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RESEARCH ARTICLE

The relationship between nutrition, gut dysbiosis, and pediatric sickle cell pain outcomes: A pilot study

Chinenye R. Dike[1,2] | Corrine Hanson[3] | H. Dele Davies[4] | Stephen Obaro[4] | Fang Yu[5] | James Harper[6] | Helen Grace[7] | Jeffrey Lebensburger[8] | Chittalsinh Raulji[6] | Jihyun Ma[5] | Peter Mannon[9]

[1]Department of Pediatrics, Division of Pediatric Gastroenterology, Hepatology and Nutrition, University of Nebraska Medical Center and Children's Hospital & Medical Center, Omaha, Nebraska, USA

[2]Department of Pediatrics, Division of Pediatric Gastroenterology, Hepatology and Nutrition, University of Alabama at Birmingham, Birmingham, Alabama, USA

[3]Department of Medical Sciences, Division of Medical Nutrition, University of Nebraska Medical Center, Omaha, Nebraska, USA

[4]Department of Pediatrics, Division of Pediatric Infectious Diseases, University of Nebraska Medical Center and Children's Hospital & Medical Center, Omaha, Nebraska, USA

[5]Department of Biostatistics, University of Nebraska Medical Center and Children's Hospital & Medical Center, Omaha, Nebraska, USA

[6]Department of Pediatrics, Division of Pediatric Hematology and Oncology, University of Nebraska Medical Center and Children's Hospital & Medical Center, Omaha, Nebraska, USA

[7]Department of Pediatrics, Division of General Pediatrics, University of Nebraska Medical Center and Children's Hospital & Medical Center, Omaha, Nebraska, USA

[8]Department of Pediatrics, Division of Pediatric Hematology and Oncology, University of Alabama at Birmingham, Birmingham, Alabama, USA

[9]Department of Internal Medicine, Gastroenterology, Hepatology and Nutrition, University of Nebraska Medical Center, Omaha, Nebraska, USA

Correspondence
Chinenye R. Dike, Department of Pediatrics, Division of Pediatric Gastroenterology, Hepatology and Nutrition, University of Alabama at Birmingham, 1600 7th Ave S, Birmingham, AL 35233, USA.
Email: Chinenyedike@gmail.com

Abstract

Background: Nutritional deficiencies are prevalent in sickle cell disease (SCD) and may be associated with worse pain outcomes. Gut dysbiosis has been reported in patients with SCD and may contribute to both nutritional deficiencies and pain.

Objectives: We tested the association of nutrition, fat-soluble vitamin (FSV) deficiency, and gut microbiome composition on clinical outcomes in SCD. Second, we measured the association between diet and exocrine pancreatic function on FSV levels.

Methods: Using case control design, we enrolled children with SCD (n = 24) and matched healthy controls (HC; n = 17, age, sex, race/ethnicity). Descriptive statistics summarized demographic and clinical data. Wilcoxson-rank tests compared FSV levels between cohorts. Regression modeling tested the association between FSV levels and SCD status. Welch's t-test with Satterthwaite adjustment evaluated associations between microbiota profiles, SCD status, and pain outcomes.

Results: Vitamin A and D levels were significantly decreased in participants with HbSS as compared to HC (vitamin A, p = < .0001, vitamin D, p = .014) independent of nutritional status. FSV correlated with dietary intake in SCD and HC cohorts. Gut microbial diversity was reduced in hemoglobin SS (HbSS) compared to hemoglobin

Abbreviations: μ;g/g, microgram per gram; μ;g/L, microgram per liter; 25-OH, vitamin D25-hydroxyvitamin D; BMI, Body Mass Index; FSV, fat-soluble vitamins; HbSC, hemoglobin SC; HbSS, hemoglobin SS; HC, healthy controls; mg/L, milligram per liter; ng/mL, nanogram per milliliter; QoL, quality of life; rRNA, ribosomal ribonucleic acid; SCD, sickle cell disease.

SC (HbSC) and HC, $p = .037$ and .059, respectively. The phyla *Erysipelotrichaceae* and *Betaproteobacteria* were higher in SCD children reporting the highest quality-of-life (QoL) scores ($p = .008$ and .049, respectively), while *Clostridia* were higher in those with lower QoL scores ($p = .03$).

Conclusion: FSV deficiencies and gut dysbiosis are prevalent in children with SCA. Gut microbial composition is significantly different in children with SCD with low QoL scores.

