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REVIEW
Micronutrients and Sickle Cell Disease, Effects on Growth, Infection and Vaso-Occlusive Crisis: A Systematic Review

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Patients with Sickle cell disease (SCD) exhibit signs of poor growth, increased susceptibility to infection and recurrent episodes of painful vaso-occlusive crises. Micronutrient deficiencies may increase susceptibility to these outcomes. We conducted a systematic review to assess the strength of evidence for improved outcomes related to micronutrient interventions. Six randomized-controlled trials of moderate quality met the inclusion criteria. Zinc supplementation was associated with improved growth and decreased incidence of infection and is a promising intervention in the management of SCD patients. Omega-3 fatty acid supplementation was associated with limited reduction in vaso occlusive crises. This review identifies key knowledge gaps, which are important research priorities for nutritional interventions. Pediatr Blood Cancer 2012;59:211–215. © 2012 Wiley Periodicals, Inc.

Key words: growth; infection; micronutrients; sickle cell disease; vaso-occlusive crisis

INTRODUCTION

Sickle cell disease (SCD) is an inherited disorder of hemoglobin, with the highest incidence in sub-Saharan Africa and the Caribbean [1] and is designated by the World Health Organization as a public health priority [2]. SCD is characterized by chronic hemolytic anemia and vascular occlusion. Patients with SCD may experience growth impairment [3,4], increased susceptibility to infection [5,6], acute painful episodes [vaso-occlusive crises (VOC)], and irreversible damage to vital organs [1,7,8]. Micronutrient deficiencies have been cited as possible contributors to the frequency and severity of these complications. However, the clinical significance of micronutrient deficiency in SCD is not well understood [7,9].

Energy and nutrient supply may be limited in SCD due to a combination of factors including: (i) reduced intake and absorption secondary to anorexia [9,10], as well as through damage to the intestinal mucosa [11,12]; (ii) a hyper dynamic circulation [7] and increased requirements from an elevated basal metabolic rate [13,14]; (iii) raised requirements secondary to increased production of red cells (e.g., folate [15], B6, and B12 [16]) and (vi), loss of nutrients through increased renal excretion [13]. Several studies have reported reduced or deficient blood concentrations of micronutrients in SCD, including vitamins B6, B12 [17], C [18], E [19], and D [20]), magnesium [21], omega-3 fatty acids [22], and zinc [23].

The NHS Sickle Cell & Thalassaemia Screening Programme advises zinc supplementation in children with SCD who are growth retarded [9]. Although micronutrient supplementation might influence SCD-related morbidity, a systematic evaluation of the effect of micronutrient supplementation has not been reported. It is essential to identify and summarize the available evidence and to assess its methodological quality. This will identify intervention priorities and potential knowledge gaps in this high-risk group of children. We have conducted a systematic literature review to address the following question: does supplementation of vitamins (A, B6, B12, C, D, and E), iron, magnesium, folic acid, omega-3 fatty acids, or zinc in patients with SCD improve their growth, reduce susceptibility to infection or reduce the frequency of VOC?

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METHODS

Literature Search and Study Identification

This systematic review was undertaken according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (referred to as ''PRISMA'') Statement [24]. The trials considered for inclusion in the current review were identified through searches in MEDLINE (National Library of Medicine, Bethesda, MD), EMBASE (Elsevier, Amsterdam, the Netherlands), Lilacs (database on Latin American and Caribbean Health Sciences), the Cochrane Central Register of Controlled Trials (CENTRAL; Wiley InterScience, Hoboken, NJ) and computerized bibliographic databases spanning the years 1966–2011. The searches were completed in June 2011. To minimize possible publication bias, searches were also conducted on databases that contained unpublished scientific trials in order to identify all available trials that were not captured in the database searches. These databases included ''ClinicalTrials.gov,'' and ''ClinicalTrialResults.org.'' In the electronic databases, a structured strategy included searches with key words including: ''sickle cell anemia,'' ''sickle cell disease,'' ''folic acid,'' ''zinc,'' ''vitamin A,'' ''vitamin B6,'' ''vitamin B12,'' ''vitamin C,'' ''vitamin D,'' ''vitamin E,'' ''iron,''

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"magnesium," "omega-3 fatty acids," and key words regarding "growth," "vaso-occlusive crisis," and "infection."

