in hair reflects long-term cortisol exposure (over several months), and can be easier to collect from research participants [13, 22].

Hair cortisol biomarker in studies of populations of African ancestry

Despite the relative ease of collection and analysis of hair cortisol content, there are limited studies which have examined hair cortisol concentration in populations of African ancestry. These studies have found higher hair cortisol concentration in African ancestry when compared with other population groups [24]. As well as associations between hair cortisol concentration and diabetes biomarkers [25], neighborhood disadvantage [26], socioeconomic status [27], traumatic life events [28], and discrimination [29] in these populations. These findings suggest a relationship between chronic stress and hair cortisol concentration in African ancestry populations. There have been no studies reporting hair cortisol as a marker for clinical phenotypes, such as decreased adrenal reserves in a population of African ancestry.

Study goal and innovation

Hair cortisol concentration has not been examined in individuals with SCD. Studies utilizing serum measures of cortisol have found that individuals with SCD have lower serum cortisol than individuals without SCD [30, 31]. Additionally, individuals with SCD who have experienced recent pain crises have higher serum cortisol than individuals with SCD who have not had any recent crises [30]. Our study is the first to examine the relationship of hematological factors associated with stress and disease severity in SCD with hair cortisol content as well as the first to identify a relationship between hair cortisol content and decreased adrenal reserves.

Materials and methods

Study population

We recruited adult patients with SCD between June 2014 and December 2017 to two different clinic locations: National Institutes of Health Clinical Center in Bethesda, Maryland, and Montefiore Medical Center in Bronx, New York. Participants were recruited for the larger ongoing INSIGHTS Study (Insights into Microbiome and Environmental Contributions to Sickle Cell Disease and Leg Ulcers Study; ClinicalTrials.gov NCT02156102). The INSIGHTS study is focused

on understanding the variation in phenotypes experienced by individuals with SCD, utilizing a holistic perspective by collecting clinical, psychosocial, environmental, genomic, transcriptomic, and microbiomic data from participants. The eligibility criteria for the INSIGHTS study was any individual who was 18 years or older with sickle cell disease, any genotype. Hair sample collection was an optional part of the study. Participants that had hair that was at least 1 cm in length were asked if they would be willing to provide a hair sample for analysis. Study personnel obtained written consent once the patient arrived at the clinic. Once there, clinical staff conducted a full medical examination of the patient, including a comprehensive history and physical exam as well as collection of clinical and research blood and urine labs. All participants were compensated once they completed their study visit.

Hair collection and cortisol content (HCC) analysis

After completion of the medical evaluation, a sample of hair was collected from patients who opted into hair collection, as previously described [22, 23]. In each case, the hair was cut from the posterior vertex of the head using clean scissors. The hair sample was secured with a tight rubber band and an initial cut was made as close to the scalp as possible. A ruler was then used to measure 3 cm or less from the cut end and a second cut was made to yield the final sample. The amount of hair taken was roughly the width of a pencil. The samples were stored in an envelope in -30°C freezers. Hair samples were processed and analyzed as described by Meyer et al [32]. Briefly, samples were weighed and then washed twice in 1 mL of isopropanol for 3 minutes each using a rotator. Washed samples were dried for at least 24 hours in a clean hood, ground to a fine powder using a bead mill, and then extracted with 1.5 mL of methanol under slow rotation for 18-24 hours at room temperature. The samples were then centrifuged, 1 mL of the methanol extract was transferred to a clean tube and the methanol was evaporated using a SpeedVac Concentrator. Finally, the cortisol was reconstituted in assay buffer and analyzed in duplicate using the Arbor Assays DetectX enzyme immunoassay. Intra- and inter-assay coefficients of variation for this assay are <10%.

Laboratory values and measures

To assess the relationship of hair cortisol to SCD biomarkers, we utilized several clinical values previously associated with SCD severity [33]. First, we analyzed the clinical severity score as calculated in Du et al [33]. Next, we analyzed individual values which contributed to the calculation of the severity score. These include genotype, self-reported iron overload, and measurement of several lab values including hemoglobin, ferritin, white blood cell count, mean corpuscular volume, lymphocytes, monocytes, platelets, reticulocytes, urea nitrogen, creatinine, uric acid, bilirubin, alkaline phosphatase, albumin, eosinophil, and fetal hemoglobin (HbF) and sickled hemoglobin (HbS) percentages. Lab values were assessed from one blood sample.

