Nutritional Status of Sickle Cell Patients: A Literature Review

Minjoon Hur

Abstract

Sickle cell disease (SCD) is a lethal, life-long condition characterized by a mutation in the gene that codes for hemoglobin. To alleviate the pain experienced by sickle cell (SC) patients, adequate nutrition levels are vital as deficiencies of vitamins or minerals may cause other symptoms. This review aims to provide an overview of the nutrient status of SC patients and propose research areas where further study is required. The review summarizes twelve primary research papers that assessed the levels of vitamins A, C, D, E, B-2, B-6, B-12, folate, magnesium, iron, zinc, and copper in SC patients. Most SC patients exhibited suboptimal levels of vitamin A, while deficiencies in vitamin D and B-2 were prevalent. Their zinc and vitamins B-6, B-12, C, and E levels were generally adequate, while elevated folate and copper levels were observed. The magnesium levels in SC patients were lower than those in the healthy population. Determining the status of iron concentration in SC patients was challenging due to the limitations associated with the measurement methods employed in the reviewed papers. There were notable differences in some of the measurement methods used for the same nutrient levels. More research is needed to find the optimal amount of vitamin A supplement for SC patients. The effect of zinc supplementation on copper levels and the cause of low zinc levels in SC patients remains unexplored.

Keywords

Sickle Cell Disease; Malnutrition; Vitamins; Minerals.

Introduction

Sickle cell disease (SCD) is a group of inherited genetic disorders characterized by a mutation in the gene that codes for hemoglobin subunit β.[1,2] The gene mutation replaces the glutamic acid with valine at position 6 in the β-globin chain.[1] The change in the primary structure of the polypeptide alters the product from Hemoglobin A to Hemoglobin S (HbS), producing erythrocytes with sickle shapes under low oxygen tension.[2] Increased sickle erythrocyte concentration causes vaso-occlusion (blockage of blood vessels), which leads to insufficient oxygen transport to tissues and organs.[2] Vaso-occlusion causes frequent pain in bones and joints, inflammatory response, and oxidative stress.[2] These symptoms lower the life expectancy of SCD patients to 54 years, making it a lethal condition as those without SCD have a life expectancy of 76 years.[4] The trait for SCD is not uncommon, as about one in twenty people in the human population carries the trait gene for hemoglobin (Hb) disorders: HbS.[3]

The severity of the SCD is determined by the concentration of HbS, which depends on the patient's genotype.[2] The most severe genotype is HbSS, followed by HbSβ^0-thalassemia, HbSβ^+-thalassemia, and HbSC.[2]

Proper nutrition is vital for overall health and in decreasing the morbidity of diseases.[6] This is particularly relevant for individuals with SCD, as maintaining optimal nutritional status can significantly enhance their quality of life with the widely accessible cure for SCD remaining as an unresolved issue.[5] This paper focuses on examining the significance of specific essential nutrients in SC patients, namely vitamins A, C, D, E, B-2, B-6, B-12, folate, magnesium, iron, zinc, and copper. When one lacks adequate intake of any or some of these essential nutrients, one may develop deficiency symptoms, which will be discussed in detail in the subsequent sections dedicated to each nutrient. To reduce the risk of an individual developing these symptoms, the Recommended Daily Allowance (RDA) has been established.[5] However, the RDA may not be sufficient for SCD patients as it is made for the general healthy population rather than individuals with specific medical conditions.[5]

To prevent SC patients from developing nutritional diseases, this paper will outline the nutrient deficiencies and elevations present in SC patients using the primary data gathered by researchers. Most of the primary data will be from papers published after 2010, as a review paper on nutrition in SCD was published that year.[5] With the latest primary data, this paper will provide an updated review of the nutrition status of SC patients.

Methods

Twelve primary research papers that investigated nutrient levels in SC patients were collected and summarized. Two of the 12 research papers collected were randomized clinical trials; the rest were cross-sectional studies. The nutrients evaluated in the papers were vitamins A, C, D, E, B-2, B-6, B-12, folate, magnesium, iron, zinc, and copper. For each nutrient, the nutrient deficiency cutoff was researched and noted. Units of the deficiency cutoff values were manually converted to match the units of the measured values in the primary research papers. The cutoff values were then compared to those obtained from SC patients to determine whether deficiencies or elevations of the nutrient were evident in SC patients.

Results & Discussion

Zinc

Zinc is essential for cellular and subcellular metabolism; it is an integral component of the catalytic sites of some enzymes and may have a specific role in many other biological proteins.[9] Zinc is also responsible for the absorption, mobilization, transport, and metabolism of vitamin A.[10] The serum zinc test is commonly used to measure zinc status in the body, with a serum zinc concentration of <60 μg/dL considered deficient.[11] This method was used by a cross-sectional study in India.[10] After recruiting 33 homozygous SC patients and 33 age and sex-matched healthy controls, the study compared the serum zinc level in SC patients to that of normal

controls.[10] The paper's main objective was to evaluate plasma vitamin A, C, and E levels and serum zinc and copper levels in adolescent patients with HbSS.[10] All subjects were aged 10-20 years and had no history of blood transfusion within three months, a factor that may significantly affect zinc concentration.[10,49] Subjects with chronic ailments, diabetes mellitus, or SCD cases with an acute crisis, together with patients on multivitamin supplementation, were excluded from the patient and control groups[10]. The serum zinc concentration in SCD and healthy controls were 83 ± 9 µg/dl and 104 ± 6 µg/dl, respectively.[10] The serum concentration in healthy controls was significantly higher than those in SCD patients ($p<0.001$).[10] Also, the values shown above suggest that no SC patients were zinc deficient because their serum zinc concentrations were greater than 60 µg/dL.[10, 11]

A cross-sectional study that took place in Saudi Arabia also concluded that there was no zinc deficiency in SC patients.[12] Using the same measurement method with 25 SC patients that have severe sickle cell anemia (SCA), the study was designed to evaluate levels of vitamins A, C, and E and elements zinc and copper.[12] The exclusion criteria of the study were individuals younger than 15 years of age, the presence of β- or α-thalassemia trait, glucose-6-phosphate dehydrogenase deficiency, regular blood transfusion, treatment with hydroxyurea, use of vitamin and trace element supplements other than folic acid, illness other than sickle cell manifestations, or pregnancy.[12] The mean measured serum zinc level was about 65 µg/dL, which is above the deficiency cutoff.[12] Nevertheless, the results still show that SCA patients have lower serum zinc levels than the controls.