KEYWORDS

children, fat-soluble vitamins, gut dysbiosis, nutrition, sickle cell disease

1 | INTRODUCTION

Sickle cell disease (SCD) is the most common inherited red cell disorder that leads to multisystemic disease and impacts over 100,000 patients in the United States.[1] Patients are at increased risk for early morbidity, mortality, and lifetime loss of income.[2] Acute pain events or vaso-occlusive crisis (VOC) is the most common cause of hospitalization in children and leads to a decreased quality of life (QoL).[3,4] Unfortunately, repeated acute pain episodes can progress to chronic, daily, and debilitating pain.[4] Current pain management strategies include both opioid and non-opioid approaches to treating acute and chronic pain in addition to prescribing daily or monthly sickle cell modifying therapies.[5] While several other disease fields have incorporated nutritional interventions to prevent or reduce pain, limited data exist on dietary interventions that could improve outcomes in children living with SCD.[6,7]

A few adult and pediatric studies suggest that sickle cell patients have an increased prevalence of fat-soluble vitamin (FSV) deficiencies: vitamin D,[7–12] vitamin A,[13–15] and vitamin E.[16,17] The prevalence of vitamin D deficiency in SCD may be as prevalent as 96%.[12] Several SCD studies link vitamin D deficiency to increased acute and chronic pain events, opioid use, and decreased QoL; furthermore, supplementation of vitamin D in deficient individuals may improve pain outcomes.[10,11,18–20] One current gap in the literature is understanding the contribution of diet, digestion, and absorption (exocrine pancreatic function) on the development of FSV deficiencies in children with SCD. A few single-center studies report that children with hemoglobin SS (HbSS) have lower vitamin D intake, suggesting a role of diet on this deficiency.[9,21] One center performed intestinal biopsies in five children with SCD and stunted growth; they did not identify fat malabsorption.[22] These limited data on the pathophysiology that contribute to increased prevalence of FSV deficiencies warrant additional studies. These studies should determine if vitamin D deficiency is due to dietary intake, maldigestion, malabsorption, or other factors yet to be defined.

Gut dysbiosis has been reported in both children and adults with SCD and may impact disease outcomes.[23–26] One study in adults with SCD did not identify a significant difference in gut microbial composition between those with and without frequent hospitalization.[23] This negative finding was attributed to the small sample size; data on gut dysbiosis in pediatric SCD are limited. Relevant to vitamin D deficiency and its supplementation are studies suggesting that vitamin D is associated with changes in the microbiome in both animal and human studies,[27–29] and the gut microbiota may supply the host with some of these essential micronutrients.[30]

Given the critical current gaps in this area, we hypothesized that FSV deficiencies will be prevalent in children with SCD despite similar dietary intake and exocrine pancreatic function to matched healthy controls (HC). Second, gut dysbiosis will be prevalent in children with SCD and be associated with worse pain outcomes. Our observational study aims to advance our knowledge related to the association of dietary intake and exocrine pancreatic function on FSV levels and the relationship between FSV status, microbiome dysbiosis, and SCD pain outcomes.

2 | METHODS

2.1 | Study design

We performed a case–control study of children aged 4—18 years with SCD (HbSS and hemoglobin SC [HbSC]) followed at the sickle cell clinic at the Children's Hospital and Medical Center (CHMC) in Omaha, NE, USA. This study was approved by the Institutional Review Board at University of Nebraska Medical Center (UNMC; with IRB protocol #: 0427-21-FB).

2.2 | Research subject enrollment

Children presenting for routine care at the SCD clinic were recruited from November 2021 to June 2022. IRB-approved age- and language-appropriate consent and assent were obtained before participation. Age-, sex-, and race/ethnicity-matched healthy children aged within 2 years of participants with SCD were recruited simultaneously from the general pediatric outpatient clinic. We excluded participants who were pregnant, breastfeeding, outside the stipulated age range (4–18 years), on multivitamin supplements, within 1 month

of completion of an antibiotics except for participants with SCD on penicillin prophylaxis and those on chronic transfusions. We stopped enrollment of HCs after June so that could match SCD participant recruitment.

2.3 | Data collection

Participant demographic information collected included age, sex, race/ethnicity, weight, height, Body Mass Index (BMI), and BMI *z* score. We used anthropometric information obtained within 3 months of enrollment for participants recruited outside a clinic visit. The BMI *z* score was used to define nutritional status: malnourished BMI *z* score (≥1 SD below the mean; i.e., ≤ −1) versus well nourished (BMI *z* score > −1). Retrospective chart review was conducted on participants with SCD to determine acute care (emergency department and urgent care visits) and hospitalizations in the past year (≥3 vs. <3). We collected fresh fecal samples from participants, and this was aliquoted into two samples (for fecal elastase and for 16S ribosomal ribonucleic acid [rRNA] sequencing) prior to freezing at −80°F. Participants who could not give a stool sample in the clinic were given containers to collect stool and return this within 4 weeks from date of recruitment. If unable to bring fecal sample within an hour of collection, they were advised to freeze the fecal sample until able to bring sample. DNA extraction was done using PowerFecal Pro DNA kit (Qiagen). Vitamin A, D (total 25-hydroxyvitamin D [25-OH vitamin D]), and E levels were measured by the hospital laboratory. Children with SCD completed a validated QoL questionnaire for children with SCD; Peds QoL SCD module.[31,32]

2.4 | Outcomes

2.4.1 | Fat-soluble vitamin levels

Vitamin D was assessed in UNMC (Nebraska Medicine) laboratory using the paramagnetic particle chemiluminescent immunoassay method with at least 0.2 mL of serum run on Beckman Coulter DXI Immunoassay System. Vitamins A and E were also assayed in the UNMC (Nebraska Medicine) laboratory using ultrahigh performance liquid chromatography.

