Low Cobalamin Levels in African Americans with and without Sickle Cell Disease

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About 7% of the adult population has subclinical cobalamin (B_{12}) deficiency. Subjects with sickle cell disease (SCD) may be at higher risk of cobalamin deficiency because of increased demand, inadequate supply, coexisting folate deficiency or malabsorption. We compared the clinical and laboratory characteristics of low serum cobalamin levels in patients with SCD with those patients without this hemoglobinopathy (non-SCD). Between 1993 and 2003, 105 SCD patients and 112 non-SCD patients who had serum cobalamin measurements were identified at our institution. The mean cobalamin level in SCD patients was significantly lower (496 ± 352 pg/ml) than that in patients without SCD (869 ± 660 pg/ml, $p<0.0001$). The frequency of low cobalamin levels, defined by a serum cobalamin level of <200 pg/ml, was 18.1% (19/105) and 9.8% (11/112) in SCD and non-SCD patients, respectively ($\chi^2=3.11$, nonsignificant). The mean age of the low-cobalamin SCD and non-SCD patients was 28.1 and 62.9, respectively, and their male:female ratios were 11:8 in SCD patients and 2:9 in non-SCD patients. None of the SCD patients had neurological manifestations, but nine of the 11 non-SCD low-cobalamin level patients did. The proportion of SCD patients with unexplained low cobalamin levels (13/19) was higher than that in non-SCD patients (4/11, $\chi^2=2.92$, nonsignificant) Our data suggest that cobalamin levels are lower in SCD patients than in subjects without SCD, and low-cobalamin SCD patients are younger and more likely to be males.

Key words: cobalamin (B_{12}) ■ sickle cell disease ■ methylmalonic acid

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INTRODUCTION

About 7% of the adult population has subclinical cobalamin (B_{12}) deficiency and it is a significant health problem, particularly among the 35 million elderly in the United States, 15% of whom have the deficiency.[1-3] Its prevalence in sickle cell disease (SCD) is not known. Al-Momen reports low vitamin cobalamin levels in 43.5% of patients with SCD in Saudi Arabia.[4] Dhar et al.[5] recently described a sickle cell patient with pernicious anemia and reviewed two additional cases from the literature. This is a retrospective study in which we compared the clinical and laboratory characteristics of low serum cobalamin in patients with SCD to those in African-American patients without this hemoglobinopathy (non-SCD).

METHODS

Institutional review board approval was obtained and medical records were reviewed. Between 1993 and 2003, 105 SCD and 112 non-SCD patients who had serum cobalamin levels done because of anemia were identified at Howard University Hospital. The diagnosis in the SCD patients was documented by hemoglobin (Hb) electrophoresis. For the purposes of this study, non-SCD patients were considered so because of their older age without any history, symptoms or laboratory findings suggestive of hemoglobinopathy. In the sickle cell patients, cobalamin levels were obtained for various reasons, such as a high red-cell mean corpuscular volume (MCV) and unexplained worsening of their anemia. Our laboratory used the solid-phase no boil (DUAL count) radioassay method initially followed by competitive immunoassay for the past few years to measure cobalamin levels. The normal range for serum cobalamin values at our hospital is 200–950 pg/ml. Medical records were reviewed for demographics. For our study purposes, a low cobalamin level was defined by a serum B_{12} lower than 200 pg/ml. We defined pernicious anemia by a low B_{12} level, along with a positive serum intrinsic factor antibody, a high serum gastrin or an abnormal Schilling's test. For statistical signifi-

cance determinations, we used Chi-squared tests for categorical variables and t tests for continuous variables. In nine of the non-SCD patients, the chart designated the cobalamin value only as "normal." In these cases we arbitrarily used 250 pg/ml as the missing value in the statistical calculations.

RESULTS

Demographics

Demographics and prevalence of low-serum-cobalamin-level patients are shown in Tables 1 and 2. The mean age of the 105 SCD patients screened for cobalamin levels was 36.4 ± 13.3 years. The 112 non-SCD patients with anemia who were screened were significantly older (mean age 58.4 ± 15.4 years). Thirty-nine SCD patients were males (mean age 34 years) and 66 were females (mean age 34 years). In SCD patients with low B_{12} levels, the Hb types were: 12 homozygous SCD (SS), four Hb SCD and three sickle-ß-thalassemia. In the non-SCD patient group, 51 were males (mean age 56 years) and 61 were females (mean age 61 years).

