Original Article *Iran J Ped Hematol Oncol. 2021, Vol 11, No 1, 11-17*

Serum zinc status in thalassemic adolescents attending Yangon Children Hospital, Myanmar

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Received: 12 May 2020 **Accepted:** 30 September 2020

Abstract

Background: Thalassemia constitutes a major public health problem causing a significant burden on children and their families. Zinc deficiency plays an important role in many thalassemia-related complications like growth retardation, hypogonadism and delayed puberty which are frequently noted in adolescent age. Although zinc is supplemented to thalassemic patients visiting Day Care Center, Yangon Children Hospital (YCH), Myanmar, a report concerning serum zinc level of these patients is still lacking. This study, therefore, aimed to assess serum zinc status in thalassemic adolescents attending Day Care Center, YCH.

Materials and Methods: This hospital-based cross-sectional study was conducted on 99 thalassemic adolescents. Mean age of diagnosis was 5.1±2.1 years. Non-fasting serum zinc concentration was determined by atomic absorption spectrophotometry. According to National Health and Nutrition Examination Survey data, zinc deficiency was defined as serum zinc concentration < 66 µg/dL (female) and < 70 µg/dL (male).

Results: Serum zinc concentration (µg/dL) was 57.35 (47.30-80.14) (median, interquartile range) with maximum, 195.05 and minimum, 28.83. Zinc deficiency was observed in 69.7% (69 out of 99; 35 males and 34 females) of the patients. The associations of zinc deficiency with gender, phenotype and the use of chelator were non-significant (P>0.05).

Conclusion: In spite of zinc supplementation, nearly 70% of the thalassemic adolescents showed zinc deficiency. Zinc deficiency in these adolescents might not be related to gender, phenotypes or the use of chelator. Poor compliance to take zinc supplementation and/or irregular blood transfusion could partly be attributable to zinc deficiency in these adolescents. Providing health education on the importance of regular intake of adequate zinc is advisable and periodic evaluation of zinc levels is recommended for thalassemic adolescents.

Key words: Adolescents, Thalassemia, Zinc

Introduction

Thalassemia, the commonest single-gene hereditary disorders in human, affects millions of people worldwide (1). Thalassemia disease constitutes a major public health problem in different regions of the world, particularly the in Middle East and Southeast Asia, generating a significant burden on children and their families as well as on the health services (2). In our country, children with Cooley's anemia-like symptoms have been observed since 1954 (3). It is estimated that, each year, 250 and 260 newborns will be affected with homozygous beta thalassemia and E-beta thalassemia, respectively according to an overview of Myanmar thalassemic children based on total population of 55,746,253 (4). Zinc, an essential trace element, is required for the activities of over 300 metalloenzymes involved in most of major metabolic pathways and consequently, many body functions are affected by zinc deficiency (5). Zinc deficiency has been reported to play a significant role in many common complications observed in thalassemic patients such as growth retardation (6), hypogonadism (6), delayed puberty (7) and depression (8) that are frequently reported during adolescent age. Zinc deficiency in thalassemic children and adolescents was reported by some previous studies (9, 10) while others reported higher

circulating zinc levels in thalassemic patients compared with control subjects (11-13).

In our country, apart from one previous local study that illustrated zinc deficiency in female adult patients with thalassemia intermedia (14), information on the serum zinc status in thalassemic adolescents is still limited. In addition, although zinc supplementation is now given to every thalassemic patient visiting Day Care Center for Thalassemia, Yangon Children Hospital (YCH), serum zinc status in these zinc-supplemented thalassemic adolescents has not been evaluated. For these reasons, this study aimed to evaluate serum zinc status in thalassemic adolescents attending Day Care Center, YCH.