We categorized and compared children with SCD and matched HC by FSV status. Vitamin D was categorized as follows: vitamin D 25-OH levels less than 12 ng/mL (deficient), greater than or equal to 12 ng/mL to less than 30 ng/mL (insufficient), and greater than or equal to 30 ng/mL (sufficient). Participants were categorized as deficient for vitamin A if their serum levels were below the reference range by age as follows: 200–430 μ;g/L (0–6 years), 250–480 μ;g/L (7–12 years), 260–720 μg/L (13–19 years). Finally, participants were categorized as deficient for vitamin E if below the reference range by age: 3.0–9.0 mg/L (0–6 years), 4.3–9.0 mg/L (7–12 years), 5.6–10.3 mg/L (13–19 years).

2.4.2 | Dietary intake

Differences in dietary intake of vitamin FSV between SCD and HC. Participants completed a 1-week recall validated food questionnaire: 2004 Block Kids Food Frequency Questionnaire (age 7—17 years) to assess both macro- and micronutrient intake,[3,33] and the intake was categorized as meeting versus not meeting dietary intake based on the recommended dietary intake for that micronutrient.

2.4.3 | Exocrine pancreatic function

We assessed the difference in percentage of children with SCD who were pancreatic sufficient (fecal elastase [>200 μg/g]) versus those who were pancreatic insufficient (≤200 μg/g) using a formed stool sample. Stool samples were transported in unpreserved media to ARUP laboratories, where tests were performed using quantitative chemiluminescent immunoassay on Liaison XL analyzer. At least 1 g of stool was required for testing. Fecal samples could be refrigerated for up to 2 weeks or frozen for up to 30 days before analysis.

2.4.4 | Gut microbiota diversity and composition

Alpha and beta diversity measures and differences in absolute and relative abundance of species and phyla between children with SCD and HC were based on 16S rRNA gene sequencing results.

We compared differences in alpha and beta diversity, specie, and phyla overabundance between children with SCD who were vitamin D deficient (vitamin D 25-OH <12 ng/mL) and sufficient (≥30 ng/mL). Second, we compared differences in alpha and beta diversity, specie, and phyla overabundance between children with SCD based on nutritional status: malnourished BMI *z* score (≥1 SD below the mean; i.e., ≤ −1) versus well nourished (BMI *z* score > −1). Third, we compared differences in alpha and beta diversity, specie, and phyla overabundance between children with SCD who have frequent hospitalizations and acute care utilization (≥3) and less frequent (<3) in the past 1 year on retrospective data review. Finally, we compared differences in alpha and beta diversity, specie, and phyla overabundance between children with SCD who have poor QoL versus those with higher QoL scores using a validated QoL questionnaire for children with SCD; Peds QoL SCD module.[31,32]

2.4.5 | Statistical analysis

We used descriptive statistics to summarize data including counts, and percentages for categorical data, and means, standard deviations, medians, minimums, and maximums for continuous data. Fisher's exact tests were used to compare the proportions of sickle cell genotypes and HCs, serum levels of vitamins A, D, and E, and nutritional status (as defined above; malnutrition vs. well-nourished) for the variables.