The mean cobalamin level of the screened SCD population was significantly lower (496 ± 352 pg/ml) than that of the screened non-SCD patients (869 ± 660 pg/ml, $p < 0.0001$). The frequency of low cobalamin levels was 18.1% (19/105) and 9.8% (11/112) in SCD and non-SCD patients, respectively ($\chi^2=3.11$, nonsignificant). The mean ages of the low-cobalamin SCD and non-SCD patients were 28.1 ± 7.8 and 62.9 ± 16.2, respectively. The male:female ratios were 11:8 and 2:9, respectively, in SCD and non-SCD patients with low cobalamin.

Neurological Manifestations

None of the low-cobalamin SCD patients had neurological manifestations. In contrast, nine of the 11 non-SCD patients with low cobalamin levels had neurological findings. Among these nine patients, four had focal neurological deficits, one had blindness, one had aphasia and two, who also had diabetes, had coexistent neuropathy. Another two patients manifested with neuropathy and dementia, two patients had urinary incontinence, one patient had a seizure disorder and two patients had no neurological manifestations (Figure 1). Of the two patients with no neurological problems, one had history of HIV and the other had coronary artery disease.

Etiology and Risk Factors of Low-Cobalamin Level Patients

Of the 19 SCD patients with low cobalamin levels, four had pernicious anemia, one had peptic ulcer and was on H2 blockers and one had pancreatic insufficiency and alcohol abuse. Among the 11 low-cobalamin non-SCD patients, four had pernicious anemia, one was on proton pump inhibitor and vitamin C, one had gastritis and one had peptic ulcer and was on H2 blockers. Thus, the proportion of SCD patients with unexplained (i.e., without risk factors) low cobalamin levels (13:19) was higher than that in non-SCD patients (4:11, $\chi^2=2.92$, nonsignificant).

Laboratory Values

The mean cobalamin levels in low-cobalamin patients with SCD and non-SCD were 150 pg/ml and 155.5 pg/ml, respectively. We found that the mean serum methylmalonic acid (MMA) levels and homocysteine levels were higher in non-SCD patients (24.18 ± 26.16 pmol/ml and 15.5 ± 12.8 micromoles/L, respectively) when compared to those in SCD patients (0.168 ± 0.084 and 8.46 ± 3.97, respectively). However, results for MMA and homocysteine levels were available only in about half of the patients. At diagnosis, the Hb levels were similar in both groups. In the SCD group, the mean Hb value was 9.42 ± 1.84 g/dl and it was 9.7 ± 1.76 g/dl in the non-SCD patients. The average MCV was 92.1 and 98.1 fl in patients with and without SCD, respectively. Mean leukocyte count was 13.4 and 5.8 x 1,000/mm^3 in SCD and non-SCD patients, respectively (Figure 2).

DISCUSSION

Our data show that serum cobalamin levels are significantly lower in SCD patients than in anemic,

Table 1. Age and sex distribution of patients with and without sickle cell disease screened for low serum cobalamin levels

	SCD (N=105)	Non-SCD (N=112)	P Value
Female	66 (63%)	61(54%)	
Mean age	36.4 ± 13.3	58.4 ± 15.4	
Mean cobalamin levels (pg/ml) (normal 200–950)	496 ± 352	869 ± 660	<0.001
Low cobalamin levels	19 (18.1%)	11 (9.8%)	NS

NS: not significant

non-SCD control subjects. Similar results were reported in a preliminary communication by Wun et al.,[6] who found a mean cobalamin level of 381 pg/ml in 18 adult SCD patients versus 577 pg/ml in 20 control subjects (p=0.02). However, other investigators have reported that serum cobalamin levels in SCD adults and in SCD children are not significantly different from those in controls.[7,8] Our data also suggest that low cobalamin is more common in SCD than in non-SCD patients. In this retrospective study, more females than male SCD patients were screened (66 out of 105). However, in SCD patients with low cobalamin, the male:female ratio was 11:8. Although this trend for gender differences between screened and low-cobalamin SCD patients was not statistically significant, we did find that the proportion of SCD males with low cobalamin levels was significantly higher than that in non-SCD low-cobalamin patients (χ^2=4.47, p<0.05). At this point, we do not know why low cobalamin seems more frequent in male SCD patients. Adult males with SS and SCD have higher hematocrits than their female counterparts[9] and it is possible that this may indicate higher cobalamin requirements. Some series also report that males have more frequent pain crises.[10] These vaso-occlusive events may be abdominal and even cause acute pancreatitis.[11] Alternative explanations might involve hormonal imbalances influencing cobalamin levels. Oktenli et al., for example, reported that (non-SCD) males with low testosterone levels had high levels of cobalamin.[12] Dr. Vincent Agbaragi, in 1997, presented data at our Howard University Hospital's Scientific Forum showing higher testosterone levels in SCD males than in controls (personal communication). It is possible that women, who normally have lower serum testosterone concentrations, might have higher cobalamin levels and thus have relative protection from this deficiency in high requirement states, such as in SCD. In the total SCD population screened, however, there were no significant gender-related differences in cobalamin levels. Clearly, additional studies are needed to validate and better explain our data.