Both papers show that SC patients have serum zinc levels lower than the normal controls but above the zinc deficiency cutoff value. Therefore, the SC patients in these studies do not have a zinc deficiency.

Vitamin A

Vitamin A is responsible for immune function, growth, development, reproduction, and, most importantly, vision.[13] The plasma retinol test commonly measures its concentration in the body, with a concentration of <20 µg/dL considered deficient and a concentration of 20-29 µg/dL considered suboptimal.[13] Vitamin A deficiency in patients may lead to night blindness, a decrease in growth rate, and a reduction in bone development rate.[14] To determine whether SC patients are also at risk of developing disorders related to vitamin A deficiency, a randomized controlled trial in the United States investigated the effect of giving supplemental vitamin A to children with SCD by measuring their plasma levels before and after the supplementation.[13]

The study aimed to determine whether three doses of supplemental vitamin A (300, 400, or 600 µg of retinyl palmitate per day for twelve months) on SCD would optimize vitamin A status in SC patients with the HbSS genotype. Subjects aged 2-13 years were enrolled.[13] The exclusion criteria were chronic transfusion therapy or a transfusion within the past two months,

hydroxyurea therapy, history of stroke, liver enzymes >3 times the reference range, height >2.0 standard deviations above the age and sex mean, participation in another intervention study, pregnancy, and other chronic conditions known to affect growth, dietary intake, or nutritional status.[13] The mean initial plasma retinol concentration of children with SCD was 26 ± 8 μg/dL: a level considered suboptimal but not deficient.[13] Though the mean plasma retinol concentration was above the deficiency cutoff value, 22 out of 96 patients were vitamin A deficient. After the twelve-month supplementation of 300/400/600 μg/d, the serum retinol values changed by -2.8 ± 2.4 μg/dL, 0.9 ± 4.1 μg/dL, and 3.6 ± 2.8 μg/dL, respectively.[13] The patients still had suboptimal vitamin levels after the supplementations, though.[13] Low vitamin A levels were also witnessed by Hasanato *et al*., as their SC patients also had a lower mean plasma retinol concentration than normal, healthy controls.[12]

Both papers lean towards the conclusion that SC patients have lower vitamin A levels than healthy controls and that there are some patients who are vitamin A deficient.[12,13]

Vitamin E

Vitamin E is the major lipid-soluble component in the cell antioxidant defense system.[15] As an antioxidant, it is effective against diseases caused by oxidation.[15,16] The most widely used method for its measurement is the serum α-tocopherol test, with a concentration of <5 μg/ml marking vitamin E deficiency.[17] This test was used by Wasnik *et al*. in 2017 to evaluate the vitamin E status of SC patients.[10] The design for this study has been thoroughly described in the subsection on zinc. The serum α-tocopherol count in SC patients and controls in the study were 9 ± 1 μg/mL and 12 ± 2 μg/mL, respectively.[10] The serum concentration in healthy controls was significantly higher than those in SCD patients ($p<0.001$).[10] Also, as the values shown were greater than 5 μg/ml, most SC patients in the study were not vitamin E deficient.[17] They were not expected to experience the symptoms of vitamin E deficiency.[17] Even so, lower levels of vitamin E should not be ignored as tissues needing α-tocopherol may be damaged when needs exceed the amounts available.[17]

Low levels of vitamin E in SC were also witnessed by Hasanato *et al*., as the mean serum vitamin E concentration of SC patients was about 2 μg/mL.[12] This value is far below the cutoff marking deficiency, and if the measurement was accurate, SC patients are at risk of experiencing increased infection, anemia, and stunting of growth.[17] The difference between the measured values may be due to their different measurement methods: the study of Wasnik *et al*. used the modified method of serum vitamin E estimation, while the study of Hasanato *et al*. used the long-term freezer storage method.[10,12,39,40] As the long-term freezer storage method may be erroneous due to the potential degradation of the sample over time, the values obtained by Wasnik *et al*. are probably more accurate.[39] Though their measurement methods differ, both papers show suboptimal vitamin E levels in SC patients.[10,12] Therefore, it is likely for SC patients to have suboptimal vitamin E levels.

Vitamin C

As an antioxidant, vitamin C is a micronutrient with pleiotropic functions.[19] It supports the human immune system and is a cofactor for a family of biosynthetic and gene-regulatory enzymes.[19] It is commonly measured by plasma ascorbic acid (vitamin C), with a cutoff value for deficiency of <11 μmol/L.[21] Hasanato *et al*. examined the vitamin C status of 25 SC patients by measuring their plasma levels of ascorbic acid.[12] The design for this study has been thoroughly described in the subsection on zinc. The study found that the mean plasma vitamin C in patients was approximately 57 μmol/L, indicating that SC patients were not vitamin C deficient.[12] However, the mean plasma level of vitamin C in the SC patients was about half that of the non-SC, healthy controls.[12]

A cross-sectional study in Nigeria also observed low vitamin C levels.[44] 80 SC patients with HbSS aged 1-15 years and 80 age and gender-matched healthy HbAA controls were recruited.[44] The exclusion criteria included the presence of febrile illness, history of blood transfusion in the past three months, history of hydroxyurea or chronic blood transfusion, and history of taking antioxidant supplements.[44] The study explored the relationship between the frequency of vaso-occlusive crises and plasma levels of antioxidant micronutrients, including vitamin C.[44] The vitamin C level of SC patients and controls in the study was 38 ± 10 μmol/L and 63 ± 17 μmol/L respectively.[44] The plasma concentration in healthy controls was significantly higher than those in SCD patients ($p<0.001$).[44] These values support the ones obtained by Hasanato *et al*. as the vitamin C levels in SC patients are above the deficiency cutoff but are lower than the controls.

