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Zinc, copper and iron and their interrelations in the growth of sickle cell patients

Josefina A. Pellegrini Braga [1], *José Kerbauy* [2], *Mauro Fisberg* [3]

Escola Paulista de Medicina, Federal University of São Paulo, Brazil.

SUMMARY. In this study we evaluated the nutritional status of 34 children with sickle cell disease (SS). Results were compared to 9 siblings with sickle cell trait (AS) and 35 eutrophic children who presented normal hemoglobin and normal hemoglobin electrophoresis (AA). All of then came from low socioeconomic level.

Analysis of the growth velocity curves revelead in SS group, tendency to increase deficit in weight and height with age. There was no relation between weight/height (W/H) and height/age (H/A) percentile and hemoglobin levels. There was no significant relation between nutritional status and severity of the disease. SS group showed significant skeletal maturation delay, the same did not occur with the siblings (AS group).

Plasma zinc levels were significantly lower in SS group than in AS and AA groups In SS group there was some association between lower plasma zinc levels and H/A percentile lower or equal to 10. Plasma copper levels were significantly greater in SS group than in AS and AA ones, and there was no relation between plasma copper levels and serum ferritin levels.

In conclusion, our patients with sickle cell disease showed indexes of malnutrition, iron deficiency, hipercupremia and low plasma zinc levels related to low stature.

RESUMEN. Zinc, cobre, hierro y su interrelación con el crecimiento de niños con anemia falciforme. El estado nutricional de 34 niños con anemia falciforme fue evaluado. El grupo estudio (SS) fue comparado a un grupo de 9 hermanos de los pacientes, portadores de trazo falciforme (AS) y con un grupo control de 35 niños eutróficos, con hemoglobina y electroforesis de hemoglobina normales (AA). Todos pertenecían a una clase socio económica baja.

El análisis de las curvas de velocidad de crecimiento reveló un standard de déficit progresivo en el peso y la talla en el grupo SS, con el incremento de la edad. No hubo relación entre los índices de percentiles peso/estatura y talla/edad con los niveles de hemoglobina. No se encontró una relación significativa entre el estado nutricional y la severidad de la enfermedad. El grupo SS presentó retraso significativo en la edad ósea, lo que no ocurrió con el grupo AS.

Los niveles plasmáticos de zinc fueron significativamente más bajos en el grupo SS que en los grupos AS y AA. Hubo alguna asociación entre bajos niveles de zinc y talla inferior al percentil 10. Los niveles de cobre fueron significativamente superiores, en el grupo SS comparados con AS y AA. No hubo correlación entre los niveles de cobre y ferritina sérica.

En conclusión, los pacientes con anemia falciforme presentaron índices elevados de malnutrición, ferropenia, hipercupremia y bajos niveles plasmáticos de zinc relacionados con baja estatura.

INTRODUCTION

The ocurrence of growth and development delay in patients with sickle cell anemia (SS) has been recognized by many authors for some years (1-10). Kramer et al (11) have reported precocius assault in the growth curves of these patients, and these findings were confirmed later on by Platt et all (4) and Stevens et al (12), when they described in these individuals a presence of progressive decreased growth velocity up to adolescence. There has been also observed incidence of delay in the bone maturation in SS children, followed by delay in the epiphysis fusion during puberty (2, 12, 14, 15); however, the

1. Physician of Department of Pediatrics, Division of Clinical Pediatric, Department of Pediatric, Federal University of São Paulo (Escola Paulista de Medicina), São Paulo, Brazil.
2. Professor and Head of Division of Hematology, Department of Medicine, Federal University of São Paulo (Escola Paulista de Medicina), São Paulo, Brasil.
3. Professor and Head of Division of Nutrition and Metabolism, Department of Pediatrics, Federal University of São Paulo (Escola Paulista de Medicina), São Paulo, Brazil.

age in which this delay starts is still not well established. In the evaluation of the post-natal growth in patients with sickle cell anemia, Kramer et al (11), observed the ocurrence of delay in bone maturation, increasing progressively with age.