Serum steroid measures

Adrenal steroid values were determined using heparin plasma and Agilent 6490 triple-quadrupole mass spectroscopy coupled with an atmospheric pressure photoionization source and Agilent 1200 Infinity series high performance liquid chromatography (HPLC) (Agilent Technologies) as described in Parikh et al [34]. Cortisol, cortisone, androstenedione, corticosterone, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), testosterone, 11-deoxycortisol, and 17-hydroxyprogesterone (17-OHP) were analyzed from participant samples.

Statistical analyses

Wilcoxon signed-rank tests were used to compare the hair cortisol content means of subgroups. Linear and logistic regression models were used to evaluate the relationship between hair cortisol levels and clinical measures. Hair cortisol levels were used as a predictor in the models, with clinical variables as outcomes. Linear regression was used for continuous clinical variables; logistic regression was used for binary clinical variables. As the hair cortisol content values were non-normally distributed, these values were log-transformed. Log-transformed values of hair cortisol content were used in subsequent regression analyses. All models adjusted for sex and age of the patient. R software version 3.4.1 was used for all analyses [35].

Table 1. Study population description

Variable	N=73 (%)
Age (in years, mean ± standard deviation)	38.9 ± 11.9
Sex	
Male	27 (37)
Female	46 (63)
Race	
American Indian or Alaska Native	1 (1)
Black or African American	66 (91)
White	4 (5)
Not provided	2 (3)
Sickle Cell Genotype	
HbSS	59 (81)
HbSC	9 (12)
HbSBO	2 (3)
HbSB+	3 (4)
Hair cortisol content (pg/mg, mean± standard deviation)	17.1 ± 41.6
Ferritin Level	
Mean (μg/L)	1384
Median (μg/L)	629
Range (μg/L)	18-10948
Over 1000 mcg/L	26 (36)
Less than 1000 mcg/L	47 (64)
Self-reported Iron Overload	
Present	23 (32)
Absent	49 (67)
Unavailable	1 (1)
On Chronic Transfusion Program	
Yes	14 (19)
No	59 (81)
Eosinophil Level (% ± standard deviation)	3.0 ± 2.2
Hemoglobin S (% ± standard deviation)	67.6 ± 20.0

The population used in this study included all individuals from the Insights into Microbiome and Environmental Contributions to Sickle Cell Disease and Leg Ulcers Study or INSIGHTS (NCT02156102) who provided a hair sample as of August 2018. All individuals have sickle cell disease.

Ethics review and data access

This study was reviewed and approved by the Institutional Review Board at the National Institutes of Health, National Human Genome Research Institute. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Results

Study population description

We collected hair from a total of 73 individuals. The study population had an average age of 39 years and contained more women (63%) than men (**Table 1**). Most individuals (81%) were sickle cell genotype HbSS. Hair cortisol concentrations ranged from 0.4 pg/mg to 280.1 pg/mg, with mean and median values of 17.1 pg/mg and 4.60 pg/mg, respectively.

Hair cortisol and hematological measures

Hair cortisol levels were not associated with the disease severity measure in a linear regression model (P=NS). Among our study population, eosinophil levels significantly increased with increasing hair cortisol content (P=0.04) (**Figure 1**). In linear regression models, higher hemoglobin S levels were associated with lower hair cortisol content (β=-5.18, SE=-3.07, P=0.003). We also observed that hair cortisol content was lower among individuals who had greater than 30% hemoglobin S, compared with those who had less than 30% hemoglobin S (W=272.5, *P*=0.01).

Lower hair cortisol content was also associated with not being on a chronic transfusion program (β=48.92, SE=13.52, P=0.0006) and higher ferritin levels (β=-0.006, SE=0.002, P=0.02). Hair cortisol levels were significantly associated with the self-reported iron overload in a logistic regression model such that individuals with iron overload had lower hair cortisol (P=0.04) (**Figure 2**). Age was also associated with iron overload; individuals with iron overload were older (P=0.0008). Hair cortisol content was not associated with hemoglobin, white blood cell count, mean corpuscular volume, differential lymphocytes, differential monocytes, platelets, reticulocytes, blood urea nitrogen, creatinine, uric acid, total bilirubin, aspartate aminotransferase, alkaline phosphatase, albumin, or fetal hemoglobin (**Table 2**).