The big difference between the ascorbic plasma levels in patients and controls made researchers conclude that SC patients have vitamin C deficiency; but as their plasma vitamin C levels are higher than the deficiency cutoff, SC patients have adequate vitamin C and are unlikely to experience the common symptoms of vitamin C deficiency.[12,20]

Copper

Copper is required in redox chemistry to produce many proteins, including enzymes[22]. The most widely used biomarker for evaluating copper status is serum copper, with a value of 64-140 μg/dL considered adequate.[23,41] By measuring this biomarker, a cross-sectional study in Ghana measured and compared copper levels in 90 SC patients aged 14-53 and 50 healthy controls aged 22-43.[22] Of the 90 SC patients, 41 had the HbSC genotype, and 49 had the HbSS genotype.[22] All 50 controls held the HbAA genotype, and any subjects with diabetes, renal disease, gastrointestinal disease, or coronary artery disease were excluded from the study.[22] As it aimed to evaluate serum iron, copper, and zinc levels in SC patients, the study also excluded patients with a history of blood transfusion three months before the study.[22] The mean serum copper levels of SC patients and controls were 221 ± 28 μg/dL and 114 ± 16 μg/dL,

respectively.[22] The serum concentration in healthy controls was significantly lower than those in SCD patients ($p<0.001$).[22] As the mean copper level of SC patients in this study was elevated, SC patients were at risk of developing stomach pain, extreme thirst, and changes in taste that may lead to decreased appetite.[23,24]

Elevated copper levels in SC patients were also seen in research done by Emokpae and Fatimehin.[45] Seeking to evaluate the serum copper and zinc levels in SC patients, 100 confirmed SC patients with a mean age of 19 ± 1 years and age-matched healthy subjects were recruited in the study.[45] The mean serum copper levels in SC patients and the controls were 184 µg/dL and 107 µg/dL, respectively.[45] The mean copper level of SC patients in this study, too, was elevated.[23,45]

Both papers show higher levels of copper in SC patients, so there was a copper elevation in SC patients in the studies reviewed.

Vitamin D

Vitamin D is crucial for calcium absorption and maintaining normal serum calcium and phosphate levels.[25] It also plays a vital role in immune function, cell proliferation, differentiation, and apoptosis.[26] These crucial tasks may not be completed efficiently when an individual is vitamin D deficient. Vitamin D deficiency (VDD) may also lead to osteoporosis (brittle bones) and increased fragility fractures.[42] VDD is commonly diagnosed by measuring the biomarker serum 25-hydroxyvitamin D (25(OH)D).[26] A serum 25(OH)D concentration of 30-60 ng/mL is optimal, while values below 30 ng/mL and 20 ng/mL are considered suboptimal and deficient, respectively.[26] By measuring and comparing the serum 25(OH)D level of 640 SC patients to the cutoff values, a cross-sectional study in Saudi Arabia evaluated the vitamin D status in SC patients.[26] All patients enrolled in the study were 12 years old and older, and there were no controls.[26] About 82% of the patients had suboptimal 25(OH)D, while 67% were deficient.[26] Middle-aged (45-65 years) and elderly (>65 years) patients had higher 25(OH)D levels than young patients, with females having slightly higher 25(OH)D levels than males.[26] Furthermore, SC patients with crisis had higher average 25(OH)D levels than those in steady state condition.[26] As most SC patients have insufficient serum 25(OH)D levels, they are also vitamin D deficient.

Folate, vitamins B-2, B-6, and B-12

Folate (vitamin B-9) is essential for producing red blood cells.[27] It is also vital in one-carbon metabolism, which is essential for synthesizing DNA and RNA.[27] Vitamins B-2, B-6, and B-12 are required to produce the universal methyl donor, S-adenosylmethionine.[27] To determine whether these metabolic reactions were carried out efficiently in SC patients, a cross-sectional study in Canada measured the folate concentrations and B-vitamin levels in the blood of Canadian children with SCD supplemented with 1 mg of folic acid daily as their regular

treatment.[27] All 11 patients were aged 2-19 years, with 8 holding the HbSS genotype, 2 holding the HbSC genotype, and 1 holding the HbSβ^0 -Thalassemia genotype.[27] The obtained mean concentration and the deficiency cutoff value of each B vitamin are listed below:

Table 1.
Obtained mean concentrations and the cutoff values for B vitamins in SC patients taking folate supplements. B-vitamin biomarker concentrations were measured in plasma (n = 8) and serum (n = 3) for folate forms, in plasma (n = 10) and serum (n = 1) for vitamin B-12, and in plasma for pyridoxal 5′-phosphate (n = 11) and vitamin B-2 (n = 11).[27]

Vitamin	Obtained mean concentration (nmol/L)	Deficiency cutoff value[27] (nmol/L)
B-2 riboflavin	15.9	26.5
B-6 pyridoxine	36.9	30
B-9 folic acid	62.0	10
B-12	0.4	0.15

As seen in Table 1, SC patients supplemented with 1 mg/d of folic acid were not deficient in vitamin B-6, B-9, or B-12, but were deficient in vitamin B-2.[27] However, without the supplement, an increase in patients with vitamin B-6 deficiency is expected as supplemental folic acid can reduce plasma levels of pyridoxal 5'-phosphate, an activated form of vitamin B-6.[27,46] None of the patients showed folate deficiency.[27] The mean total folate level (62.0 nmol/L) exceeded the limit for normal folate (45.3 nmol/L), indicating that the patients had elevated folate after the supplement.