In the last two decades, some studies have emerged trying to explain what would determine the growth delay in individuals with sickle cell disease. Hormonal, metabolic, intestinal absorption and nutritional evaluations, were performed (16-21).

Due to certain clinical similarities between individuals suffering from sickle cell anemia and those with zinc deficiency described by Prasad et al (22) since the 70's decade, it was suggested thy hypotehisis of occurring deficiency of this trace element in the sickle cell individuals.

Although not all studies are considered unanimous, several works have confirmed the existence of zinc deficiency in these patient (23-28).

The relation between the state of zinc and the growth delay in these patients was studied by Phebus et all (27), being related the presence of lower levels of serum zinc in patients who presented height-for-age below 5th percentil than those with normal growth. Others authors have also observed that there is a relation between lower levels of carbonic anhydrase (zinc-dependent enzyme) and the growth delay, in individuals with sickle cell anemia (29).

However, Russel et al (30) have not found an evident correlation between zinc concentration and the individual's growth, although they have observed lower levels of plasmatic zinc in SS patients with age below 12 years.

The interrelations between zinc and trace elements copper and iron, although very known, have been scarcely studied in these patients. Some works report the finding of hypercupremia in individuals with sickle cell anemia (14, 31, 32, 33). Some studies have demonstrated that the iron deficiency is not rare in sickle cell anemia (26, 34, 35) and 'the excessive iron accumulation can be related or not to the number of transfusions received (6, 36, 37, 38, 39).

So, this study was done aiming the evaluation of a group of children with sickle cell anemia, their growth and the zinc, copper and iron status and their possible interrelation.

MATERIAL AND METHODS

The population studied consisted of 34 children suffering sickle cell anemia (SS), whose ages varied from 1 to 11 years, 16 of them (47%) were male. All were attended in the Hematology Sector, Federal University of São Paulo (Escola Paulista de Medicina), in São Paulo, Brazil.

The control group consisted of two distinct groups. One of then consisted of 9 sibling of the patients with sickle cell trait (AS), whose ages varied from 3 to 11 years. The other one consisted of 35 eutrophic children (AA), from the same environment, whose ages varied from 1 to 11, all of them with normal dosages of hemoglobin and electrophoresis hemoglobin.

The diagnosis was established through the electrophoresis of hemoglobin in cellulose acetate, pH=8,0. Fetal hemoglobin was assayed by method of Betke (40).

All patients were supplemented with folic acid (5 mg per day) from the diagnosis (41), the patients did not received any blood transfusion in the last three months.

Anthropometry: Weight and stature were measured by one observer. The children were without shoes and wearing light clothes To evaluate nutritional status, weight/height (W/H) and height/age (H/A) indices were analysed, using the NCHS tables for reference (42), considering as malnourished children presenting W/H below P 10 and stunded the one presenting H/A below P10.

The stature was measured with the stadiometer. The child was put standing erect, with knees together and the line of vision directed horizontally. All children were weight in anthropometric scale, always after previous checking.

Socio-Economical level: The socio-economical level was evaluated through Graffar' method (43), modified by Grunberg et al (44).

Dietary intake analyses: A dietary intake analysis was performed through the 24 hours recordatory method average of three records (45) and analysed later on in relation to the energetic, iron, zinc and copper intake.

Disease's severity: The severity rate was established by the number of hospital admittance occurred caused by the disease's complications until now.

Bone age: The bone ages of the patients and their brothers were obtained through the left wrist and hand radiography, analysed under the Grewlich & Pyle Criteria (46).

Hematological evaluation: the ferritin levels in children with sickle cell disease were analysed in serum by kits (Abbott® Laboratories).

Laboratory: Sediment urinary and stools exams were done in order to evaluate blood loss. Electrophoresis of proteins were done in the patients. In the three studied groups, the zinc and copper levels were determined in plasma. The analysed blood was collected in polypropilen tubes, metal free, followed by the plasma separation in which was stocked to -20 °C until the trace elements could be determined by atomic absorption spectrophotometry (Perkin-Elmer, 5100 PC pattern) (47).