Materials and Methods

This hospital-based cross-sectional study was carried out at Day Care Center for Thalassemia, YCH, Dagon Township, Myanmar. Male and female patients between 13-15 years of age (completed years) with thalassemia major or intermedia were included in our study. Mean age of diagnosis was 5.1±2.1 years. Thalassemic adolescents with current febrile condition, with documented history of diabetes mellitus or liver diseases or HIV infection or those taking corticosteroid therapy were excluded. Since there are the recommended set values for zinc deficiency with regard to age and gender, age- and sex-matched control group did not include in our study.

The aim and detailed procedures of the study were thoroughly explained to those fulfilling inclusion criteria as well as to their parents/guardians. Written informed consent was taken from parents/guardians and assent from the thalassemic adolescents. Within the study period, from March 2014 to December 2016, 99 thalassemic adolescents were eligible and evaluated for zinc deficiency. This study was approved by Research and Ethics Committee, University of Medicine-1, Yangon (001/UM1, REC.2013).

Sample collection

Blood sample was collected few days before blood transfusion or on the morning of receiving blood transfusion. Three mililiters (mL) of non-fasting blood was withdrawn between 8:30 and 10:30 am. After separating serum within two hours of sample collection, serum was poured into Eppendorf tubes instead of aspiration in order to prevent hemolysis. When the sample had visible hemolysis, it was not used for analysis and the blood sample was taken for the second time on the next follow-up of the patients. Sera were stored with code numbers at-20ºC.

Within three months of sample collection, serum zinc level was determined with atomic absorption spectrophotometry (Model-AA 6650, Shimadzu, JAPAN) at Water and Chemical Department, National Health Laboratory, Yangon. One mL of serum was diluted with five mL of distilled water. The diluted serum was then aspirated and read by atomic absorption spectrophotometer at 213 nm wavelength using air-acetylene flame with 0.2 nm slit.

Identification of zinc deficiency was based on the suggested cut-offs of serum zinc concentration (morning, non-fasting) proposed by National Health and Nutrition Examination Survey (NHANES); 66 µg/dL for females and 70 µg/dL for males, respectively (15).

Statistical analysis

Statistical Package for the Social Sciences-version 11 was used for data entry, cleaning, summarization and analysis. Numerical variables of the subgroups were presented as median (interquartile range) as they showed skewed distribution. Comparisons between the subgroups were analyzed by non-parametric test, Mann-Whitney U test. Pearson's chi-square test was used to determine any association of zinc deficiency with gender, phenotypes and the use of chelator. Statistical significance level was set at $P< 0.05$.

Results

Our study included 31 adolescents with thalassemia major (TM) and 68 adolescents with thalassemia intermedia (TI). Mean age of the thalassemic adolescents was 14.4 ± 1.4 years. The general characteristics of the thalassemic adolescents are shown in Table I.

The median serum zinc concentration was 57.35 (47.3-80.14) µg/dL, maximum was 195.05 µg/dL and minimum, 28.83 µg/dL. For evaluation of zinc deficiency, we used different cut-offs for male and female thalassemic adolescents (15). Thirty four out of 55 female thalassemic adolescents (61.8%) and 35 out of 44 male thalassemic adolescents (79.5%) were found to have zinc deficiency. The overall percentage of zinc deficiency of the thalassemic adolescents participated in our study was 69.7% (69 out of 99). Among these 69 patients, some clinical features of zinc deficiency such as delayed puberty (82.6% of the patients), growth retardation (88.4%) and depression (32.2%) were noted.

In addition, we did the subgroup division and compared serum zinc concentrations between these three pairs of subgroups; female patients vs. male patients; TM patients vs. TI patients, and patients taking iron chelator vs. those not taking iron chelator, respectively. No statistical significant difference in serum zinc concentrations was detected within these subgroups (Figure 1).

Zinc deficiency was observed in 23 out of 31 TM children (74.2%) and in 48 out of 68 TI children (70.6%). Regarding the third group, 42 out of 61 (68.9%) in the thalassemic adolescents taking iron chelator and 27out of 38 (71.1%) in those not taking iron chelator showed zinc deficiency. There was no significant association between these categorical variables (gender, phenotype and use of chelator) and zinc deficiency (Table II).