The various causes of cobalamin deficiency in the general population include pernicious anemia, infections such as HIV and parasites, gastrointestinal surgery, Crohn's disease, bacterial overgrowth, small intestine malabsorption, low dietary intake, chronic pancreatitis, multiple sclerosis and drugs, such as proton pump inhibitors, H2-blockers and metformin.

Patients with SCD could be more prone to cobalamin deficiency because of increased requirement and/or decreased dietary intake or absorption. Factors contributing to the latter mechanism may include decreased production of intrinsic factor by the stomach and decreased cobalamin absorption from the terminal ileum due to recurrent sickling crisis[13,14] and concurrent folate deficiency.[15] These factors also might explain low cobalamin levels occurring in SCD patients at a younger age than in those without SCD.

Since the introduction of fortification of folic acid in the U.S. diet, cobalamin deficiency is more common than folic acid deficiency. Therefore, physicians have to be alert to detect cobalamin deficiency in early stages: folic acid can reverse the megaloblastic features of anemia due to cobalamin deficiency

Figure 1. Neurological manifestations in non-SCD patients with low serum-B_{12} levels

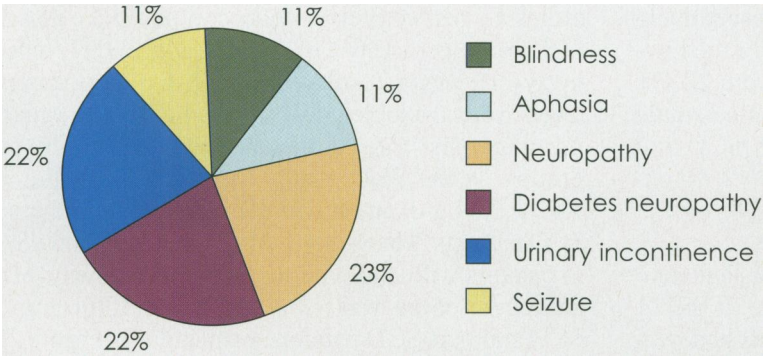


Table 2. Characteristics of low serum cobalamin patients with and without SCD

	SCD (N=19)	Non-SCD (N=11)	P Value
Male	11 (58%)	2 (18%)	<0.05
Mean age (years)	28.1 ± 7.8	62.9 ± 16.2	
Mean cobalamin levels (pg/ml) (normal 200–950)	150	155.5	NS
MMA levels (pmol/ml) (normal <0.41)	0.168 ± 0.084 (N=7)	24.18 ± 26.16 (N=5)	
Homocysteine (micromoles/L) (normal 4–15)	8.46 ± 3.97 (N=6)	15.5 ± 12.8 (N=5)	NS

NS: not significant

while neurological manifestations may worsen.[16,17] At least one of the three reported patients with SCD and pernicious anemia had neuropsychiatric manifestations.[5] Fortunately for our SCD patient group, neurological manifestations of low cobalamin levels were not seen despite routine folate supplementation.

We do not know why MMA levels were lower in SCD patients with low cobalamin levels than in their non-SCD counterparts (MMA levels were available in only 7/19 SCD and 5/11 non-SCD patients). One obvious explanation is the small sample size, since MMA and homocysteine levels were available in only a few patients. In any case, elevated MMA is seen not only in cobalamin deficiency but also in renal insufficiency, volume contraction states, infancy, severe enzyme defects (mutase deficiency) and laboratory errors.[18] Patients with SCD are more prone to sickle cell nephropathy, which might lead to increase in MMA levels. However, none of our patients had renal impairment. In a study by Donaldson on "Hallelujah" (pure vegetarian) diet, urinary MMA levels were more useful in detecting those at risk of cobalamin deficiency and in monitoring improvement in cobalamin status.[19] Since our study is limited by its retrospective design, we were unable to assess the urinary MMA levels. In patients with low cobalamin levels who also have clinical manifestations of cobalamin deficiency as seen in our non-SCD counter parts, MMA levels are not necessary to establish the diagnosis. Although MMA assay is expensive, it will be very helpful in studies of high-risk populations, such as those with SCD.