Data analysis: In the statistical analysis, depending on the variables the following tests were used: Fisher's Exact, Mann-Whitney, Kruskall-Wallis, Mc Nemar (48), Statistical significance was set at $p<0,05$, and when significant it will be should by an. *

RESULTS

Through the socio-economical level it was observed that 73.53% of the patients were in the levels IV and V of Graffar, confirming their low socio-economic situation and 27% of the patients were in the level III.

The SS children presented percentile W/H and H/A significantly lower than the control group AA and was not significant with group AS (Table 1). The malnourishment percentage in the studied patients was 3.9 times higher than the percentage showed by their siblings (Table 2). There was not any significant association between the disease's severity and nutritional state. There was no relation between the hemoglobin values and the W/H and H/A percentiles (Table 3).

TABLE 1
CHILDREN WITH SICKLE CELL ANEMIA (SS) AND CONTROL GROUP (AA), ACORDING WEIGHT -FOR- AGE (W/A) AND HEIGHT -FOR- AGE (H/A) PERCENTILE

Groups	W/H percentile		H/A percentile	
	<or=10	>10	<or=10	>10
SS	11	23	9	25
AA	0	35*	0	35*
AS	1	8	0	9

Fisher's test *P= 0.0002 (significant)

TABLE 2
CHILDREN WITH SICKLE CELL ANEMIA (SS) PAIRED WITH THEIR SIBLINGS (AS) AND CORRELATED WITH NUTRITIONAL STATUS

	AS (n=12)		
SS (n=12)	Eutrophic	Malnourished	Total
Eutrophic	3 (25%)	1 (8%)	4 (33%)
Malnourished	7 (58%)	1 (8%)	8 (67%)
Total	10 (83%)	2 (17%)	12 (100%) (a)

Mac Nemar' test *p = 0.038 (significant)

HbSS-W/H <P10= 8/12= 0,666= 67%
HbAS-W/H ≤P10= 2/12= 0,166= 17%
(a) It was possible to compare 12 patients whithin their families. The 12 patients were paired with their siblings (9 siblings), resulting in 12 pairs- SS X AS.

TABLE 3
HEMOGLOBIN LEVELS AND PERCENTILE HEIGHT - FOR- AGE (H/A) AND WEIGHT -FOR- AGE (W/A) IN CHILDREN WITH SICKLE CELL ANEMIA

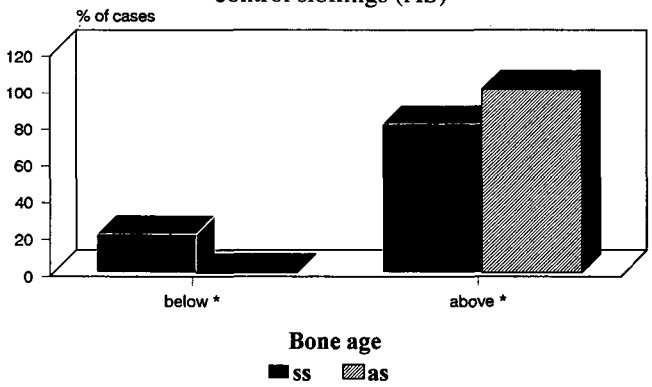
	Hb g/dl mean (sd)	
Percentil	W/H	H/A
<or=P10	7.06 (1,05)	7.14 (0.99)
>P10	7.56(1,21)	7.51(1,2)
Mann-Whitney'test	p=0,14	p=0,25

In the dietary intake analysis (measured by three days recordatory survey), it was concluded that the diet of the patients and their siblings were qualitatively and quantitatively inadequate in energy, iron, zinc and copper intake. Food which could interfere in the absorption of these trace elements (fibers) was consumed in very small quantities (data will be presented in another publication).

All patients presented serum albumin dosage within the normal limits (>3,5 g/dl). Level of serum ferritin lower than 25 ng/ml were observed in 13,3% of the patients. Blood loss was not noticed through urine and stools exams.