Table I. General characteristics of the thalassemic adolescents participated in the study

General characteristics	Frequency (%)	Mean ± SD
Age (years)		14.4 ± 1.4
Gender		
• Male	44 (44.4)	
• Female	55 (56.6)	
Height (m)		1.4 ± 0.2
Weight (kg)		28.8 ± 5.9
BMI (kg/m^2)		15.2 ± 2.1
Phenotype		
• Thalassemia major	31 (31.3)	
• Thalassemia intermedia	68 (68.7)	
Genotype*		
• β-thalassemia	39 (39.4)	
• E- β thalassemia	56 (56.6)	
• α- thalassemia	4 (4.0)	

***** Diagnostic genotyping was done by gel electrophoresis.

Table II. Associations of zinc deficiency with gender, phenotypes and use of chelator

Subgroups (Number)	Zinc deficiency (%)	Chi-square	P value
Gender			
• Male (44)	79.5		
• Female (55)	61.8	3.6	0.06
Phenotype			
• Thalassemia major (31)	74.2		
• Thalassemia intermedia (68)	70.6	0.17	0.74
Use of chelator			
• Yes (38)	71.1		
• No (61)	68.9	0.55	0.82

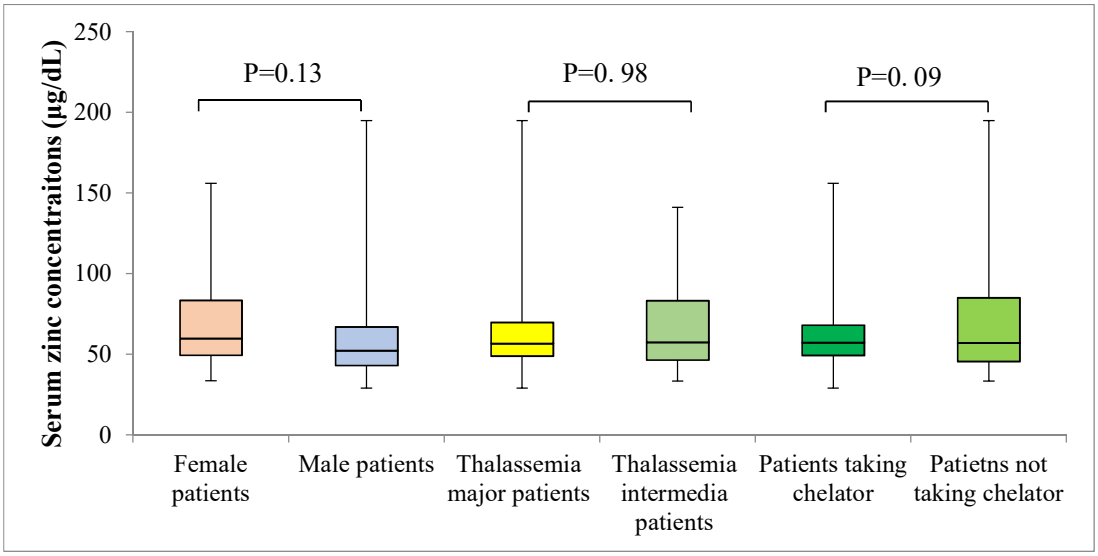

Figure1.Comparisons of serum zinc concentrations between the subgroups; female patients vs. male patients, TM patients vs. TI patients, and patients taking chelator vs. those not taking chelator (No significant difference; P> 0.05)