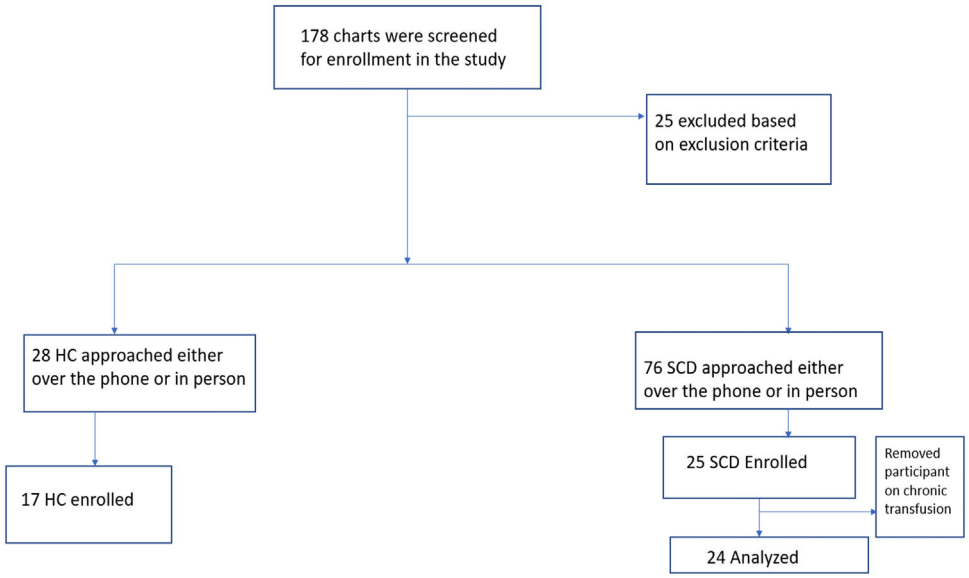

FIGURE 1 Flowchart of participants approached for enrollment, consented, and finally included in the analysis for both the SCD and HC cohorts. SCD: sickle cell diease includes HbSS and HbSC; HCs: healthy controls; HbSC: hemoglobin SC; HbSS: hemoglobin SS.

Wilcoxson-rank tests were used to compare the serum vitamin levels as continuous measures between cohorts. Kruskal–Wallis tests with DSCF tests for multiple comparisons were used to compare FSV levels between cohorts. A linear regression model was used to investigate the association between serum vitamin levels and SCD status while controlling for the dietary intake and malnutrition of that specific vitamin. The alpha diversity (Shannon diversity index) was compared between groups using ANOVA. The beta diversity, including Bray–Curtis dissimilarity, was calculated to measure the community composition. The PERMANOVA[34] method was used to test the association of the microbial community compositions with treatments. The relative abundances of species at various taxonomic levels were compared among groups using Welch's *t*-test with Satterthwaite adjustment. The *p*-values were adjusted using the Benjamin–Hochberg method to control false discovery rates (FDR). SAS version 9.4 and R version 4.0.4 were used for analyses, and *p*-value less than .05 was considered significant.

3 | RESULTS

3.1 | Cohort demographics

We enrolled 25 participants with SCD and 17 matched age, sex, and race/ethnicity HCs. One of the 25 enrolled SCD participants was excluded for being initiated on chronic transfusion therapy (Figure 1). Among the 24 SCD participants included in the final analysis, 16 participants were diagnosed with HbSS and eight were diagnosed with HbSC. Twelve of these SCD participants were prescribed hydroxyurea with one known to be nonadherent, three were on penicillin prophylaxis (with only one submitting stool for 16S rRNA sequencing), 15 were on folic acid, and 16 were on as needed laxatives. Two of the enrolled HCs (2/17) documented a sickle cell trait carrier status. Three participants (two in the SCD group and one in the HC cohort) documented receiving a multivitamin in their dietary questionnaire cohort, although they had responded "No" in the prescreening questionnaire. Fifty-four percent of the cohort were female and 73% of the cohort self-identified as Black/African American. The median age of participants was 12 years. Seventy-three percent of the cohort were enrolled in the winter, while 27% were enrolled in the spring (Table 1). For the 16S rRNA gene sequencing result, only reads with appropriate depth were utilized for analysis.

TABLE 1 Cohort demographic and clinical characteristics between the participants with SCD and matched age, sex, and race/ethnicity HC.

Characteristics	ALL ($n = 41$)	SCD ($n = 24$)	HC ($n = 17$)	*p*-Value
Gender, *n* (%)				.75[a]
Male	19 (46)	12 (50)	7 (41)	
Female	22 (54)	12 (50)	10 (59)	
Age, median (IQR)	12 (8)	12 (8)	12.0 (9)	.83[b]
HbS type				-
HbSC	–	8 (33)	–	
HbSS	–	16 (67)	–	
Consented months				.15[a]
11/12/1	30 (73)	20 (83)	10 (59)	
2/3/4	11 (27)	4 (17)	7 (41)	
Race and ethnicity				
Black	30 (73)	17 (71)	13 (76)	.74[a]
Hispanic	11 (27)	7 (29)	4 (24)	

Abbreviations: HbSC, hemoglobin SC; HbSS, hemoglobin SS; HC, healthy controls; SCD, sickle cell disease includes HbSS and HbSC.
[a]Fisher's exact test.
[b]Wilcoxon rank sum test.

