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Hematological Impact of Vitamin A and Zinc Supplementation in School Children

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Abstract

Introduction: Anemia is one of the most important public health problems in developed and developing countries. It is present in 800 million people, and 273 million are children, it is estimated that approximately 50% of children under 5 years and 25% of children 6-12 years of the world population suffer from it.

Objective: To evaluate the effect of the supplementation with Vitamin A and Zinc on hematological values in school children belonging to an educational center in Zulia State, Venezuela.

Methodology: An experimental, prospective study. The population was 202 school children, only 80 selected from the Maracaibo Municipality, Zulia State, subdivided into 3 subgroups,

- Group 1:Vitamin A+ Zinc 29 school children,
- Group 2:Vitamin A, 25 school children and
- Group 3: Zinc 26 school children

Clinical, biochemical and nutritional evaluation was performed pre and post supplementation to each school child. The average values before and after supplementations were compared, the ANOVA test was applied, and the percentage values were compared with Fisher's test. Results: The results showed a higher frequency of male children (55%), eutrophics between 6.06 ± 12.6 years of age, considered to have some degree of anemia. Group 2 (n=25 children) supplemented only with Vitamin A, achieved better results of HGB with (12.54 ± 0.64), followed by the group supplemented with only Zn (12.12 ± 1.05), that although they started with values before being supplemented considered as low (11. 49 ± 0.79 and 11.35 ± 0.74), respectively achieved normality (12.54 ± 0.64 and 12.12 ± 1.05), after supplementation, both the group supplemented with only Vitamin A and only Zinc.

Discussion: when supplementing with Vitamin A, hemoglobin levels improved from 47.2% to 27.2%. This effect was similar to that found in the group of girls supplemented with Vitamin A alone, but also with Zn alone.

Conclusions: The study evidenced the beneficial effect of Vitamin A supplementation as a single dose, also Zn alone in reducing the prevalence of anemia present in the children studied.

Keywords: Anemia; Supplementation; Vitamin A; Zinc; Hemoglobin

Introduction

Anemia is one of the most important public health problems in developed and developing countries. According to data from the World Health Organization (WHO), the latest reports (2011), reveal that more than 2 billion people have iron deficiency, which represents almost 25% of the world's population. It is present in 800 million people, and 273 million are children. It is estimated that approximately 50% of children under 5 years of age and 25% of children 6-12 years of age in the world population suffer from iron deficiency [1,2]. The etiology of anemia is multifactorial and may be associated with iron deficiency, infectious or chronic diseases, genetic defects, or deficiencies of folate, cobalamin and vitamin A [3,4].

Developing countries in Africa, Asia and Latin America and the Caribbean have always reported the highest rates of anemia in children under three years of age (between 60.0 % and 78 % of the population under three years of age), with the highest peaks in sub-African countries such as Angola, Botswana, Cameroon, Gambia and Maputo, and, in Latin America, countries such as Bolivia, Peru, Ecuador and Venezuela [5].

In Latin America and the Caribbean, according to international agencies, hunger has recently shown a slow and progressive increase, even though it is still below 7%, in many middle-income countries where the economy has slowed down or contracted, including Venezuela. Food insecurity in Venezuela plays an important role as a determinant of malnutrition affecting the population. This situation generates the increase of malnutrition in all its forms and hidden hunger in the most vulnerable population, children, pregnant women and older adults, with a greater impact on the poorest [6].

Micronutrient Deficiencies (MND), known as "Hidden Hunger" represents the most widespread form of malnutrition in the world. The most frequent are iron, iodine and vitamin A deficiencies, which mainly affect children and women. It is estimated that more than two billion people in the world suffer from various deficiencies [7].

CMD is a global problem that has serious consequences for the health of the population. When its prevalence is high, it negatively affects the economy of a country, since it contributes significantly to increasing the global burden of disease estimated in the number of annual deaths and years of life lost due to disability. Therefore, their prevention is imperative. It is estimated that more than 2 billion people in the world are deficient in some of the following micronutrients: Iron, vitamin A, iodine and zinc, which are considered the main micronutrient deficiencies in terms of frequency and health consequences [8].