Discussion

In our study, about 70% of thalassemic adolescents were found to have serum zinc levels below the cut-offs set for zinc deficiency. There was neither a significant association of zinc deficiency with gender, genotype and the use of chelator nor a significant difference in serum zinc concentrations within the subdivided groups. The finding of zinc deficiency in the majority of the participants in our study is in tune with reports of a previous local study (65%) (14), a study from Iran (64%) (9) and a study from India (65%) (10). Even though the findings of our study and other previous studies are compatible, percentage of zinc deficiency found in our study is still higher and this could partly be due to difference in consideration of cut-offs for deficiency. We defined zinc deficiency separately for males (≤ 70 µg/dL) and females (≤ 66 µg/dL) while these studies applied a single cut-off, 70 µg/dL for both gender groups. Zinc supplement is now freely available to all thalassemic adolescents visiting the Day Care Center. The usual recommended dose of zinc syrup varied from 5 mL one time per day to 3 times per day in which each 5 mL of syrup contains 10 mg of elemental zinc. It is quite shocking that why nearly 70% of the patients in our study showed hypozincemia despite the administration of zinc supplement. One plausible answer is poor

compliance of the patients. The supplement provided by the center usually lasts for about one month and after that, they have to buy themselves for remaining period till next visit. However, in practice, the majority of parents/guardians seem to underestimate the importance of daily zinc intake and make their children omit supplementation until they received the zinc syrup again on the next visit to the center. Additionally, serum zinc concentration itself reflects short term changes in zinc intake. Serum zinc level can return to basal value within four to five days following discontinuation of zinc supplementation (16). Therefore, irregular taking of zinc supplements and short term changes of zinc in circulation could be contributable to the finding of low serum zinc level. Health education is of great importance for regular zinc intake.

If there were no providence of zinc supplementation from the center, additional number of thalassemic adolescents in our study could suffer zinc deficiency. A report from Iran by Mashhadi et al. (2014) study (12) supported our suggestion because in that study, the researchers already excluded thalassemic children taking zinc supplement and noted that all 333 children suffered zinc deficiency.

Many researchers consistently suggest that zinc deficiency in thalassemic children can be attributed to insufficient dietary zinc intake (9, 10). Red meat is one of the rich sources of zinc. Thalassemic patients usually avoid taking red meat and such avoidance may lead to nutritional zinc deficiency. One limitation of our study is unavailability of data concerning dietary pattern and so it could not be predicted whether individual thalassemic adolescents took sufficient or inadequate zinc in their daily meal.

One more important matter is that zinc deficiency in our thalassemic adolescents could also be due to our daily diet which itself contains less zinc content. The staple food in Myanmar is a grain and it is reported that meal based largely on staple grains such as rice can be considered a prime contributor to micronutrients deficiency including zinc (17). International Zinc Nutrition Consultative Group (IZiNCG) also states that the global prevalence of zinc deficiency ranges from 4% to 73% and the highest prevalence is seen in South and South-East Asia (34-73%) (18). A population-based study is required to determine zinc status of general population in our country.

Some previous studies found higher serum zinc levels in TM children compared with control subjects and recommended reconsidering zinc supplementation for these children (11, 12). On the contrary, Keikhaei et al. (2010) (19) and Naji et al. (2012) (20) gave a common conclusion that TM children were suffering from hypozincemia. In our study, nearly 75% of TM adolescents showed zinc deficiency and, at the same time, about 70% of TI adolescents exhibited the deficiency. Moafi et al. (2008) (8) stated that zinc deficiency was not dependent on phenotypes but on the condition whether blood transfusion was regular or not. At the Day Care Center for Thalassemia, YCH, although transfusion regime is well established, many patients fail to do regular follow up and transfusion has to be given only when there is symptomatic anaemia. Hence, another possible reason for hypozincemia detected in both TM and TI adolescents in our study could be irregular blood transfusion.

Although serum zinc concentrations between gender were not significantly different, deficiency of zinc was more frequent in male thalassemic adolescents and this finding was in accordance with that of Bekheirnia et al. (2004) (21). In addition, there was no association between gender and zinc deficiency of thalassemic adolescents and this finding also agrees with that of a study performed by Mansi et al. (2009) (11). In contrast, a significant gender difference in serum zinc levels was reported by studies of Keikhaei et al. (2010) (19) and Mashhadi et al. (2014) (12).