Hair cortisol and serum steroids

Furthermore, hair cortisol content was significantly correlated with several blood steroid measures including serum cortisol (r_s=0.26,

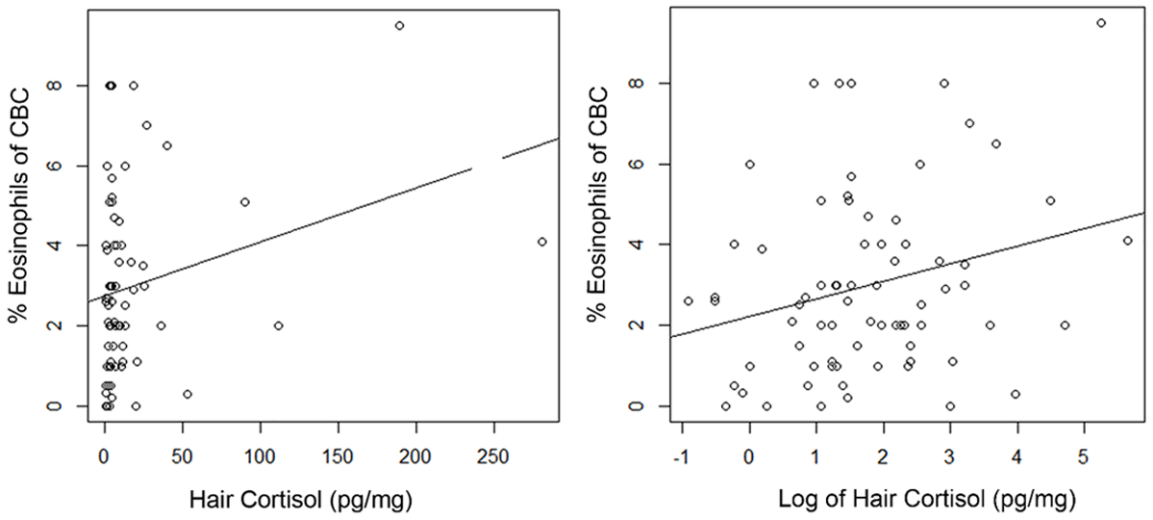

Figure 1. Hair cortisol levels significantly increased with increasing eosinophil levels. Within the study population (n=73), increasing log hair cortisol levels were associated with increasing eosinophil levels (β=0.41, SE=0.19, P=0.04).

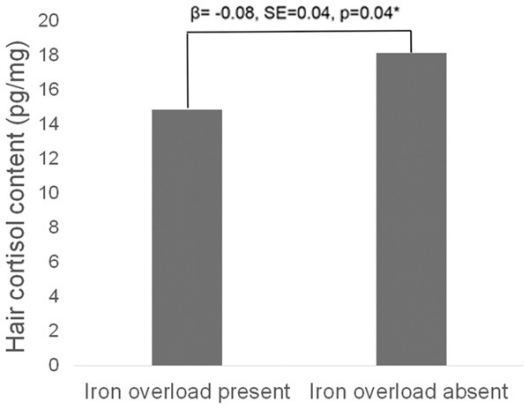

Figure 2. Hair cortisol is lower in individuals with iron overload. Of the total study population, 23 individuals had self-reported iron overload. The average hair cortisol among individuals with iron overload was 14.8 pg/mg. 49 individuals did not have iron overload and the average hair cortisol among this group was 18.1 pg/mg. Log hair cortisol content was significantly lower in individuals with iron overload as assessed by logistic regression where the outcome was self-reported iron overload and the predictor was the log of hair cortisol content, adjusted for participant age and sex (β=-0.08, SE=0.04, P=0.04).

P=0.03), corticosterone (r_s=0.29, P=0.01), and progesterone (r_s=0.26, P=0.03) (**Table 3**). We observed a consistent pattern of low steroid levels among our population (**Figure 3**).

Discussion

To our knowledge, this is the first study of hair cortisol content (HCC) in individuals with sickle cell disease (SCD). We did not observe a positive association between stress and HCC as expected. Based on the multiple significant associations with serum steroids and consistent low cortisol levels we observed, we conclude that HCC may be a marker of cortisol deficiency in SCD. We observed a positive correlation between eosinophil levels and HCC; further exploration is needed to determine if any clinical significance arises from this relationship.

Our results suggest that individuals with iron overload and higher hemoglobin S, markers of severe disease, may have hypoadrenalism, as indicated by their lower hair cortisol levels. This is consistent with previous research outside of SCD, where dysfunction of the endocrine system was reported in individuals with iron overload [36]. Our findings are consistent with the relationship we observed between higher hair cortisol levels in individuals who are on a chronic blood transfusion program, who typically have increased quality of life and reduced clinical complications and therefore potentially better adrenal function.