In Venezuela, the prevalence of iron deficiency in preschoolers, school children and adolescents varies between 9% and 34.66% [9,10]. There are several causes of iron deficiency, including insufficient intake, inadequate absorption, or increased requirements due to growth, the prevalence of anemia is usually variable, ranging from 14.92% to 78% [11,12]. Although the most common causes of anemia are associated with multi-and micronutrient malnutrition (particularly nutritional deficiencies of iron, folic acid, vitamin B12 and vitamin A), anemia also occurs as a consequence of prolonged infectious or inflammatory processes or chronic blood loss [13].There is worldwide evidence that interventions for the control of anemia have managed to avoid its complications in the physical and mental development of children.

Among the interventions, in addition to specific treatment with iron, the strategy of supplementation with multi micronutrients has been developed, which has proven to be an effective intervention to reduce the levels of anemia [14]. Among the effective interventions to reduce stunting, micronutrient deficiencies, infant morbidity and mortality the most commonly performed are vitamin A supplementation, therapeutic zinc supplementation and the use of micronutrient powders [15].

Vitamin A has been shown to improve hemoglobin concentrations and increase the efficacy of iron supplementation. The mechanisms are not fully understood but are suggested to operate through effects on transferrin receptors that affect the mobilization of iron stores, increasing iron absorption, stimulating erythroid receptors in the bone marrow, and reducing susceptibility to infections [16]. Vitamin A plays a vital role in the functioning of the human body in supporting immune function, vision, eye health, and reproduction [17].

On the other hand, there is evidence that plasma zinc (Zn) is a strong predictor of hemoglobin, independent of iron status, in 2 previous studies [18,19]. Because of the above, the research was framed to evaluate the hematological effect of Vitamin A and Zinc supplementation in school children belonging to an educational center in the State of Zulia, Venezuela. It should be noted that among the Sustainable Development Goal 2 (SDG 2) is to eradicate the global burden of micronutrient malnutrition, improve maternal health and reduce child mortality [19]. A challenge for many countries, mainly developing countries [20].

Materials and Methods

This is an experimental, prospective, descriptive and comparative study. The population consisted of 202 female and male school children who attended the National Bolivarian School "Catatumbo", located in the Amparo Sector, Maracaibo Municipality, Zulia State, located in the Amparo sector, Cacique Mara parish, from September to July 2016 (2nd cut). A total of 122 school children (40 girls and 82 boys) were excluded from the total population because they did not meet the inclusion criteria after prior informed consent of the parents or legal representatives; children of Venezuelan nationality, asymptomatic, without active infectious processes. Children under 6 years old and over 12 years old, with evident clinical signs of active infectious processes, with associated pathologies and children who received nutritional therapy or vitamin and mineral supplements two months before the collection of the first blood sample were excluded. As described in the Charts of the Venezuela Project and those of the Longitudinal study of the Metropolitan area of Caracas [21,22] taken care by a specialist in clinical nutrition. The study complied with the provisions of the international ethical standards established by the WHO for research on human subjects and the Declaration of Helsinki [23]. A pediatric specialist was in charge of the clinical evaluation and the deworming process, administering a single dose of anthelmintic (Albendazole), provided by the Maracaibo Health Unit. A dietary interview was conducted directly with the mother and/or legal representative of the selected children responsible for their feeding, and the 24-hour reminder method was applied before and after supplementation. The blood collection was carried out taking care of adequate quality control to obtain accurate and reliable results and complete hematology was taken before and after the study by a specialized bioanalyst, in the first hours of the day, the children subject of the studies had to be fasting, these days were carried out during five continuous days, the blood sample was taken from a forearm vein, blood (5 ml), by peripheral venous puncture. The blood was collected in two tubes, one with anticoagulant for the determination of hematological parameters, hemoglobin (HGB), hematocrit (HTC) and erythrocyte indices were measured in an automated electronic hematological counter Sysmex K-800. The reference values for classifying hemoglobin were those recommended by the World Health Organization (WHO). The cut-off point for defining anemia in children aged 6 to 14 years is 11.5 g/dL. The degree of anemia was classified as mild (<11.0 g/dL in children under 6 years and 8 years and 11.5 g/dL between 8 to 14 years), moderate (<10 g/dL) and severe (<7 g/dL); all data were corrected for altitude above sea level (17.45). Reference hematocrit values in children aged 5 to 15 years: 37%-47%. They were randomly grouped into three groups.