Inclusion Criteria

Located studies were subject to the following inclusion criteria: randomized and controlled; human participants which were diagnosed with homozygous SCD (Hb-SS) or sickle beta-zero-thalassaemia (Hb-S/β0); data on micronutrient status at baseline and following supplementation; the outcome variable was a validated measure of growth, infection and/or VOC. Studies that did not meet these inclusion criteria were excluded. When more than 20% of participants included had other forms of SCD [e.g., sickle-hemoglobin C disease (Hb-SC), sickle beta-plus-thalassaemia (Hb-S/β+) or were carriers of SCD (Hb-SA)], the study was excluded. After removal of duplicates, titles were reviewed and screened for relevance. Empirical trials that appeared potentially relevant were then identified independently by two of the authors (KF, LD) by checking the abstracts. Of those trials that seemed relevant, full articles were obtained and were then read to ensure that they met inclusion criteria. Bibliographic citations for each of the articles ultimately selected for inclusion in the analysis were also examined, with the use of ISI Web of Knowledge database, to identify any other trials that were not captured by the initial searches.

Data Extraction and Assessment of Methodological Quality

To ensure that appraisals were drawn from methodologically sound trials and to reduce bias at the study level, each article was rigorously analyzed for methodological quality using the Consolidated Standards of Reporting Trials (CONSORT) statement [25], which concerns the following items: method of randomization, concealment of allocation, blinding of investigators, patients, and outcome assessor, follow-up and intention-to-treat analysis. Data on study characteristics was extracted by a standardized data extraction form. Three authors (KF, MB, LD) assessed the methodological quality of the trials by modified quality assessment scales (Table I in the online issue). Based on the CONSORT statement, trials were divided into three categories: (A) all quality criteria met (low risk of bias), (B) it was uncertain whether quality criteria are met (moderate risk of bias), and (C) one or more criteria not met (high risk of bias). Discrepancies between the authors in terms of data extracted, choice of articles meriting inclusion and quality assessment were resolved by discussion and consensus.

RESULTS

Description of Trials and Study Participants

Our initial search yielded 2,099 literature citations (Fig. 1). After exclusion of 361 duplicates, 1,738 unique references remained. Of these, 1,549 were excluded after scanning titles or reading the abstracts. Of the remaining 189 unique articles, 183 were excluded after full article review. Six randomized-controlled trials (RCTs) remained and were included in the final selection [23,26–30]. As shown in Table I, these trials were conducted between 1983 and 2008. Four trials were from the USA [23,27,28,30], one from India [29] and one from Jamaica [26]. The trials varied in sample size (10–130 participants), and the age of participating SCD patients ranged between 6 months to 49 years. The effects of zinc, folic acid, and omega-3 fatty acid supplementation on SCD morbidity were evaluated. No trials were found studying the effect of vitamin E, D, and/or C, iron and magnesium supplementation in relation to the selected biological outcomes among SCD patients.

Quality Assessment

Quality was assessed by classification according to risk of bias. Five studies were classified as a moderate risk of bias (Table II). One trial had a high risk of bias as the authors did not report whether, or how, randomization had taken place, follow-up was insufficient and no intention-to-treat analysis was performed [27]. Except for one trial [28] allocation was unclear, and therefore indication bias was possible in most trials (score "B"). Performance bias by unequal provision of care in the two groups, influencing their treatment response was a concern in three trials (score "B") [23,27,30]. The percentage of dropouts ranged from 0% to 20% and was equally divided among intervention and control groups.

Growth

Zemel et al. [23] reported that children supplemented with 10 mg/day elemental zinc showed a significantly increased mean (±SE) height after 1 year of supplementation (0.66 ± 0.29 cm/year, ($P = 0.004$), but height-for-age z-scores did not differ between zinc and control groups ($P = 0.07$). In a subgroup of 24 children, whose initial height status was low (height-for-age z-score < -0.15), zinc-supplemented children showed better height gain, which was 1.3 cm/year more than that of the control group ($P < 0.0001$). An RCT with folic acid supplementation (5 mg/day) observed no differences in growth between intervention and control groups after a 1 year of follow-up [26].

Infections

Bao et al. [28] reported that supplementation with 50 mg zinc for 3 months to adult patients with SCD reduced the total number of infections from seven to one ($P = 0.022$) over a 6-month follow-up period. Prasad et al. also observed a reduction in respiratory infections among a zinc deficient group receiving zinc supplementation (from mean ± SD:1.3 ± 1.1 to 0.4 ± 0.6) compared to a (placebo-) control group (0.90 ± 0.9 to 0.60 ± 0.8, *P*-values not provided) [30]. Another trial among patients aged 12–27 year old [29] reported a reduced number of infection episodes in SCD patients who were supplemented with 22 mg zinc sulphate three times daily. The number of infective episodes was 204 in the control group compared to 108 in the intervention group ($P < 0.01$), after a 1.5-year follow-up period. A RCT on the effect of folic acid supplementation on infection incidence reported no differences compared to controls [26].