Magnesium
Magnesium is key in many cellular processes, including intermediary metabolism, DNA replication and repair, and transporting potassium and calcium ions.[28] Serum magnesium measures magnesium levels in the body, with a deficiency cutoff value of 0.85 mmol/L.[29] Using serum magnesium as an indicator of magnesium status in subjects, a cross-sectional study measured the magnesium content in 120 SC patients with HbSS and HbSC genotypes and 48 healthy controls with the HbAA genotype.[32] Patients with coronary artery disease, diabetes mellitus, hypertension, renal failure, pregnancy, and a history of blood transfusion within three months before the study were excluded.[32] The patients and the controls had serum magnesium levels of 0.80 ± 0.24 mmol/L and 0.90 ± 0.11 mmol/L, respectively.[32] As the mean serum magnesium level of SC patients is below the deficiency cutoff value, SC patients in the study were magnesium deficient.

The lower serum/plasma magnesium content in SC patients was also shown by a randomized trial that measured the plasma and red blood cell (RBC) magnesium content in 10 HbSS treated with oral magnesium supplements (0.6 meq/kg per day of magnesium pidolate).[30] The study aimed to evaluate whether magnesium supplementation to SC patients reduces the number of sickled erythrocytes. The trial enrolled 45 patients who were 19 years and older, along with 17 non-SC controls with a mean age of 32 ± 9 years.[30] The inclusion criteria were normal renal and liver function, 70% or greater performance status, and no blood transfusions during the preceding three months.[30] Using plasma magnesium and erythrocyte magnesium as the biomarkers, the authors measured the magnesium content in HbSS and HbSC patients before the supplementation, 14 days after the supplementation, and 28 days after the supplementation.[30] The initial erythrocyte magnesium levels in HbSS and HbSC patients and the controls were 7 ± 1 mmol/kg Hb, 6 ± 1 mmol/kg Hb, and 9 ± 1 mmol/kg Hb respectively ($p<0.05$).[30] The paper does not show the data on plasma magnesium as there were no significant differences between the plasma magnesium levels of HbSS patients, Hb SC patients, and normal controls.[30] The magnesium RBC content after 14 and 28 days of supplementation was only measured on 10 HbSS patients, and this data is represented in the table below.[30]

Table 2.
Mean plasma and erythrocyte (RBC) magnesium concentrations in HbSS patients on oral magnesium supplementation at baseline, 14, and 28 days after the supplementation

Time (days)	Plasma Magnesium (mmol/L)	Erythrocyte Magnesium (mmol/kg Hb)
Baseline	0.86 ± 0.06	5.18 ± 0.24
14	0.95 ± 0.11	9.34 ± 2.30
28	0.94 ± 0.09	11.40 ± 1.20

As shown in Table 2, there was a significant increase in erythrocyte magnesium content in HbSS patients ($p<0.05$, $p<0.005$ for days 14 and 28, respectively).[30] There was no significant change in the plasma magnesium level, but this may be due to the fact that it generally takes three months to see the rise in plasma magnesium level for patients who took oral magnesium therapy.[30,51]

The first paper shows that SC patients have a magnesium level below the deficiency cutoff, while the other shows that SC patients have a magnesium level above the deficiency cutoff.[30,32] However, both papers agree that the mean serum magnesium value of SC patients is below or

very close to the deficiency cutoff value. This indicates that SC patients are at risk of developing chronic diseases, insulin resistance, and type-2 diabetes.[33]

Iron

Iron is an essential element as it is responsible for blood production as it is a component of hemoglobin.[34] It is also an essential component for electron transport and DNA synthesis.[43] An individual's iron status is commonly assessed by measuring the biomarker serum ferritin, with a reference range of 12-300 ng/mL.[35]

Two studies with differing opinions regarding iron deficiency in SC patients were studied. The first study was a cross-sectional study that took place in Nigeria.[36] The authors investigated whether iron was deficient in patients with SCD by measuring serum ferritin concentration in 43 HbSS patients aged over 14 years and 43 age and sex-matched HbAA controls.[36] Exclusion criteria included a history of blood transfusion in the previous three months or any form of SC crisis within two weeks of the study. The results showed that only 7% of the SC patients were iron-deficient and that the mean serum ferritin in SC patients and in the healthy controls was 559 ± 428 ng/mL and 185 ± 120 ng/mL, respectively.[36] The serum ferritin concentration in healthy controls was significantly lower than those in SCD patients ($p<0.001$).[36] Also, the value from SC patients was even higher than the upper limit of the reference range (300 ng/mL). This indicates iron elevation, which may be caused by vitamin B 12 deficiency, folate deficiency, myelodysplastic syndrome, or malignancy in SC patients.[37]

The other study was also cross-sectional, with 40 SCD patients and 30 age-matched controls enrolled.[38] All subjects were 3-18 years old and had hemoglobin levels <11 g/dL.[38] The study measured the level of transferrin soluble receptors (sTfR) to determine whether iron was deficient. As the body increases the production of sTfR in response to low iron levels, iron deficiency is marked by an sTfR value greater than 1.8 mg/L.[47,48] The authors of the cross-sectional study found that 97.5% of the patients had higher sTfR values than the threshold indicative of iron deficiency.[48] Still, it is difficult to say that SCD is solely responsible for this increase, as hemolysis (destruction of red blood cells) could also affect sTfR values.[38,48]

The differences in the results and conclusions of the two papers confirm the limitations of usual biochemical parameters in the diagnosis of iron deficiency in homozygous drepanocytosis.

Overview, Limitations

Table 3. Overview of Nutrient Status of SC patients.