Individuals with chronic iron overload experience an accumulation of iron in vital organs, such as the heart and liver. This accumulation leads to eventual dysfunction, as well as morbidity and mortality [37]. Furthermore, individuals with iron overload often have increased pain crises and hospitalizations [37]. Due to

Table 2. Clinical variables as predictors of the log of hair cortisol content

Predictor variable	Beta	Std. Error	*P* value
Chronic Transfusion (yes/no)	1.42	.044	0.002
Ferritin	-0.0002	0.00008	0.006
Severity score	-0.0007	0.002	0.70
Hemoglobin	0.019	0.02	0.36
White blood cell count	0.026	0.041	0.53
Mean corpuscular volume	-0.0004	0.009	0.97
Lymphocytes	-0.018	0.014	0.19
Monocytes	-0.07	0.039	0.076
Platelets	-0.001	0.0009	0.27
Reticulocyte percent	-0.005	0.018	0.79
Blood urea nitrogen	0.017	0.016	0.29
Creatinine	0.008	0.13	0.95
Bilirubin	0.03	0.08	0.72
Aspartate aminotransferase	-0.007	0.007	0.34
Alkaline phosphatase	-0.0007	0.002	0.73
Albumin	0.47	0.41	0.25
Fetal hemoglobin	-0.01	0.02	0.47

Linear regression models were used. Model: log of hair cortisol content = Predictor + age + sex.

Table 3. Serum adrenal steroid Spearman correlation with hair cortisol content in total sample (n=73)

Steroid	Correlation Coefficient	*P* value
Cortisone	0.19	0.10
Cortisol	0.26	0.026
Corticosterone	0.29	0.013
11-deoxycortisol	0.15	0.21
Androstenedione	0.02	0.88
Testosterone	-0.02	0.88
17-hydroxyprogesterone (OHP)	0.17	0.16
Progesterone	0.26	0.028
Dehydroepiandrosterone (DHEA)	0.07	0.56
Dehydroepiandrosterone Sulfate DHEAS*	0.07	0.57

Serum cortisol, corticosterone, and progesterone were all significantly correlated with hair cortisol content. **Only 71 individuals had lab values for DHEAS.*

this relationship with disease severity, we anticipated that individuals with iron overload would be chronically stressed and therefore have higher cortisol than those without iron overload. However, we observed the opposite. This was different from prior studies of HCC in African ancestry populations, where increased stress was associated with increased HCC [25-29]. This suggests that hair cortisol may not be functioning as a marker of stress in the sickle cell population, as it has in other populations, perhaps due to disease-related compromise of adrenal function [27]. Instead, hair cortisol may be a marker of reduced adrenocortical hormonal secretion in patients with more severe disease.

Adrenal insufficiency is defined as the inability of the adrenal cortex to produce sufficient glucocorticoids, which can affect energy, salt, and fluid homeostasis [38]. It is associated with increased morbidity and mortality [39]. Additionally, individuals with adrenal insufficiency are at risk of adrenal crisis, a life-threatening complication caused by insufficient cortisol production [40]. Previous studies have observed an increased prevalence of adrenal insufficiency among individuals with SCD when compared with the general population [3, 41, 42]. This is potentially due to a dysfunction in the hypothalamus-pituitary-adrenal axis in individuals with SCD [4]. Our findings support the use of hair cortisol as a potential measure of diminished adrenocortical reserves in individuals with SCD and possible tool for following patients with SCD who may be at risk for developing adrenal insufficiency.

As adrenal insufficiency may be more prevalent in SCD [3, 41, 42], we recommend that clinicians treating patients with sickle cell disease follow the Endocrine Society clinical guidelines for testing and treating adrenal insufficiency [38]. We suggest evaluation of primary adrenal insufficiency in all patients with SCD, who may have indicative clinical symptoms or signs of adrenal insufficiency. This can be done with a standard dose (250 μg for adults and children >2 year of age) corticotropin stimulation test (30 or 60 min) [34]. Peak serum cortisol levels <18 μg/dL at 30 or 60 minutes indicate adrenal insufficiency. If a corticotropin stimulation test is not available, a morning cortisol (<5 μg/dL) in combination with plasma adrenocorticotropic hormone ACTH as a preliminary test suggestive of adrenal insufficiency [35]. Patients with SCD and adrenal insufficien-