- Group 1: Supplemented with vitamin A (DU)+Zn=Multivitamin, composed of 29 children.
- Group 2: Supplemented with only Vitamin A (DU); integrated by 25 children.
- Group 3: Supplemented only with Zinc, integrated by 26 children.

The Zinc (Zn) supplemented group of 26 school children was given liquid zinc sulfate (dose:12.5 mg) continuously for eighty days. Zinc sulfate (AG) 120 ml bottle (oral solution). 2 mg Zinc/ml. Zn doses per child/day of 12.5 mg, i.e. 12.5 cc, were administered, considering an acceptable and tolerable supplementation dose between 12 and 23 mg/day for children whose ages ranged from 4 to 13 years [24,25]. Zinc sulfate was

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given to the children in the morning before the first meal of the day (breakfast) to ensure absorption. The group supplemented with VA+Zn consisted of 29 school children, who received Vitamin A (single dose: 100,000 IU)+Zinc (12.5 mg of zinc sulfate, continuously for eighty days of treatment). The data were analyzed with the SAS statistical program. Normal distribution tests were applied for the variables studied. The results were presented in tables in number (n), percentages (%) and as mean ± Standard deviation (m ± SD) as appropriate. Mean values between groups were compared before and after supplementation with the ANOVA test. A value of $p<0.05$ was considered statistically significant. Percentage values were compared with Fisher's test.

Results

Hematological variables in study participants, according to a group, before and after the intervention as shown in **Table 1**.

Table 1: Mean hematological variables in study participants, according to a group, before and after the intervention.

Hematologic al	**Group 1 (n=29) VA+Zn**		**Group 2 (n=25) VA**		**Group 3 (n=26) Zn**	
Variable	Before	After	Before	After	Before	After
HGB	11.48 ± 0.70	11.98 ± 0.88	11.49 ± 0.79	12.54 ± 0.64	11.35 ± 0.74	12.12 ± 1.05
HCT	36.94 ± 2.52	38.84 ± 2.98	36.88 ± 3.06	41.02 ± 2.45	36.18 ± 2.10	38.72 ± 3.21
MCV	92.46 ± 4.78	86.30 ± 6.37	90.84 ± 4.06	84.54 ± 5.69	91.90 ± 6.05	85.52 ± 6.06
MCH	28.74 ± 1.94	26.79 ± 2.24	28.32 ± 1.44	25.80 ± 2.19	28.83 ± 2.47	26.78 ± 2.03

Analysis of results

The group of eutrophic children consisted of 80 children. The results showed a higher frequency of male (55%) and female (45%) children between 6.06 ± 12.6 years of age, with an average age of 8 years and 9 months for girls and 8 years and 5 months for boys, which classifies as school population (6 to 12 years old), with an average weight of 25.21 kg and an average height of 125.75 cm for boys/girls. The results of weight and average height were similar both in the initial stage and after supplementation in both sexes, detecting no significant differences between genders. To show if there was any variation in the biochemical results of the schoolchildren, hematological analysis was performed before and after the supplementation intake by extracting a 5 ml blood sample. It was found that all the schoolchildren studied had HGB levels below 11.49 ± 0.79 (g/dL) before being supplemented with Vitamin A and Zinc multivitamin, being considered as mild anemia (<11.0 g/dL in children under 6 and 8 years old and 11.5 g/dL between 8 and 14 years according to the WHO 2001 classification and HTC values below 36.94% ± 2.52 before supplementation is considered below (37%-47% normal values for children between 5 to 15 years). It is important to note from this study that deals with hematological levels in school children that hemoglobin is an iron protein found inside red blood cells that transports oxygen throughout the body. Hematocrit is a measure of the percentage of blood represented by red blood cells. Low hemoglobin or hematocrit value is a sign of anemia. The WHO estimates that 700 to 800 million of the world's population is anemic, that in industrialized countries the percentage is between 2% to 8%, and that the figures in the underdeveloped world are alarming. Although the causes of anemia are multifactorial, iron deficiency resulting from nutritional deficiency is considered to be the most frequent cause of anemia, together with a deficit of other important micronutrients as shown in Figures 1 and 2.