There was significantly delay in bone age in the SS group in which 23,5% of the children presented bone age below second standard deviation of normality. In the AS group, all presented bone maturation within the normal standards (Graphic 1).

GRAPHIC 1
Bone ages, below and above the 2nd standard deviation of normatily, of children, with sickle cell anemia (SS) and control sibilings (AS)



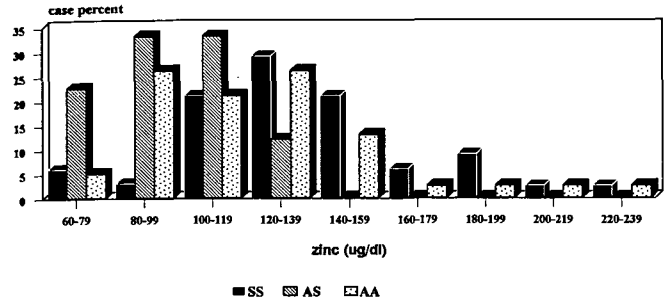
* Second of interior

When we compare the plasmatic levels of the zinc and copper trace elements in the three studied groups, we could verify that in the SS group (98.68 mcg/dl), the zinc level were significantly lower than the AS (123,33 mcg/dl) and AA (145,71 mcg/dl) groups (Graphic 2); and the copper levels

were significantly higher in the SS group (133,24 mcg/dl) than in the other ones (AS-94,44 mcg/dl; AA-117,43 mcg/dl) (Graphic 3).

GRAPHIC 2

Plasma zinc in children with sickle cell anemia (SS), sibilings (AS), and control group (AA)

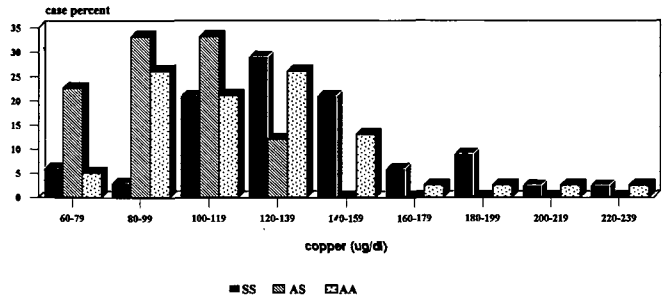


	SS	AS	AA
Zinc ug/dl (media)	98,68	123,33	145,71

KRUSKAL-WALLIS: SS < AS and AA

GRAPHIC 3

Plasma copper in children with sickle cell anemia (SS), sibilings (AS), and control group (AA)



	SS	AS	AA
Copper ug/dl (media)	98,68	123,33	145,71

KRUSKAL-WALLIS: SS > AS and AA

The statiscal analyses (Mann-Whitney) showed a significant relation between lower zinc levels and low stature. There was no significant correlation between the copper and ferritin levels.

DISCUSSION

In this study, the SS patients presented significant deficit in height when compared to the controls groups (AS e AA), which increased with age. These results agree with the observations previously related by Phebus et al (3); Platt et al (4); Stevens et al (12), Gray et al (49).

It could be expected a high rate of malnourishment in these patients, since a low energetic intake in the diet's family was observed and 73,53% of these families belonged to less privileged socio-economical levels (Graffar IV and V). However, this finding is dissonant when we compare the patient's nutritional state with their sibling's. This is because only 1 of their siblings presented malnourishment (W/H<10 percentile).

Then, considering that the two groups have the same genetic patterns and are submitted to be same socio-economic conditions and diet, there must be other factors involved in the malnutrition of the SS children, like chronic anemia, the state of chronic disease itself, a greater requirement of caloric intake or even specific deficit of nutrients.

It is interesting to observe that even though chronic anemia can affect the growth, in the sickle cell anemia little correlation has been found (6,50), and this fact was not observed in our study either.