VOC

Gupta et al. reported that the number of VOC episodes was significantly less in the group that received 22 mg zinc sulphate three times daily compared to the control group after 1 year

TABLE I. Characteristics of Trials Included in this Systematic Review

Lead author (year), Country	Type of SCD	Mean baseline age[a] (year)	Male[a] (%)	No.[b] in intervention/ Patient-control groups	Mean follow-up (year)	Study design		Adjustments	Micronutrient (s) assessment				Biological outcome (s) assessed			Results in supplemented group
						Cases	Controls		Zn	Folic acid	Vit A	ω-3 FA	G[c]	I[d]	VOC[e]	
Bao (2008), USA; [28]	All Hb-SS	33.6	61.1	18; 18	0.25	25 mg Zn/2× day	Placebo	—	√				√	√	√	Decreased incidence of infections
Zemel (2002), USA; [23]	All Hb-SS	6.8	52.4	18; 20	1	10 mg Zn/day	Placebo	Age	√			√			√	Significantly greater mean increase in linear growth
Prasad (1999), USA; [30]	26 Hb-SS, 3 Hb-SC, 3 Hb-S β+/− thalassemia	19–49[f]	50	11; 10	1	Zn def +50–75 mg Zn/day	Zn def + placebo	—	√				√	√	√	Significant decrease in incidence of infections. No difference in VOC
Gupta (1995), India; [29]	All Hb-SS	12–27[f]	46.0	65; 65	1,5	22 mg Zn/3× day	Placebo	—	√				√	√	√	Reduced mean number of VOC
Tomer (2001), USA; [27]	Not specified	—(adult)	—	5; 5	1	0.1 g/kg/day ω-3 FA[g]	Olive oil				√				√	Reduced frequency of VOC
Rabb (1983), Jamaica; [26]	All Hb-SS	0.5–4[f]	—	41; 39	2	Folic acid	Placebo	Age, sex		√		√	√	√		No significant difference in growth characteristics, minor or major infections and VOC

[a]In intervention group. [b]Effective sample size. [c]Growth. [d]Infection. [e]Vaso-occlusive crisis. [f]Range as provided by the authors. <30 μg/dl; > = 30 μg/dl; Defined as <10.7 μmol/L; Defined as 10.7–23.0 μmol/L. [g]Dietary n-3FA therapy (0.1 g/kg/day) was provided as menhaden fish oil (0.25 g/kg/day) containing 12% eicosapentaenoic acid (EPA), and 18% docosahexaenoic acid (DHA).

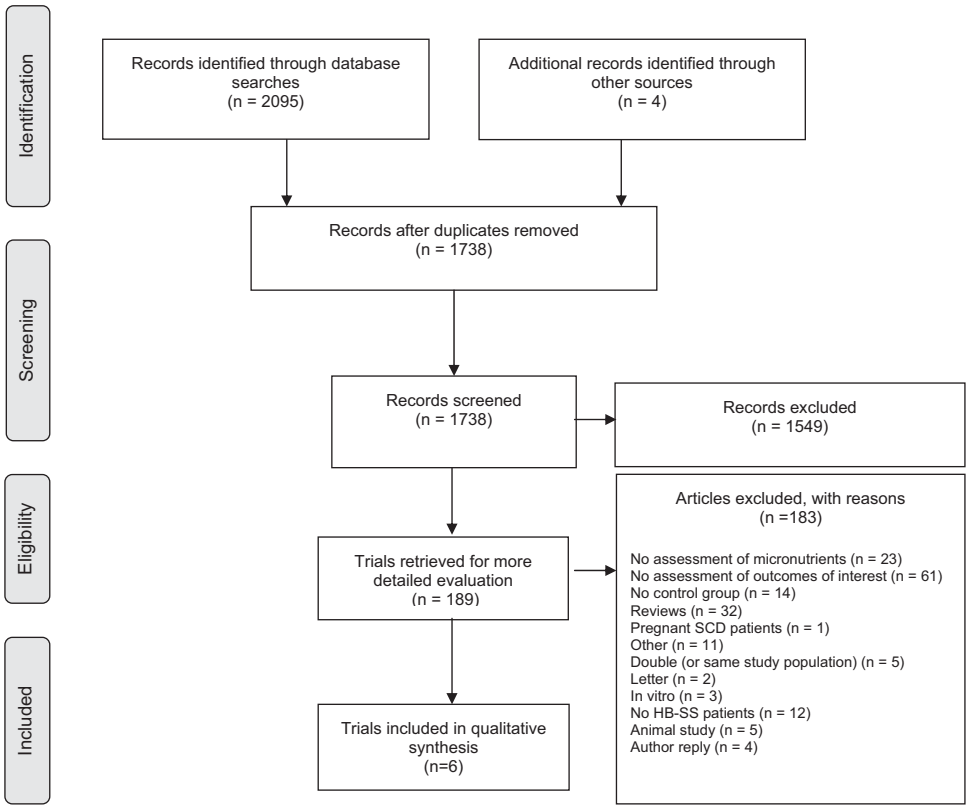

Fig. 1. Prisma flow diagram of study selection and review.

follow-up (mean ± SD: 2.5 ± 1.0 and 5.3 ± 2.6, respectively, $P < 0.025$) [29]. However, Boa et al. [28] and Prasad et al. [30], found no differences in number of (hospital admissions for) VOC episodes between the zinc supplemented group and the control group.