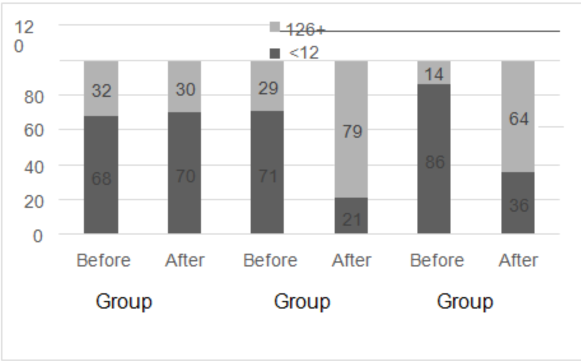

Figure 1: Distribution of participants, according to intervention group, according to normal hemoglobin values (girls).

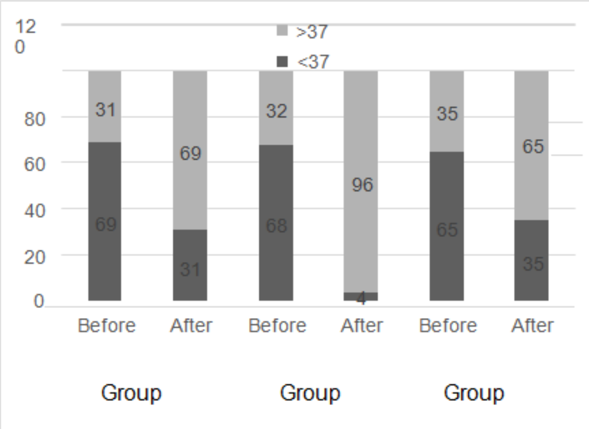

Figure 2: Distribution of participants, according to intervention group, according to normal hematocrit values.

It was observed that all the study groups of children before supplementation presented a Hemoglobin (HGB) value (<11.49 ± 0.79), considered according to the classification (<11.5 g/dL between 8 to 14 years) as mild anemia according to WHO, 2001. Group 2 (n=25 children) supplemented only with Vitamin A, achieved better results of HGB with (12.54 ± 0.64), followed by the group supplemented with only Zn (12.12 ± 1.05), which although they started with values before being supplemented considered as low (11.49 0.79 and 11.35 0.74), respectively achieved normality (12.54 ± 0.64 and 12.12 ± 1.05), after supplementation, both the group supplemented with only Vitamin A and only Zinc. About hematocrit levels (HCT), for all groups levels (<36.94 ± 2.52) were found to be low, according to the classification (37%-47% normal values for children between 5 to 15 years). Group 2 (n=25) supplemented only with Vitamin A achieved normal values of HTC (41.02 ± 2.45) according to the classification. Using as a reference value the hematological results of hemoglobin in blood, a tendency in the suffering of anemia was evidenced in boys more than in girls, observing that in group 1 (Vit A+Zn) and 2(Vit A) 100% of the studied boys suffered from anemia concerning the girls of which (68%) versus (71%) respectively were obtained. The results of the graph show that the group of boys improved their hemoglobin levels after supplementation of Vit A+Zn and Vit A alone, going from 100% to 50% improvement in HGB. On the contrary, the group of girls had a better performance in HGB, when they were supplemented with Vit A and Zn alone, going from 71% and 86% to 21% and 36% improvement in HGB, respectively. With HTC levels, it was observed that for all groups before supplementation the levels of these were found (<37%) in an important percentage according to classification, and that after supplementation these values improved above (37%) considered as normal, in a good percentage of the children studied. These results are contradictory, so further research is warranted.

Discussion

Anemia is a global public health problem that may be related to many causes, including vitamin A deficiency, and other micronutrients. Vitamin A deficiency alters iron mobilization, but studies are inconclusive, considering the interaction between iron and vitamin A metabolisms and that vitamin A deficiency can cause anemia.

No effect on Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCH) values probably because, during periods of Fe insufficiency or failure in its utilization (anemia), Zinc (Zn) becomes an alternative metal substrate for ferrochelatase, leading to increased formation of Zinc Protoporphyrin (ZnPP), i.e., a heme group containing Zn instead of Fe and therefore not utilized for HGB synthesis.