Nutrient	Measurement Method/Biomarker used	Measured value in SC patients	Measured value in healthy controls	Cutoffs	Deficient/Suboptimal/ Elevated for SC patients
Vitamin A	Plasma Retinol	26 ± 8 µg/dL	No controls used	- Suboptimal if 20-29 µg/dL - Deficient if <20 µg/dL	Suboptimal
Vitamin B-2	Plasma Riboflavin	15.9 nmol/L	No controls used	- Deficient if <26.5 nmol/L	Deficient
Vitamin B-6	Plsma PLP	36.9 nmol/L	No controls used	- Deficient if <30 nmol/L	Adequate
Vitamin B-12	Serum Vitamin B-12	405 pmol/L	No controls used	- Deficient if <150 pmol/L	Adequate
Vitamin C	Plasma Vitamin C	38.36 ± 9.81 µmol/L	63.20 ± 16.96 µmol/L	- Deficient if <11 µmol/L	Adequate
Vitamin D	Serum 25-hydroxyvitamin D	14.3 ng/mL	No controls used	- Optimal if 30-60 ng/mL - Suboptimal if <30 ng/mL - Deficient if <20 ng/mL	Deficient
Vitamin E	Serum Tocopherol count	8.662 ± 1.137 µg/ml	11.762 ± 1.848 µg/ml	- Deficient if <5.168 µg/ml	Adequate
Folate (vitamin B-9)	Total Folate level	62.0 nmol/L	No controls used	- Deficient if <10.0 nmol/L - Elevated if >45.3 nmol/L	Elevated
Magnesium	Serum Magnesium level	0.80 ± 0.24 mmol/L 0.86 ± 0.06 mmol/L	0.90 ± 0.11 mmol/L	- Deficient if <0.85 mmol/L	Unable to Determine
Copper	Serum Copper level	220.9 ± 27.8 µg/dL	114 ± 16.3 µg/dL	- Optimal if 83.7-140.12 µg/dL	Elevated
Zinc	Serum Zinc level	83.09 ± 9.26 µg/dl	104.06 ± 6.27 µg/dl	- Deficient if <60 µg/dl - Optimal if ≥80 µg/dl	Adequate
Iron	Serum Ferritin	559.33 ± 427.61 ng/mL	184.53 ± 119.74 ng/mL	- Elevated if >300 ng/mL	Elevated
Iron	Transferrin Soluble Receptors (sTfR)	Not reported	Not reported	- Deficient if >1.8 mg/L	Deficient

As shown by Table 3, it was clear that most SC patients had suboptimal vitamin A levels and were vitamin D and B-2 deficient. However, their zinc and vitamins B-6, B-12, C, and E levels were adequate, while their folate and copper levels were elevated. It is not possible to conclude the iron status of SC patients as the results of the papers agreed on the limitations of usual biochemical parameters in the diagnosis of iron deficiency in SC patients.[35,38] The same goes for magnesium, as the papers had different conclusions and results.[30,32]

There were some limitations in some of these research papers: sample sizes were often small,[12,27] the most accurate method may have yet to be used,[30,36] and exact numerical values were not recorded.[12] For instance, for iron, two studies used different biomarkers to assess iron status in SC patients,[36,38] one used the serum ferritin method, while the other used sTfR. Ironically, both authors favored the other method over the method they used. The author, who used serum ferritin, stated that measuring sTfR is a more reliable index of iron status in SC patients than serum ferritin due to the low sensitivity of serum ferritin in SCA.[36] The author who used sTfR, on the other hand, stated that serum ferritin is a better biomarker for iron deficiency as sTfR could be affected by hemolysis and therefore has limits in accuracy.[22,38]

The differences in methods used were also seen in papers that measured magnesium content in SC patients.[30,32] Both papers measured serum magnesium,[30,32] but only one measured erythrocyte magnesium.[30] It has been suggested that measuring the erythrocyte magnesium content is not the most accurate way to represent magnesium content in one's body,[31] so data on serum magnesium could be considered more reliable. Despite the difference in their

measurement methods, both papers claimed that SC patients had a mean serum magnesium value that is very close to the deficiency cutoff value.[30,32]

There were limitations in other reviewed studies as well. The study that measured vitamin D did not analyze other factors that may have affected the vitamin D levels in the subjects.[26] These factors include nutritional status, physical activity, lack of sun exposure, medications that alleviate SCD crises, and other illnesses.[26]
For the measurement of the vitamin C level in SC patients, none of the studies used the most accurate method, which is measuring vitamin C in lymphocytes.[8] This may be because the equipment required to ensure vitamin C in lymphocytes is not widely available. Therefore, researchers who conducted the studies used the plasma vitamin C level instead to determine whether SC patients were vitamin C deficient.[12,44]

For vitamin B-2, its status is usually determined using the erythrocyte glutathione reductase activation coefficient (EGRac), which was not available in the study conducted by Williams *et al*.[27] Consequently, the alternative value of 26.5 nmol/L has been used instead of the EGRac of 1.25, which indicates vitamin B-2 deficiency.

For the majority of the studies, SC patients with a history of blood transfusion months before the study were excluded.[10,12,13,22,30,36,44] For studies that measure the concentrations of iron, zinc, and copper, patients with a history of blood transfusion were excluded as blood transfusion may heavily alter the levels of these elements.[7,10,12,22,36,49] This may also mean that the studies did not include the sickest of the SC patients who are transfusion-dependent, indicating that the serum status of these nutrients may be worse among those with a more severe state of the disease.[7,49] Blood transfusion does not alter the levels of folic acid and vitamin B-12, so the study of Williams *et al.* may not have had a history of blood transfusion as an exclusion criterion due to this reason.[50] The effect of blood transfusion on antioxidant levels and magnesium seems to be a field yet to be explored, and Dougherty *et al.*, De Franceschi *et al.,* and Smith *et al.* may have excluded patients who have received a blood transfusion to eliminate this risk factor.[13,30,44]