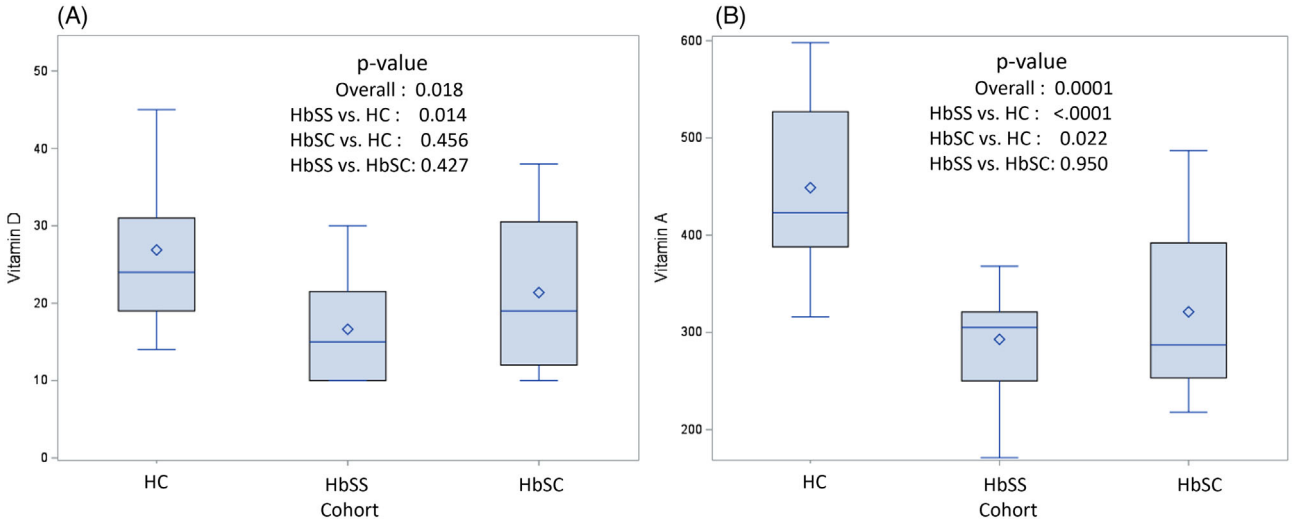

FIGURE 2 (A) Serum vitamin D levels between sickle cell diease (SCD) (HbSS and HbSC) and HC. Legend: Boxplot of serum vitamin A levels with interquartile range at the ends of the boxplots (25th and 75th), and whiskers extending to the maximum value above and minimum value below. The thick bar inside the boxplot represents the median, and the diamond represents the mean. *p*-Values were calculated using the median. This shows a significant difference between HC and HbSS participants. (B) Serum vitamin A levels between SCD (HbSS and HbSC) and HC. Legend: Boxplot of serum vitamin A levels with interquartile range at the ends of the box plots (25th and 75th), and whiskers extending to the maximum value above and minimum value below. The thick bar inside the boxplot represents the median, and the diamond represents the mean. *p*-Values were calculated using the median. This shows a significant difference between HC and HbSS and between HC and HbSC. HCs: healthy controls; HbSC: hemoglobin SC; HbSS: hemoglobin SS.

3.2 | Outcomes

3.2.1 | Vitamin D

The overall prevalence of vitamin D deficiency in the SCD cohort was 33% (8/24) participants as compared to 6% (1/17) HC participants ($p = .09$). Among SCD participants, vitamin D deficiency was identified in six participants (38%) with HbSS and two participants (25%) with HbSC. Comparing HbSS to HC, participants with HbSS were identified with significantly lower serum levels of vitamin D when compared to the HC cohort ($p = .01$) (Figure 2A). Further, the lower serum vitamin D levels persisted in the well-nourished SCD cohort when compared to the well-nourished HC cohort ($p = .01$). Next, this significant difference in serum vitamin D levels between the SCD cohort and HC persisted when adjusting the model for dietary vitamin D intake, which appeared similar between groups. This difference was most pronounced when we restricted the comparison to HbSS participants as compared to HC participants ($p = .02$). Finally, this difference in vitamin D levels persisted between HbSS and HC participants when adjusting the model for malnutrition; $p = .01$ and after adjusting the model for age, sex, and log-BMI ($p = .01$).

3.2.2 | Vitamin A

The vitamin A levels were measured on 24 participants with SCD, and 15 participants in the HC cohort. Five (21%) participants with SCD were vitamin A deficient versus 0% of HC ($p = .14$) comparing serum vitamin A status as a categorical variable (deficient vs. not deficient). However, the serum vitamin A levels were lower in the HbSS cohort, and the HbSC cohort when compared to the HCs ($p < .001$ for HbSS vs. HC; $p = .02$ for HbSC vs. HC) as a continuous measure (Figure 2B). After adjusting the model for malnutrition, the difference in vitamin A levels persisted with a statistical significance of both HbSS ($p < .001$) and HbSC ($p \leq .01$) when compared to the HC cohort. Finally, the difference in vitamin A levels identified between HbSS and HC and between HbSC and HC persisted after adjusting the model for age, sex, and log-BMI with *p*-values of less than .001 and .03, respectively.