In a community trial study, of prophylactic Vitamin A supplements alone, or multiple micronutrient supplements, and another group supplemented with Vit A+Zn, administered to three cohorts of children starting at 6 months and continuing up to 24 months found that the group of uninfected children had improved HGB levels, decreasing the number of children with anemia following Vitamin A supplementation alone from (76.6%-59%) these values were surpassed by the group supplemented with multivitamins. Results very similar to those found by the cohort group where children with anemia after supplementation of vitamin A alone, went from (76.5% to 58.8%), this value was surpassed by the group after supplementation of multivitamins including Zn and vitamin A, going from (83% to 58.5%). These results are similar to the study after Vit A supplementation where the children improved HGB levels, decreasing the anemic picture in both girls and boys, observing similar results to those obtained after Zn supplementation only in girls, while in boys a decrease of 50% in the anemic picture was observed after Vit A+Zn supplementation.

In a study conducted by in children, the iron zinc vitamin A group, children between 6 and 12 months of age received a single dose of 100. 000 IU vitamin A as retinol palmitate; those aged 1 year received 200 000 IU The dose of zinc was 3 (mg/kg) 1 of zinc sulfate providing 0.7 (mg/kg) 1-d1 of elemental zinc, children who received iron, vitamin A and zinc after 18 weeks of treatment, the mean increase (SD) of hemoglobin and zinc group was (24.0 ± 9.95 g/L), and that in the iron, zinc and Vitamin A group was (23.8 ± 7.72 g/L), the hemoglobin increase in iron and zinc and iron, zinc and vitamin A. Of the 22.5% of anemic children at the end of iron, zinc and vitamin A supplementation treatment, only 9.4% of the children remained anemic. In the study, it was found that both children improved their HGB levels and therefore their anemia after supplementation with Vit A alone, Zn alone and Vit A+Zn, results that do not make it clear which micronutrient had the best effect: Vit A alone, Zn alone, or Vit A+Zn.

Chen selected 186 anemic pre-school anemic children aged 3 to 6 years [20]. They were randomly divided into three groups; group 2 received albendazole alone treatment, 3 received 60000 µg vitamin A capsule combined with 400 mg single dose albendazole, treated for 6 months, they showed that albendazole alone treatment and albendazole+vitamin A administration showed improvement in anemia. The HGB levels of children in group 3 at 3 and 6 months were statistically higher than those of children in group 2, reducing the number of children with anemia at 3 months of treatment to 56.7% and at 6 months to 38.3%. These results are similar to the study where the group of girls after supplementation of Vit A alone managed to decrease their anemic picture from (71% to 21%), but the same effect occurred in the group supplemented with Zn alone, as well as a similar effect in the group of children supplemented with Vit A+Zn.

Al-Mekhlafi, et al. studied 126 children, 124 girls aged 7 to 12 years a mean age of 10 years, received Vitamin A supplementation, The prevalence of anemia was higher among children aged ≤ 10 years, than children>10 years the findings indicated that changes in HGB and other indices of iron status after three months were significantly higher in children supplemented with Vitamin A compared to those supplemented with placebo. At 3 months of vitamin A treatment, hemoglobin levels improved from 47.2% to 27.2%, thus decreasing the anemia picture. This effect was similar to that found in the group of girls supplemented with vitamin A alone, but also with Zn alone. In contrast to these findings, a previous study found no effect for weekly Vit A supplementation on HGB concentration among school children in rural and urban areas of East Java, Indonesia. This could be due to the short duration and low dose of vitamin A (10,000 IU) used. The benefits of vitamin A supplementation appear to be more pronounced among anemic children. However, the interaction of vitamin A with other micronutrients is still under debate. Most of the effects of vitamin A on zinc have been demonstrated in animal models. Human trials have failed to show a consistent relationship between zinc and vitamin A.

Conclusion

The role of micronutrients, including vitamin A and zinc, play an important role in iron metabolism and thus in the levels of HGB, HTC, MCV and MCH, the study evidenced the beneficial effect of vitamin A supplementation as a single dose in reducing the prevalence of anemia present in the children studied, given these results, further studies related to this research are recommended to be able to detect anemia figures in the child population, to develop micronutrient supplementation programs to solve this public health problem for the benefit of our children.

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Conflict of Interest

The authors report having no conflicts of interest in the development of the study and the writing of the manuscript.

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