Conclusion

Though many nutrient deficiencies in SC patients were initially suspected, SC patients were only deficient in vitamin D and B-2. Instead: they had elevated levels of vitamin B-9 and copper. Low levels of magnesium in the patients were shown,[27] and it is impossible to confirm iron deficiency as the results of the papers highlight the limitations of usual biochemical parameters in the diagnosis of iron deficiency in SC patients.[35,38] The patients had adequate levels of vitamins B-6, B-12, C, and E, and zinc, as none of the measured values of these nutrients were below the deficiency cutoffs. However, though they had adequate levels of these nutrients, SC patients generally had lower levels than the healthy population. As both lower and elevated nutrient levels impede normal body function, SC patients risk experiencing various toxic effects. More

research is required to understand the sodium/magnesium transporter to find the cause of reduced magnesium content in SC patients.[30] There is also a need for another study involving age and sex-matched healthy control to determine whether SCD is solely responsible for VDD, not other factors. Furthermore, further research is needed to confirm whether VDD worsens chronic pain in SC patients.[26] With no improvement of vitamin A status in those taking 600 µg of vitamin A daily, a study that tests the effect of a daily supplemental dose of retinyl palmitate of over 600 µg on those aged 14-18 years with SCD must be conducted. Such an age group is selected based on the National Institutes of Health's recommendation that individuals aged 14 years and above not exceed a daily intake of 900 µg of supplemental vitamin A.[18] The effect of zinc supplements on the vitamin A levels of SC patients must also be explored. The confirmation of whether low zinc levels in SC patients are due to excessive destruction of red blood cells or excessive excretion is required.[10] The effect of zinc supplementation on copper levels in SC patients must also be studied.[22] As shown by Table 3, SC patients generally have a lower level of micronutrients. This supports the claim made by Hyacinth *et al.*: the RDAs for the essential nutrients are only for healthy people and that these requirements for optimal intakes may be higher for SC patients.[5] As the studies reviewed by this paper only focus on patients with SCD and not those in a carrier state, there is more research needed to confirm whether low micronutrient levels are also observed in populations with only the sickle cell trait but not the disease. This literature review analyzed the existing studies that measured the nutrient status in SC patients.

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References

1. Kavanagh, P. L.; Fasipe, T. A.; Wun, T. Sickle Cell Disease: A review. *JAMA*. **2022**, 328 (1), 57-68. DOI: 10.1001/jama.2022.10233
2. Tebbi, C. K. Sickle Cell Disease, a Review. *Hemato*. **2022**, 3 (2), 341-366. DOI: 10.3390/hemato3020024
3. Xu, J. Z. and Thein, S. L. The carrier state for sickle cell disease is not completely harmless. *Haematologica*. **2019**, 104 (6), 1106-1111. DOI: 10.3324/haematol.2018.206060
4. Lubeck, D.; Agodoa, I.; Bhakta, N.; Danese, M.; Pappu, K.; Howard, R.; Gleeson, M.; Halperin, M.; Lanzkron, S. Estimated Life Expectancy and Income of Patients with Sickle Cell Disease Compared with Those Without Sickle Cell Disease. *JAMA Network Open*. **2019**, 2 (11), e1915374. DOI: 10.1001/jamanetworkopen.2019.15374
5. Hyacinth, H. I.; Gee, B. E.; Hibbert, J. M. The Role of Nutrition in Sickle Cell Disease. *Nutr. Metab. Insights*. **2010**, 3, 57-67. DOI: 10.4137/NMI.S5048
6. Ohlhorst, S. D.; Russell, R.; Bier, D.; Klurfeld, D. M.; Li, Z.; Mein, J. R.; Milner, J.; Ross, A. C.; Stover, P.; Konopka, E. Nutrition research to affect food and a healthy lifespan. *Adv Nutr*. **2013**, 4 (5), 579-584. DOI: 10.3945/an.113.004176

