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ORIGINAL RESEARCH PAPER

Zinc and ferritin in haemoglobinopathies an observational study

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ABSTRACT

Introduction: *It has been estimated that with a population of 1000 million at the millennium year 2000 and a birth rate of 25 per thousand, there would be about 45 million carriers and about 15,000 infants born each year with haemoglobinopathies in India. The present study is taken up with special emphasis to paediatric patients with thalassemia major and sickle cell anaemia.* ***Objective****: To measure serum zinc and ferritin levels in paediatric thalassemia major and sickle cell anemia patients.* ***Materials and methods****: Serum zinc estimated by colorimetric method and ferritin by immunoradiometric method.* ***Result:*** *Mean ± S.D. of zinc in thalassemia major found to be lower than sickle cell disease with zinc being in lower side of normal range in sickle cell anemia cases suggesting hypozincemia in both groups whereas Mean ± S.D. of ferritin found to be higher in both the groups indicating iron overload in both the groups.* ***Conclusion****: Decreased zinc level and increased ferritin level found in the study may be caused by disease itself or may be consequence of repeated transfusion which has to be ruled out by further study in larger patient groups and meanwhile nutritional supplement of zinc and iron chelation therapy must be mandatory in these group of patients.*

Keywords*: Transfusion overload; nutritional supplement; chelation; Assam.*

INTRODUCTION

Hemoglobinopathies are a group of inherited disorders characterized by abnormal structure or production of haemoglobin caused by gene mutations. While iron deficiency is the most common cause of acquired anemia, haemoglobinopathies have emerged as the most common cause of hereditary anemia. The carrier frequency of haemoglobinopathy varies from 3 to 17% in different population groups of India.[1]

The cumulative gene frequency of the three most predominant abnormal haemoglobins, i.e., sickle cell, haemoglobin D and haemoglobin E has been estimated to be 5.35% in India.[2] Thus, there is a tremendous amount of burden of haemoglobinopathies in India .

Historically, the majority of children who were carriers of these diseases died during their first 10 years of life from complications. However, recent important advances have extended the average life of patients and significantly improved their quality of life. Improved understanding of the etiology and mechanisms of anemia, earlier diagnosis, new therapeutic approaches and better management of transfusion related iron overload have dramatically improved the clinical picture.[3]

Trace elements play an important role in many biological systems because they act as activators or inhibitors, hence competing with other elements and protein for binding site, influences the permeability of membrane.[4] Iron and zinc are essential trace elements in human body and are often altered in patients with thalassaemia and sickle cell anaemia in which

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they may play a role in pathogenesis. The alteration of these elements combined with excess amounts of haemoglobin subunits enhance the generation of oxygen radicals after a chain of reactions leading to early death of the red cells and haemolysis.[5] Iron overload is an unavoidable complication suffered by thalassemia major patients as a consequence of excessive number of blood transfusions. It is so common that it has been referred to as a "second disease" during treatment of first.[6]

Iron overload from chronic transfusion therapy can be extremely toxic. Excess transfusional iron is deposited in the liver, heart, and other organs as free iron, which can cause organ dysfunction and damage over time. Zinc (Zn) is an essential nutrient for all forms of life and its importance lies in the fact that many body functions are linked to zinc containing enzymes.[7]

Zn has an indispensible role in human health and diseases. It has been insufficiently recognised by a number of experts as an important public health issue, especially in developing countries. It is the most abundant intracellular metal ion found in cytosol, vesicles, organelles and in the nucleus.[8] However, even a small deficiency is a disaster to human health, so as such the number of biological functions, health implications and pharmacological targets that are emerging for zinc has evoked further interest regarding its status in human health and nutrition.[9]

The present study aims to measure serum zinc and ferritin and their co-relation in patients with haemoglobinopathies.

METERIALS AND METHODS

100 cases diagnosed as haemoglobinopathies, including both outdoor patients and patients admitted in the Department of Paediatrics of Assam Medical College and Hospital, were taken for the study. The study group was further subdivided into two groups one as thalassemia and other as sickle cell disease. Thalassemic group was again subdivided into homozygous thalassemia major, heterozygous E-thalassemia and S-thalassemia. Similarly sickle cell disease group was also subdivided into homozygous sickle cell disease and heterozygous E-sickle cell disease.

Inclusion criteria: 100 cases, both male and female, in the age group 1-16 years were included on the basis of detailed history and clinical diagnosis. Newly diagnosed patients of haemoglobinopathies (thalassemia and sickle cell anaemia) without transfusion and those coming for follow up and transfusion therapy. Diagnosed patients of haemoglobinopathies without having any oral medication that contain iron, zinc preparation for at least 2 months duration.

Exclusion criteria: Haemoglobinopathies associated with other haemolytic disorder (e.g.G-6PD deficiency), severe malnutrition, repeated respiratory infection, thalassemia minor and intermedia.