The Day Care Center for Thalassemia, YCH, is currently using an oral iron chelator, Deferiprone, for the reduction of iron overload. Depending on serum ferritin concentrations, Deferiprone is administered to those having iron overload and doses ranges from 250 mg one time per day to 500 mg two times per day. Evidences shows that low zinc concentration is not a common problem in thalassemic children treated with Deferiprone (22) and that long-term Deferiprone intake causes only a slight decrease in zinc concentration and the mean value found remained within the normal reference range (23, 24). It could explain the finding of no association between the use of iron chelator and zinc deficiency in our patients.

Milne et al. (1984) studied the effect of folic acid supplementation on some trace elements (zinc, copper and iron) absorption and excretion and concluded that supplementation of folic acid influences zinc homeostasis, perhaps through formation of an insoluble chelate and the impairment of absorption (25). This could add as another factor responsible for zinc deficiency found in the patients of our study because all of them get folic acid therapy continuously.

Conclusion

In conclusion, nearly 70% of the thalassemic adolescents showed zinc deficiency in spite of zinc supplement. Low serum zinc status in our patients could not be related to gender, phenotypes and chelator use. Irregular blood transfusion and intermittent zinc intake could partly be responsible for this finding of zinc deficiency. Moreover, daily intake of folic acid supplementation and nutritional deficiency may also partly cause zinc deficiency in these patients. Nevertheless, additional studies are required to verify these possibilities.

It is recommended to give health education regarding the importance of regular zinc supplement. Periodic evaluations of zinc status in zinc-supplemented thalassemic patients are also necessary and reevaluation of dosage of zinc supplements should be individualized based on degree of zinc deficiency.

Acknowledgments

We want to show special thanks to Professor Aye-Aye-Khaing, paediatric-haemato-oncologist, Yangon Children Hospital, for her kind permission to recruit thalassemic adolescents at Day Care Center for Thalassemia, Yangon Children Hospital.

Funding

This research received no specific grant from the funding agencies in the public, commercial, or not-for-profit sector.

Conflict of Interest

The authors declare that they have no conflicts of interest.

References

1.Weatherall DJ, Clegg JB. The thalassaemia syndromes: 4th ED. John Wiley & Sons, United Kingdom, Blackwell Science Ltd. ISBN 0470695943; 2008.

2. Weatherall DJ. The inherited diseases of hemoglobin are an emerging global health burden. Blood 2010; 115 (22): 4331-4336.

3. Aung-Than-Batu, Hla-Pe, Khin-Kyi-Nyunt. The thalassemia in Burma. Union of Burma JLife Sci 1968; 1: 241-247.

4. Aye-Aye-Khaing. Overview of thalassemia in Myanmar Children. MMJ 2015; 57 (2): 53-62.

5.Gibson RS, Hess SY, Hotz C, Brown KH. Indicators of zinc status at the population level: a review of the evidence. Br J Nutr 2008; 99 (S3): S14-S23.

6.Srisukh S, Ongphiphadhanakul B, Bunnag P. Hypogonadism in thalassemia major patients. J Clin Transl Endocrinol 2016; 5: 42-45.

7.Kyriakou A, Skordis N. Thalassaemia and aberrations of growth and puberty. Mediterr J Hematol Infect Dis 2009; 1 (1): 1-9.