With supplementation of 5 mg folic acid, Rabb et al. [26] did not find a difference between the proportion of children with VOC compared to controls (31% and 37%, respectively, $P > 0.05$).

Tomer et al. [27] assessed the effect of omega-3 FA on VOC in a small trial of 10 adult patients. After one year of supplementation, the frequency of VOC was reduced to 50% in subjects receiving dietary omega-3 fatty acids compared to control subjects receiving olive oil (7.1 and 3.8 episodes/year, $P < 0.01$). As this was a very small study that was classified to have a high risk of bias the results should be interpreted with caution.

TABLE II. Quality Appraisal

	Risk of Bias Assessment						
	Randomization (selection bias)	Concealment allocation (indication bias)	Blinding 1 (performance bias)	Blinding 2 (detection bias)	Follow-up (attrition bias)	Intention-to-treat analysis	Category
Randomized-controlled trials							
Bao et al. [28]	A	A	A	A	B	Done	B
Zemel et al. [23]	B	B	B	B	A	Done	B
Tomer et al. [27]	B	C	B	A	B	Not done	C
Prasad et al. [30]	B	B	B	A	A	Done	B
Gupta and Chaubey [29]	B	B	A	A	A	Done	B
Rabb et al. [26]	B	B	A	A	A	Done	B

Categories used: A = all quality criteria met (low risk of bias); B = when it is uncertain whether quality criteria are met (moderate risk of bias); and C = one ore more criteria not met (high risk of bias).

CONCLUSION

In this systematic review of six RCTs assessing the role of micronutrient supplementation on SCD morbidity zinc supplementation was identified as resulting in significant improvement of growth, as well as reducing infectious episodes. The effect on VOC was less convincing. Conversely, folic acid had no effect on these three outcome variables.

We used a systematic review method and appraised the quality of all included trials to estimate risk of bias. There was a moderate to high risk of bias for individual trials because information was lacking on essential items such as: type of randomization, selection procedures, blinding methods, and potential adjustments. The power of all trials was limited by the small number of participants that were included in these studies.

Randomized-controlled trials are the optimal design to determine the effect of an intervention. Yet, none of these trials were reported according to the CONSORT statement [25], which is a minimum set of recommendations for reporting randomized controlled trials [31]. Drawing general conclusions regarding benefits of supplementation of specific nutrients in the management of SCD is challenging as different target populations were enrolled in this review (e.g., children vs. adults) and because the definition of the various outcome variables differed across trials (e.g., duration of VOC varying from 2 to 6 hours). As we have limited our review to studies including participants with Hb-SS (in order to reduce heterogeneity) the results should be applied with caution to other types of SCD, such as HbSC. Due to this heterogeneity among the included trials and the small number of trials identified it was not possible to perform a quantitative meta-analysis. This is unfortunate, as this also precluded analysis on geographical and dose aspects of micronutrient supplementation.

Zinc supplementation is a promising therapeutic modality for SCD patients as it may improve growth and reduce incidence of infections. The effect on the number of VOCs may only occur after a certain minimum period of supplementation. No effect is seen when it is given for a shorter period of 3 months, whereas a clear reduction in VOCs occurs when it is given for up to a year [28,30]. As the number of RCTs is limited, further evidence for the association between micronutrient status and SCD morbidity may be found in cross-sectional studies. An association between zinc deficiency and growth impairment in SCD was observed by Leonard et al.[32] and Phebus et al. [11]. However, this could not be confirmed by three other studies [33–35].

In conclusion, it is clear that the possible association between SCD morbidity and micronutrient deficiency has received little research attention. Nutritional interventions may play an important role in improving survival in SCD patients, but specific interventions need to be appropriately tested before any recommendations can be made. Currently, available data indicates that zinc supplementation is potentially a promising strategy for morbidity reduction in SCD. Larger prospective trials confirming the beneficial effects of zinc supplementation in children with SCD are required. This review highlights the huge knowledge-gap with respect to the role of micronutrients in SCD-related morbidity and the need for well designed, properly powered RCTs. Improving our understanding of the interaction between micronutrients and SCD morbidity may allow early nutritional interventions, especially as neonatal SCD screening is now being performed in many countries. This will be especially important in low resource settings where these children have the highest mortality.

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