7. Rasel, M. and Mohammad R. Transfusion Iron Overload. In *StatPearls [Internet]*, Year 2023 ed.; StatPearls Publishing, 2023. https://www.ncbi.nlm.nih.gov/books/NBK562146/
8. Emadi-Konjin, P.; Verjee, Z.; Levin, A. V.; Adeli, K. Measurement of intracellular vitamin C levels in human lymphocytes by reverse phase high performance liquid chromatography (HPLC). *Clin Biochem.* **2005,** 38 (5), 450-456. DOI: 10.1016/j.clinbiochem.2005.01.018.
9. Hambidge, M. Human zinc deficiency. *J. Nutr*. **2000**, 130 (5S Suppl), 1344S-9S. DOI: 10.1093/jn/130.5.1344S
10. Wasnik, R. R. and Akarte, N. R. Evaluation of Serum Zinc and Antioxidant Vitamins in Adolescent Homozygous Sickle Cell Patients in Wardha, District of Central India. *J. Clin. Diagn. Res*. **2017**, 11 (8), BC01-BC03. DOI: 10.7860/JCDR/2017/30855.10320
11. Yokokawa, H.; Fukuda, H.; Saita, M.; Miyagami, T.; Takahashi, Y.; Hisaoka, T.; Naito, T. Serum zinc concentrations and characteristics of zinc deficiency/marginal deficiency among Japanese subjects. *J. Gen. Fam. Med.* **2020**, 21 (6), 248-255. DOI: 10.1002/jgf2.377
12. Hasanato, R. M. W. Zinc and antioxidant vitamin deficiency in patients with severe sickle cell anemia. *Ann. Saudi. Med.* **2006**, 26 (1), 17-21. DOI: 10.5144/0256-4947.2006.17
13. Dougherty, K. A.; Schall, J. I.; Kawchak, D. A.; Green, M. H.; Ohene-Frempong, K.; Zemel, B. S.; Stallings, V. A. No Improvement in suboptimal vitamin A status with a randomized, double-blind, placebo-controlled trial of vitamin A supplementation in children with sickle cell disease. *Am. J. Clin. Nutr*. **2012**, 96 (4), 932-940. DOI: 10.3945/ajcn.112.035725
14. *World Health Organization-Vitamin A deficiency*. https://www.who.int/data/nutrition/nlis/info/vitamin-a-deficiency. Accessed on 23rd December 2022
15. Rizvi, S.; Raza, S. T.; Ahmed, F.; Ahmad, A.; Abbas, S.; Mahdi, F. The Role of Vitamin E in Human Health and Some Diseases. *Sultan Qaboos Univ. Med. J.* **2014**, 14 (2), e157-e165
16. Miyazawa, T.; Burdeos, G.C.; Itaya, M.; Nakagawa, K.; Miyazawa, T. Vitamin E: Regulatory Redox Interactions. *IUBMB Life*. **2019**, 71 (4), 430-441. DOI: 10.1002/iub.2008
17. Traber, M. G. Vitamin E inadequacy in humans: causes and consequences. *Adv. Nutr*. **2014**, 5 (5), 503-514. DOI: 10.3945/an.114.006254
18. *National Institutes of Health-Vitamin A and Carotenoids.* https://ods.od.nih.gov/factsheets/VitaminA-HealthProfessional/. Accessed on 13th February 2023
19. Carr, A. C. and Maggini, S. Vitamin C and Immune Function. *Nutrients*. **2017**, 9 (11), 1211. DOI: 10.3390/nu9111211
20. McCall, S. J.; Clark, A. B.; Luben, R. N.; Wareham, N. J.; Khaw, K. T.; Myint, P. K. Plasma Vitamin C and Functional Health: Results from the European Prospective Investigation into Cancer-Norfolk. *Nutrients*. **2019**, 11 (7), 1552. DOI: 10.3390/nu11071552
21. Rowe, S.; Carr, A. C. Global Vitamin C Status and Prevalence of Deficiency: A Cause for Concern? *Nutrients*. **2020**, 12 (7), 2008, DOI: 10.3390/nu12072008
22. Antwi-Boasiako, C.; Dankwah, G. B.; Aryee, R.; Hayfron-Benjamin, C.; Doku, A.; N'guessan, B. B.; Asiedu-Gyekye, I. J.; Campbell, A. D.. Serum Iron Levels and

Copper-to-Zinc Ratio in Sickle Cell Disease. *Medicina*. **2019**, 55 (5), 180. DOI: 10.3390/medicina55050180

23. Kennelly, P. J.; Murray, R. K.; Jacob, M. Plasma Proteins and Immunoglobulins. *Harper's Illustrated Biochemistry*, 30th ed.; Cenveo Publisher Services. 2011. https://accessmedicine.mhmedical.com/content.aspx?bookid=1366&sectionid=73247095#1106060414
24. Royer, A. and Sharman, T. Copper Toxicity. In *StatPearls [Internet]*, Year 2022 ed.; StatPearls Publishing, 2022. https://www.ncbi.nlm.nih.gov/books/NBK557456/
25. Lee, H. C.; Chang, S. W. Vitamin D and health - The missing vitamin in humans. *Pediatr. Neonatol*. **2019**, 60 (3), 237-244
26. AlJama, A.; AlKhalifah, M.; Al-Dabbous, I. A.; Alqudaihi, G. Vitamin D deficiency in sickle cell disease patients in the Eastern Province of Saudi Arabia. *Ann. Saudi. Med*. **2018**, 38 (2), 130-136. DOI: 10.5144/0256-4947.2018.130
27. Williams, B.A.; Mayer, C.; McCartney, H.; Devlin, A. M.; Lamers, Y.; Vercauteren, S. M.; Wu, J. K.; Karakochuck, C. D. Detectable Unmetabolized Folic Acid and Elevated Folate Concentrations in Folic Acid-Supplemented Canadian Children With Sickle Cell Disease. *Front. Nutr*. **2021**. DOI: 10.3389/2021/642306
28. Blaszczyk, U.; Duda-Chodak, A. Magnesium: its role in nutrition and carcinogenesis. *Rocz. Panstw. Zakl. Hig.* **2013**, 64 (3), 165-71.
29. Rosanoff, A.; West, C.; Elin, R. J.; Micke, O.; Baniasadi, S.; Barbagallo, M.; Campbell, E.; Cheng, F.; Costello, R. B.; Gamboa-Gomez, C.; Guerrero-Romero, F.; Gletsu-Miller, N.; Ehrlich, B. V.; Iotti, S.; Kahe, K.; Kim, D. J.; Kisters, K.; Kolisek, M.; Kraus, A.; Maier, J. A.; Maj-Zurawska, M.; Merolle, L.; Nechifor, M.; Pourdowlat, G.; Shechter, M.; Song, Y.; Teoh, Y. P.; Touyz, R. M.; Wallace, T. C.; Yokota, K.; Wolf, F. Recommendation on an updated standardization of serum magnesium reference ranges. *Eur. J. Nutr*. **2022**, 61 (7), 3697-3706. DOI: 10.1007/s00394-022-02916-w
30. De Franceschi, L.; Bachir, D.; Galacteros, F.; Tchernia, G.; Cynober, T.; Alper, S.; Platt, O.; Beuzard, Y.; Brugnana, C. Oral magnesium supplements reduce erythrocyte dehydration in patients with sickle cell disease. *J. Clin. Invest*. **1997**, 100 (7), 1847-52. DOI: 10.1172/JCI119713
31. Arnaud, M. J. Update on the assessment of magnesium status. *Br. J. Nutr.* **2008**, 99 (3S Suppl), S24-36. DOI: 10.1017/S000711450800682X
32. Antwi-Boasiako, C.; Kusi-Mensah, Y. A.; Hayfron-Benjamin, C.; Aryee, R.; Dankwah, D. B.; Kwawukume, L. A.; Darkwa, E. O. Total Serum Magnesium Levels and Calcium-To-Magnesium Ratio in Sickle Cell Disease. *Medicina*. **2019**, 55 (9), 547. DOI: 10.3390/medicina55090547
33. Gröber, U.; Schmidt, J.; Kisters, K. Magnesium in Prevention and Therapy. *Nutrients*. **2015**, 7 (9), 8199-226. DOI: 10.3390/nu7095388
34. Naigamwalla, D. Z.; Webb, J. A.; Giger, U. Iron deficiency anemia. *Can. Vet. J*. **2012**, 53(3), 250-256.
35. Pagana, K. D.; Pagana, T. J.; Pagana, T. N. Mosby's Diagnostic & Laboratory Test Reference, 14th ed.; Elsevier. 2019.
36. Sani, M. A.; Adewuyi, J. O.; Babatunde, A. S.; Olawumi, H. O.; Shittu, R. O. The Iron Status of Sickle Cell Anaemia Patients in Ilorin, North Central Nigeria. *Adv. Hematol*. **2015**. DOI: 10.1155/2015/386451.