8.Moafi A, Mobaraki G, Taheri SS, Heidarzadeh A, Shahabi I, Majidi F. Zinc in thalassemic patients and its relation with depression. Biol Trace Elem Res 2008; 123 (1): 8-13.
9.Mahyar A, Ayazi P, Pahlevan A-A, Mojabi H, Sehhat M-R, Javadi A. Zinc and copper status in children with Beta-thalassemia major. Iran J Pediatr 2010; 20 (3): 297-302.
10.Nidumuru S, Boddula V, Vadakedath S, Kolanu BR, Kandi V. Evaluating the role of zinc in beta thalassemia major: a prospective case-control study from a tertiary care teaching hospital in India. Cureus 2017; 9 (7): 1-7.
11.Mansi K, Aburjai T, Barqawi M, Naser H. Copper and zinc status in Jordanian patients with β-thalassemia major treated with Deferoxamine. Res J Biol Sci 2009; 4 (5): 566-572.
12.Mashhadi MA, Sepehri Z, Heidari Z, Shirzaee E, Kiani Z. The prevalence of zinc deficiency in patients with thalassemia in South East of iran, sistan and baluchistan province. Iran Red Crescent Med J 2014; 16 (8): 1-4.
13.Alhillawi Z, Al-Hakeim H, Moustafa S, Maes M. Increased Zinc and Albumin but Lowered Copper in Children with Transfusion-dependent Thalassemia. Preprints 2020; 1-26.
14.Win-Yu A, Thandar M, Khine Z, Mra R, Phone-Kyaw M. Effect of zinc supplementation on red cell deformability in beta thalassemic patients. MMJ 2010; 53 (1): 26-35.
15.Hotz C, Peerson JM, Brown KH. Suggested lower cutoffs of serum zinc concentrations for assessing zinc status: reanalysis of the second National Health and Nutrition Examination Survey data (1976–1980). Am J Clin Nutr 2003; 78 (4): 756-64.
16.Wessells KR, Jorgensen JM, Hess SY, Woodhouse LR, Peerson JM, Brown KH. Plasma zinc concentration responds rapidly to the initiation and discontinuation of short-term zinc supplementation in healthy men. Nutr J 2010; 140 (12): 2128-2133.
17.Chaparro C, Oot L, Sethuraman K. Overview of the nutrition situation in seven countries in Southeast Asia: Washintong, DC:FHI 360/Food and Nutrition Technical Assistant. 2014; Chapter 2: 4-16.
18.Brown K, Rivera J, Bhutta Z, Gibson R, King J, Lonnerdal B, et al. International Zinc Nutrition Consultative Group. International Zinc Nutrition Consultative Group (IZiNCG) technical document 1. Assessment of the risk of zinc deficiency in populations and options for its control. Food Nutr Bull 2004; 25 (1): S99-S203.
19.Keikhaei B, Badavi M, Pedram M, Zandian K, Rahim F. Serum Zinc Level in Thalassemia Major. Pak J Med Sci 2010; 26 (4): 942-945.
20.Naji ZM. Serum trace elements (zinc, copper and magnesium) in Iraqi patients with thalassemia major receiving desferrioxamine and its relation with growth state. Iraqi J Med Sci 2012; 10 (4): 375-382.
21.Bekheirnia R, Shamshirsaz AA, Kamgar M, Bouzari N, Erfanzadeh G, Pourzahedgilani N, et al. Serum zinc and its relation to bone mineral density in β-thalassemic adolescents. Biol Trace Elem Res 2004; 97 (3): 215-224.
22.Galanello R. Deferiprone in the treatment of transfusion-dependent thalassemia: a review and perspective. Ther Clin Risk Manag 2007; 3 (5): 795-805.
23.Cohen AR, Galanello R, Piga A, De Sanctis V, Tricta F. Safety and effectiveness of long-term therapy with the oral iron chelator deferiprone. Blood 2003; 102 (5): 1583-1587.
24.Taher A, Aoun E, Sharara A, Mourad F, Gharzuddine W, Koussa S, et al. Five-year trial of deferiprone chelation therapy in thalassaemia major patients. Acta Haematol 2004; 112 (4): 179-183.
25.Milne D, Canfield W, Mahalko J, Sandstead H. Effect of oral folic acid supplements on zinc, copper, and iron absorption and excretion. Am J Clin Nutr 1984; 39 (4): 535-539.