Only six participants in the SCD cohort and two participants in the HC cohort met recommended dietary allowance of vitamin A for their age. Dietary intake of vitamin A was significantly associated with serum vitamin A level ($p < .001$). Therefore, children who consumed higher levels of vitamin A in their diet had higher vitamin A levels and those with lower vitamin A dietary intake had lower serum levels of vitamin A.

3.2.3 | Vitamin E

We identified no difference between serum vitamin E levels between the SCD cohort and HC cohort under univariate analysis or additional analysis with adjustment for malnutrition. Dietary intake of vitamin E did not differ between groups.

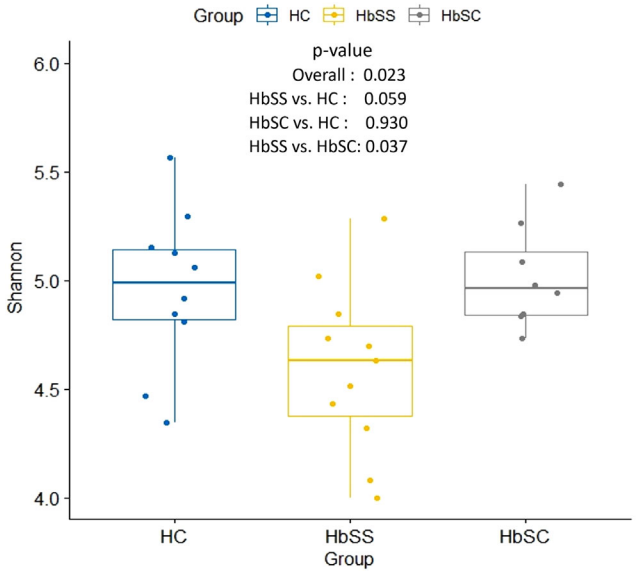

FIGURE 3 Alpha diversity (Shannon index) SCD (HbSS and HbSC) and HC. Legend: This boxplot with interquartile range and median shows a significant decrease in the alpha diversity in the HbSS cohort when compared to the HbSC participants. The diversity is also decreased in participants with HbSS when compared to HC, but this did not achieve statistical significance. HCs: healthy controls; HbSC: hemoglobin SC; HbSS: hemoglobin SS.

3.2.4 | Presence of exocrine pancreatic insufficiency

All children in our study who completed evaluation of exocrine pancreatic insufficiency by fecal elastase (17 children in SCD cohort and nine children in HC cohort) had levels higher than 200 μg/g. Most of these children (76% in the SCD cohort and ~78% in the HC) had fecal elastase levels of higher than 800 μg/g. The lowest fecal elastase level seen in the SCD cohort was 306 μg/g, while that in the HC cohort was 499 μg/g.

3.2.5 | 16S rRNA gene sequencing results

The alpha diversity described as specie richness, measured with faith phylogenetic diversity was significantly different between the SCD cohort and HC cohort ($p = .02$). This difference was particularly seen between the HbSS cohort and HC cohort ($p = .01$), with the HC cohort being more diverse than the HbSS cohort. Additionally, alpha diversity as measured by Shannon entropy was significantly different between HbSS and HbSC and marginally significant between HbSS and HC. The Shannon alpha diversity was significantly reduced in the HbSS when compared to the HbSC cohort ($p = .02$) (Figure 3), while this diversity trended toward significance when HbSS is compared to HCs ($p = .05$). This significant difference persisted when the model was adjusted for age, sex, and log-BMI with p-value = .03 when HbSS is compared to HC and $p = .02$ when HbSS is compared to HbSC. Beta diversity, defined as the evenness between samples, was not significantly different between groups.

Certain species, including *Veillonella dispar*, *Eubacterium dolichum*, *Eggerthella lenta*, *Streptococcus anginosus*, were more abundant in the SCD cohort when compared to the HC cohort while *Dorea formicigenerans* was more abundant in the HC cohort. However, these differences did not remain statistically significant after controlling the FDR for multiple comparisons.

3.2.6 | Quality-of-life questionnaires

All participants in the SCD cohort completed the QoL questionnaires. All participants below 8 years had the parent version of the PedsQoL SCD module completed by a parent or caregiver. There were two participants in the SCD cohort who were 8 years old. One of the participants completed the self-version of the PedsQoL SCD module (8–12 years), while the parent of the other 8 years old completed the parent version.