37. Lanier, J. B.; Park, J. J.; Callahan, R. C. Anemia in Older Adults. *Am. Fam. Physician*. **2018**, 98 (7), 437-442.
38. Lopez-Sall, P.; Diop, P. A.; Diagne, I.; Cissé, A.; Mahou, C. M. S.; Sylla-Niang, M.; Guéye, P. M.; Diarra, M. Transferrine soluble receptors' contribution to the assessment of iron status in homozygous drepanocytic anemia. *Ann. Biol. Clin (Paris)*. **2004**, 62 (4), 415-421.
39. Comstock, G. W.; Alberg, A. J.; Helzlsouer, K. J. Reported effects of long-term freezer storage on concentrations of retinol, beta-carotene, and alpha-tocopherol in serum or plasma summarized. *Clin. Chem*. **1993**, 39 (6), 1075-1078.
40. Jargar, J. G.; Hattiwale, S. H.; Das, S.; Dhundasi, S. A.; Das, K. K. A modified simple method for determination of serum α-tocopherol (vitamin E). *J. Basic Clin. Physiol. Pharmacol*. **2012**, 23 (1), 45-48. DOI: 10.3390/ma14133691
41. Olivares, M.; Méndez, M. A.; Astudillo, P. A.; Pizarro, F. Present situation of biomarkers for copper status. *Am. J. Clin. Nutr.* **2008**, 88 (3), 859S-862S. DOI: 10.1093/ajcn/88.3.859S
42. Sizar, O.; Khare, S.; Goyal, A.; Givler, A. Vitamin D Deficiency. In *StatPearls [Internet]*, Year 2022 ed.; StatPearls Publishing, 2022. https://www.ncbi.nlm.nih.gov/books/NBK532266/
43. Lieu, P. T.; Heiskala, M.; Peterson, P. A.; Yang, Y. The roles of iron in health and disease. *Mol. Aspects Med.* **2001**, 22 (1-2), 1-87. DOI: 10.1016/s0098-2997(00)00006-6
44. Smith, O. S.; Ajose, O. A.; Adegoke, S. A.; Adegoke, O. A.; Adedeji, T. A.; Oderinu, K. A. Plasma level of antioxidants is related to frequency of vaso-occlusive crises in children with sickle cell anaemia in steady state in Nigeria. *Pediatric Hematology Oncology Journal*. **2019**, 4 (1) 17-22. DOI: 10.1016/j.phoj.2019.03.003
45. Emokpae, M. A. and Fatimehin, E. B. Copper-To-Zinc Ratio as an Inflammatory Marker in Patients with Sickle Cell Disease. *Sci*. **2020**, 2 (4), 89. DOI: 10.3390/sci2040089
46. Tsang, B.; Sandalinas, F.; De-Regil, L. M.; Folate supplementation in women of reproductive age. *Cochrane Database Syst Rev*. **2015**, 2015 (6), CD011766. DOI: 10.1002/14651858.CD011766
47. Bermejo, F. and García-López, S. A guide to diagnosis of iron deficiency and iron deficiency anemia in digestive diseases. *World J Gastroenterol*. **2009**, 15 (37), 4638-4643. DOI: 10.3748/wjg.15.4638
48. Oustamanolakis, P.; Koutroubakis, I. E.; Messaritakis, I.; Niniraki, M.; Kouroumalis, E. A. Soluble transferrin receptor-ferritin index in the evaluation of anemia in inflammatory bowel disease: a case-control study. *Ann. Gastroenterol*. **2011**, 24 (2), 108-114.
49. Alhillawi, Z. H.; Al-Hakeim, H. K.; Moustafa, S. R.; Maes, M. Increased zinc and albumin but lowered copper in children with transfusion-dependent thalassemia. *J Trace Elem Med Biol*. **2021**. DOI: 10.1016/j.jtemb.2021.126713
50. Ho, C. H. The effects of blood transfusion on serum ferritin, folic acid, and cobalamin levels. *Transfusion.* **1992**, 32 (8), 764-765. DOI: 10.1046/j.1537-2995.1992.32893032107.x.
51. Zhang, X.; Li, Y.; Gobbo, L.C. D.; Rosanoff, A.; Wang, J.; Zhang, W.; Song, Y. Effects of Magnesium Supplementation on Blood Pressure: A Meta-Analysis of Randomized Double-Bline Placebo-Controlled Trials. *Hypertension.* **2016**, 68 (2), 324-333. DOI: 10.1161/HYPERTENSIONAHA.116.07664.

Author

The author, Minjoon Hur, is a high schooler at Raffles Christian School in Indonesia passionate about nutrition science and biomedical sciences. He hopes to become a pharmaceutical researcher or a nutritionist and plans to conduct quantitative primary research in the near future.