The presence of a more accelerated metabolism could increase the nutritional necessities in these patients (17) and since the diet of these children has been related as diminished or normal (18), these factors could contribute to the growth deficit However, in this study, individual reports of some patients refer to normal or increased food intake.

The disease's severity influence on the growth was evaluated by Lowry et all (50), when they observed a trend towards low weight in those patients who presented more hospital admissions. In our study, we did not observe any association between malnutrition and past hospital admission.

The iron deficiency can alter the performance and growth in children. Some works have observed higher prevalence of iron deficiency anemia in children who live in countries in development (35,37,51). The lack of iron observed in our patients was probably due to an inadequate diet concerning iron, since there was no blood loss through stools and urine. There was not any association with iron level and malnutrition, since 100% of these individuals with iron deficiency, were eutrophic.

The observation of significant delay in bone age in SS patients and the fact that this has not happened in their siblings reafirm the hypothesis that the cause of the growth retardation observed in these individuals probably does not depend on the hereditary and socio-economical factors. So, in the last decades, some studies have emerged trying to correlate the growth delay with zinc deficiency observed in these individuals (27, 52).

The reasons which would determine the presence of low levels of zinc in sickle cell anemia are still not determined. The causes are still questioned, a generalized lack of intake which would cause in these patients, an appetite and/or taste alteration, with a possible worsening of malnourishment, is a possibility.

In our patient's diet evaluation we could notice a low zinc intake, but only this fact could not justify the low levels obtained in our patients since their siblings are also submitted to the same diet and do not present a significant deficit of this trace element.

However, it is also possible that the daily recommendations used in the diet to the healthy population can not be appropriate to children suffering from sickle cell anemia (27,28), since the existence of chronic anemia, increased of reticulocytosis and the state of chronic disease itself could require an increase of caloric requeriments and other nutrients.

Another probable mechanism for the zinc deficiency could be the diminution of zinc absorption in diet through the usage of fibres, but when we have evaluated our children's diet we did not notice this factor.

Zinc deficiency in sickle cell anemia may be secondary hyperzincuria. The reason for hyperzincuria in SS patients is not clear, may be resulted of a increased filtration of zinc by the glomeruly, or defect in tubular reabsorption of zinc, or chronic hemolysis of erytrocytes rich in zinc (23,31).

The possibility of the decrease of zinc levels be related to albumin was put aside since the dosage of seric albumin was within the normal patterns, also observed by Phebus et all (27).

Finally, it is important to underline that plasmatic zinc represents only a little part of the total of zinc in the body, so, for a better evaluation of the nutritional state of this element in the organism, it is necessary dosages in the hair, in the erythrocytes, urinary, neutrophilis and the zinc dependent enzymes, which is of extreme importance, because this can be the main factor for the disagreement of findings among several authors, since in the majority of the studies it is done only plasmatic or seric zinc dosage.

The finding of elevated levels of plasmatic copper in our patients makes us question a possible interrelation between the iron and copper metabolism in this patients, making a hypercupremia state possible. This hypothesis was also metioned by Kapu et all (32), however, we have not observed this correlation. It must be considered that other elements also present an interrelation with copper, making possible interference in the levels of this element in the organism, like ascorbate acid and zinc.

The elevation of copper levels could result from decrease of zinc level. Antagonist interaction between zinc and copper were demonstrated in nutritional studies in human beings (53,54). Since zinc and copper compete in the intestinal level through the same place of absorption, the deficit of one of then could promote an elevation of the other and vice-versa.

Since in our patients the other causes that could promote elevations in copper levels (external contamination, inflammatory processes, tobacco, oral contraceptives, estrogens, infection and the usage of corticosteroid in initial phase) were put aside, the elevated levels of copper must probably be related to the interactions of it with zinc and/or iron metabolism.

So we could verify that children with sickle cell anemia present their nutritional state compromised, characterized by growth delay, bone age delay, plasmatic zinc levels diminution and increased in the levels of plasmatic copper. The finding of depletion in zinc levels confirm this trace element can be one of the probable responsible factor for the growth retardation in SS children.

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