Certain bacteria phyla, family, genera, and species were seen in relative abundance in children with SCD with lower overall mean QoL scores and QoL scores in the pain domains when compared with higher scores (Figure 4 and Table S1). *Ruminococcus lactaris* specie had higher relative abundance in children with SCD who had lower mean overall QoL scores when compared to those with higher scores. Additionally, the gut microbiota profile was different in children who had less than three hospitalizations when compared to those with three or more hospitalizations. However, statistical testing was not done given the limited number of participants with three or more hospitalizations (only two participants). Of the two participants in the SCD cohort who had three or more hospitalizations in the past year, one had HbSC genotype and had normal vitamin D levels, while the other had HbSS and had insufficient vitamin D levels. Both participants also had malnutrition (as defined by BMI z score ≤ -1), but had normal vitamin A levels. Additionally, malnutrition was associated with lower mean QoL scores in the pain domain ($p = .02$).

3.3 | Hospitalizations

Only two (~11%) of the 19 children with SCD who had 16S rRNA gene sequencing results completed had three or more hospitalizations and acute care visits for pain. One (50%) out of these two had vitamin D insufficiency (HbSC), while the other was vitamin D sufficient (HbSS phenotype). Both participants were malnourished.

4 | DISCUSSION

Our study provides additional evidence to support screening for FSV deficiency regardless of nutritional status. Further, we identified that FSV deficiencies in children with SCD occurred in the setting of normal exocrine pancreatic function. These FSV deficiencies may impact clinical outcomes, particularly pain outcomes.[10,11,18,20] A few studies have suggested that decreased dietary intake may contribute to

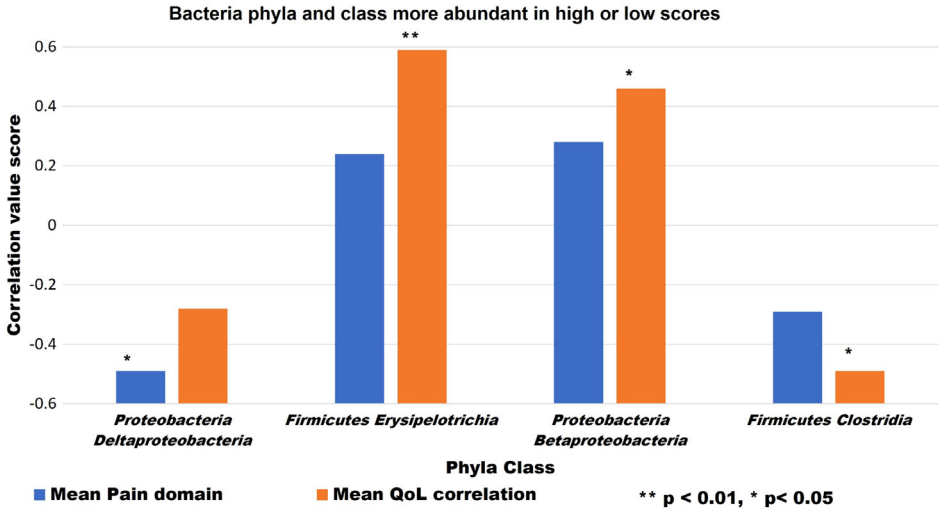

FIGURE 4 Bar chart of bacterial phyla and class that were differentially expressed in participants with sickle cell diease (SCD) who have higher and lower mean pain domains and mean overall quality-of-life (QOL) scores.

this deficiency,[9,21] with one case series of five children with SCD who had growth retardation showing they had normal fat absorption and intestinal biopsies.[22] Similarly, all the fecal elastase levels obtained from our SCD cohort were above 200 μg/g, with more than 75% being above 800 μg/g. Our study also showed that dietary intake of specific vitamins correlated with the vitamin levels. However, dietary intake of specific micronutrients was similar in both SCD and HC cohorts. This suggests that children with SCD may require a higher dietary vitamin intake to maintain normal serum vitamin levels.

Poor nutritional status has been shown to be prevalent in individuals with SCD.[35–38] Limited studies also show that malnutrition and undernutrition affect QoL and are associated with frequent hospitalizations.[35,39] Similarly, our study showed significant associations between nutritional status and worse QoL scores in the pain domains and overall mean QoL score in the SCD cohort.

Furthermore, HbSS was associated with significant gut dysbiosis (reduced alpha diversity) when compared to the HbSC cohort and reduced alpha diversity compared to HC, but this did not achieve statistical significance. Gut microbiota taxonomic profile was different in children with decreased mean QoL scores. Malnutrition defined by BMI *z* score less than or equal to −1 was also associated with decreased QoL scores. Although, statistical testing was not done on children with more frequent hospitalizations given their limited number (two participants), their gut microbiota was different from those with fewer hospitalizations. To our knowledge, this is the first study to demonstrate an association between gut microbial profile and pain outcomes in children with SCD. It is also the first study to show that gut microbial diversity is similar in children with HbSC when compared to HC.