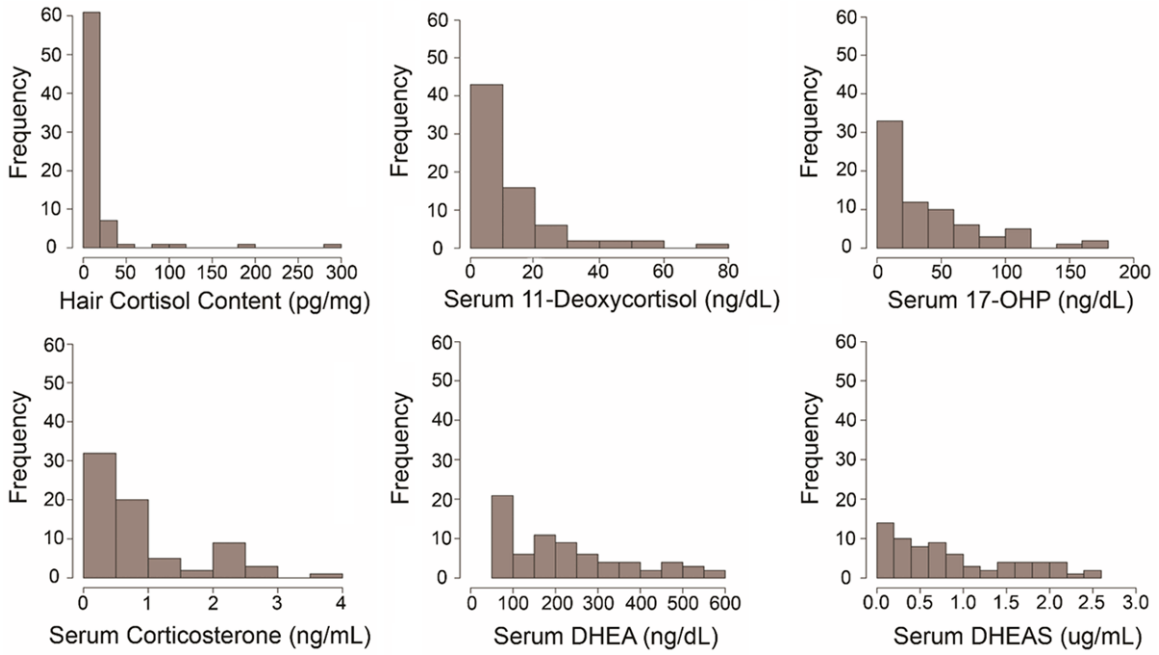

Figure 3. High prevalence of low serum steroid values among sickle cell disease study population. With the exception of cortisol and cortisone, the distributions of steroid values were skewed to the left, indicating a large portion of the study population with low steroid values.

cy may need to be educated about stress dosing and possibly prescribed glucocorticoid for emergency intramuscular administration. Future studies should continue to examine the prevalence and effects of adrenal insufficiency in SCD, as well as the effects of the standard of care treatment on this patient population.

Our study has some limitations. The study is limited by sample size. While our sample size is comparable to other published studies of hair cortisol in individuals of African descent [24-27], it is a small sample. Many individuals within the INSIGHTS cohort did not have long enough hair (3 cm) to provide a sample and short hair was more prevalent among men within the cohort. The second limitation of our study is the lack of adrenal function measures. Future studies should analyze adrenal steroids within the population to determine if SCD population shows a high prevalence of adrenal insufficiency. Participants' reports of iron overload were supported with ferritin levels measured at a CLIA certified clinical laboratory. However, iron overload was not measured with MRI imaging (FerriScan®) or a liver biopsy. Finally, our study is cross-sectional, so we are unable to follow our participants over time.

Despite these limitations, this study is the first to examine hair cortisol content in individuals with sickle cell disease. Other studies have examined serum cortisol [30, 31], but serum cortisol is affected by the time of day and is not reflective of a long time period. Our hair cortisol values are reflective of cortisol content over an average of three months [43, 44], making it a more stable indicator of adrenal function. Secondly, our study has abundant clinical phenotype data, allowing us to fully investigate the relationship between variation in SCD and hair cortisol content.

In conclusion, hair cortisol may be a potential measure of adrenal insufficiency in individuals with SCD and is not functioning as a typical measure of stress as seen in other populations [27]. Future studies should continue to examine the prevalence and effects of adrenal insufficiency in SCD, as well as the effects of the standard of care treatment on this patient population.

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Disclosure of conflict of interest

None.

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