Laboratory investigations:

- R/E Blood, Complete haemogram
- Hb typing by cation exchange high performance liquid chromatography (BIO-RAD D-10)[10]
- Sickling test using Na metabisulphite method (if necessary)
- Serum Zinc measured by colorimetric method (in semiautoanalyzer, microlab 300 MERK)[11,12]
- Serum Ferritin measured by MAG-16 kit, which is a immunoradiometric assay kit.[13]

Students 't' test was used for comparison of quantitative variables. Co-relation between serum zinc and ferritin were evaluated using Pearson Co-relation Co-efficient. All tests were considered statistically significant if the p-value was <0.05.

To see the correlation between two variables co-efficient of correlation (r) is applied.

All statistical analysis were done in Microsoft Excel and Graphpad instat.

RESULT

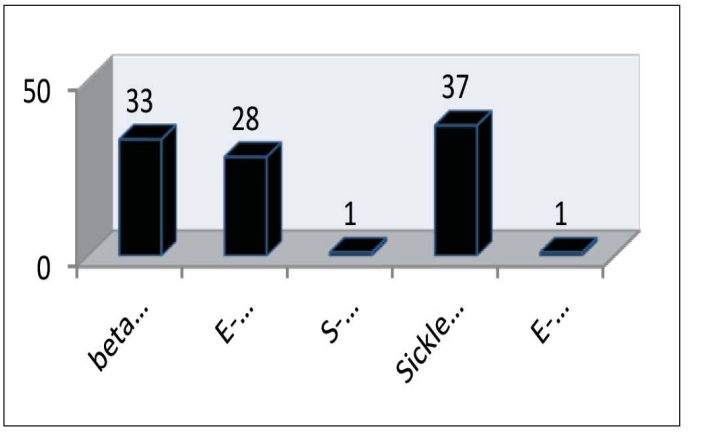

Figure 1 Distribution of cases according to HPLC result

It is seen from **Figure 1** that homozygous beta thalassemia, heterozygous E-thalassemia and homozygous sickle cell anemia cases are more prevalent in this study.

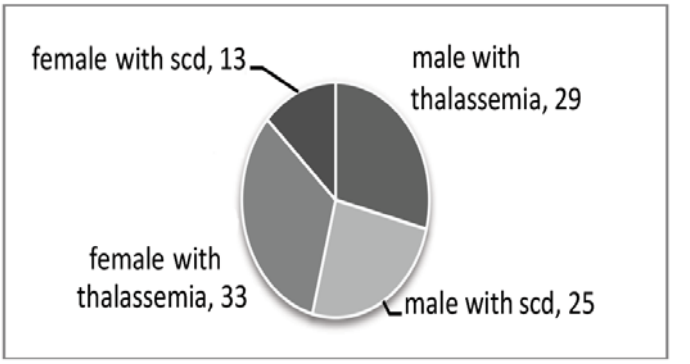

Figure 2 Distribution of cases according to gender

As seen in **Figure 2** majority of the thalassemic patients in the present study were female (53.23%) whereas majority of the sickle cell disease patients were male (65.78%).

Table 1 Patients based on level of serum ferritin (ng/ml)

STUDY GROUP	< 100		100-500		500-1000		>1000	
	n	%	n	%	n	%	n	%
THALASSEMIA	1	1.61	6	9.68	20	32.26	35	56.45
SICKLE CELL ANEMIA	2	5.26	13	34.22	18	47.37	5	13.15

as seen in **Table 1** ferritin levels are more than 1000 in majority of thalassemia cases whereas it is within 1000 in sickle cell anemia cases. It may be due to variation in number of transfusion in both groups.

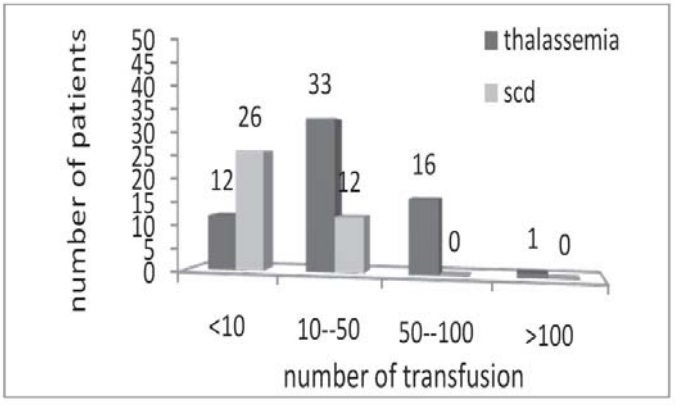

Figure 3 Distribution of cases according to number of transfusion received

As seen in **Figure 3** maximum 33 number of thalassemic patients (i.e.53.22%) have received transfusion 10-50 times and in sickle cell disease maximum 26 number of patients (i.e.68.42%) have received transfusion <10 times. Mean number of transfusion in thalassemia and sickle cell disease patients are found to be 34.20 ± 29.10 and 6.5 ± 5.7 respectively .