Few studies have shown prevalence of gut dysbiosis in individuals with SCD.[23–26] Most of the studies done were in adults. While Lim and colleagues included children from ages 10 and above, the median age in their cohort was 31 years and they recruited individuals with sickle cell trait as controls.[26] They observed differences at the microbial composition when they modeled the top 15 bacterial genera, but did not find differences at the phylum level or in diversity. Delgadinho and colleagues showed that Angolan children (3–14 years)[40] had higher Actinobacteria phylum, and *Clostridium* were more prevalent in the SCA population. In our study, the gut microbial diversity was similar between the HbSC (less severe form of sickle cell disease) and HC, but the alpha diversity was different between the SCA (HbSS) cohort and HC, although the difference seen did not achieve statistical significance. This was not seen for beta diversity. It is possible that our sample size may have contributed to this. It is also possible that antibiotic exposure seen in the SCD population could have contributed to this. Children with SCD are usually placed on prophylactic penicillin to prevent invasive pneumococcal infections until around age 5.[41] Although, this may have played a role, the youngest ages for the 16S rRNA gene sequencing results were 7 years for HbSS (SCA), 6 years for HbSC, and 5 years for HC. Additionally, children with SCD are frequently exposed to antibiotics when they have acute chest syndrome,[42] which could also contribute to the dysbiosis seen. We attempted to reduce the effect of antibiotic exposure on the gut microbiome, by excluding children within 30 days of completion of antibiotics. Furthermore, alterations in gut perfusion, oxidative stress, and other factors present in the gut of individuals with SCD may contribute to the gut dysbiosis seen in these individuals. *Ruminococcus* species, which has been implicated in pediatric inflammatory bowel disease,[43] were seen in overabundance in children with SCD with decreased QoL when compared to those with higher QoL scores. *Veillonella* species, which have been seen in patients with concomitant primary sclerosing cholangitis and inflammatory bowel disease,[44] were also seen in overabundance in the SCD cohort compared to the HC. Therefore, although our sample size is small, this warrants further study.

Some of the limitations of our study include a small sample size, and that it is a case–control study completed in a single institution. We used a retrospective chart review to conduct acute pain assessment, and this could potentially have missed daily pain episodes. Both the food frequency and QoL questionnaires are subject to recall bias.

Additionally, only 16S rRNA gene sequencing was done, and shot gun metagenomic sequencing was not done. However, we hope that this study will serve as a premise for larger studies in this area that would utilize metagenomics to show both taxonomic and functional profiles and how they affect pain outcomes in individuals with SCD. Additionally, we decided to concentrate on the FSV that have been shown to be associated with worse pain outcomes in this pilot study. Further studies in this area should include estimates of past antibiotic exposure and assessment of vitamin K, given that reduced gut microbiota diversity seen in the HbSS population may also affect these levels from decreased production of vitamin K2 (menaquinone) by gut bacteria.[45]

In conclusion, our study showed decreased serum levels of vitamin A and D in the cohort of participants with SCD despite having similar intake of these micronutrients as the HC with normal exocrine pancreatic function (measured with fecal elastase). This suggests that children with SCD may require a higher intake of FSV than their peers to maintain normal FSV levels. Additionally, reduced gut microbial diversity exists in the HbSS cohort when compared to HbSC and HC cohorts. This may explain the worse outcomes seen with HbSS genotype compared to HbSC genotype. It is possible that manipulation of the gut microbiome with pre- or probiotics in patients with HbSS may decrease their disease severity. Certain bacterial taxonomic profiles correlate with lower QoL scores (both overall mean and pain domain scores) in the SCD cohort. This pilot, single-center study provides preliminary data to design larger, multicenter trials investigating these findings and the potential of manipulating the gut microbiome as a possible treatment strategy for SCD pain outcomes.

AUTHOR CONTRIBUTIONS

The authors made significant contributions as follows. Chinenye R. Dike: conception, design, data acquisition, data interpretation, drafted the initial manuscript, critically reviewed and revised the manuscript. Corinne Hanson: design, data interpretation, and critically reviewed and revised the manuscript. H. Dele Davies: design, data interpretation, writing, and critically reviewed and revised the manuscript. Stephen Obaro: conception, design, data interpretation, critically reviewed and revised the manuscript. Fang Yu: design, data analysis, data interpretation, critically reviewed and revised the manuscript. James Harper and Chittalsinh Raulji: design, data acquisition of participants with sickle cell disease, critically reviewed and revised the manuscript. Helen Grace: design, data acquisition of HCs, critically reviewed and revised the manuscript. Jeffrey Lebensburger: design, writing, critically reviewed, and revised the manuscript. Jihyun Ma: data analysis, data interpretation, critically reviewed and revised the manuscript. Peter Mannon: conception, design, data analysis, data interpretation, writing, critically reviewed and revised the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflicts of interest relevant to this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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