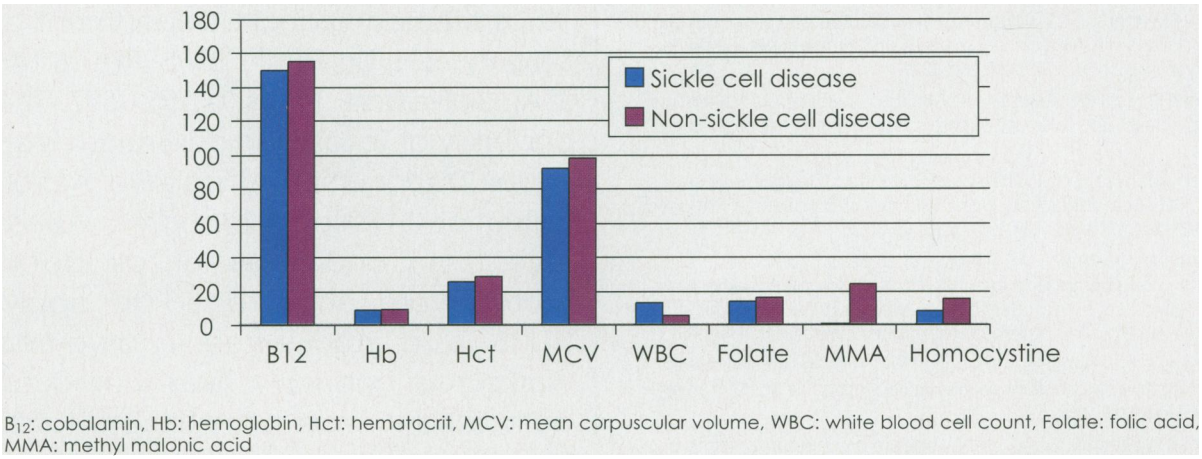
Homocysteine levels were also lower in SCD patients with low cobalamin levels when compared to their non-SCD counterparts. As described above, this finding may be related to small sample size. On the other hand, the lower levels of homocysteine in our patients with SCD could partly be explained by folic acid supplementation. These normal levels of MMA and homocysteine could explain the lack of neurological manifestations in our SCD patients. The study done by Carmel et al.[20] shows that changes in metabolic pathways differ in cobalamin deficient patients with and without neurological manifestations. Furthermore, Carmel's study also demonstrated that patients who had neurological disease had elevated levels of S-adenosyl methionine (AdoMet), cysteine, cysteinyl glycine (cys-gly) and folate metabolites, compared to those in subjects without neurological findings. Associations between high folate levels and neurological manifestations have been shown in other studies of cobalamin deficiency.[21,22] Because all our sickle cell patients were on folate supplements, we could not investigate such correlations. In our sickle cell patients with low cobalamin levels, metabolites other than MMA and homocysteine could play a role. About 20-30% of cobalamin deficient patients have high serum folate levels despite low red-cell folate. This is due to trapping of methyl tetrahydrofolate as a result of impaired methionine synthetase activity.[23]

Our retrospective study is limited by incomplete data sets (MMA levels were available in seven SCD and five non-SCD patients and homocysteine levels were available in six SCD and five non-SCD patients). It is also not an age- and gender-matched control study. SCD patients develop anemia at a young age and have a shortened survival when compared to the non-SCD counterparts. So we were not able to calculate the statistical significance for these parameters.

CONCLUSIONS

Our study suggests that low serum cobalamin levels are more common in SCD patients than in those

Figure 2. Laboratory parameters in low-serum-cobalamin patients with and without SCD



B_{12}: cobalamin, Hb: hemoglobin, Hct: hematocrit, MCV: mean corpuscular volume, WBC: white blood cell count, Folate: folic acid, MMA: methyl malonic acid

without this hemoglobinopathy. Low-cobalamin SCD patients are younger and more likely to be males when compared to non-SCD low-cobalamin level patients. Additional prospective studies need to be done to validate our findings. However, in view of the frequency of low cobalamin levels in SCD patients, it would seem prudent to perform baseline screening for this vitamin in all SCD patients before prescribing folic acid supplementation.

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