Table 2 Comparison of mean serum zinc (µg/dl) level between the study groups

STUDY GROUP	ZINC (MEAN ± S.D.)	p value
THALASSEMIA	71.85 ± 16.40	0.98
SCD	72.17 ± 14.97	

In **Table 2** it is observed that mean serum zinc level in thalassemic patients is lower in comparison to sickle cell disease patients but the difference is statistically not significant.

Table 3 Mean serum ferritin (ng/ml) level between the study groups

Study Group	Ferritin (mean ± S.D)	p value
THALASSEMIA	1130.53 ± 457.60	<0.0001
SCD	620.92 ± 359.76	

In **Table 3** it is observed that the mean serum ferritin (ng/ml) level in thalassemic patients is higher than the sickle cell disease patients and the result is statistically highly significant.

Table 4 Co-relation of serum zinc with ferritin in thalassemia major patients

Parameter	Correlation co-efficient (r)	p value
Zn vs Ferritin	-0.39	<0.0001*

*statistically significant

Table 4 shows zinc has negative co-relation with ferritin in thalassemia cases and it is statistically highly significant.

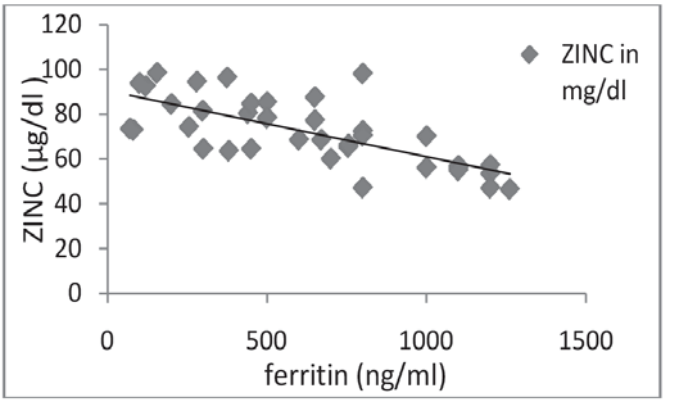

Figure 4 Co-relation of zinc with ferritin in sickle cell anemia patients

Figure 4 shows zinc has negative co relation with ferritin in sickle cell anemia cases and it is statistically highly significant

DISCUSSION

In the present study, mean ± S.D. of serum zinc level in thalassemia patients is found to be 71.85 ± 16.40 µg/dl and that of sickle cell disease found to be 72.17 ± 14.97 µg/dl. The mean level of zinc is within normal reference interval but on lower side in both the study groups indicating hypozincemia. In thalassemia patients zinc level is numerically found to be less than sickle cell disease patients, but this difference is statistically not significant (p=0.98).

Zahraa MA Naji in his study on "Serum Trace Elements (Zinc, Copper and Magnesium) in Iraqi Patients with Thalassemia Major Receiving Desferrioxamine and its Relation with Growth State" found that patients with thalassemia major showed lower levels of serum zinc as compared to that of control subjects (p<.05), which indicates that most of patients had hypozincemia; he concluded this may be related to dietary

insufficiency of zinc in those patients in addition to the effects of disease and desferrioxamine administration without dose adjustment for each patient.

Mahyar et al., found that the mean concentrations of serum zinc was 67.35±20.38 ìg/dl . Their study revealed that hypozincemia is common in thalassemic patients .

Although many studies revealed low serum zinc in thalas-semia patients at least one study by Mehdi-zadeh M et al revealed significantly higher serum zinc in the thalassemic group with no significant correlation between serum zinc level and serum ferritin level, so indicates zinc deficiency in thalassemic patients ,who are on regular blood transfusion is rare.

Bot Y.S et al[14] study on "Analyses of Cu and Zn in serum of sickle cell disease patients in Jos" found that a significantly low zinc concentration was obtained from the general comparison of sickle cell disease patients with control subjects.

Zemel et al., 2002; Singhi et al., 2003 in their study found that the biochemical evidence for zinc deficiency in patients with SCD includes low zinc concentrations in plasma, erythrocytes, hair lymphocytes and granulocytes.

In another report by Parad, et al. (1975), low activities of zinc dependent enzymes such as carbonic anhydrase, alkaline phosphate and thymidine kinase found in SCD patients.

A higher than normal activity of plasma ribonuclease in patients with SCD is also seen because zinc is known to inhibit the activities of this enzyme (Parad et al., 1975).

Zinc deficiency can also be the result of the adverse effect of hydroxyurea which increase zinc excretion as reported by Silliman et al., (1993).

Kaur M et al (2013) in their study on "the haemoglobinopathies and ratio of copper and zinc in sindhi community of Bhopal" found that zinc deficiency is common in haemoglobinopathic patients (thalassemia and sickle cell anaemia).

In the present study mean serum ferritin (ng/ml) level in thalassemia patients is more than sickle cell disease patients and are statistically highly significant (p<.0001). The mean ± S.D. of ferritin (ng/ml) in thalassemia patients is found to be 1130.53 ± 457.60 ng/ml and that of sickle cell disease cases is 620.92 ± 359.76 ng/ml.

In a study conducted by Nadeem Ikram et al., (2004) on "Ferritin Levels in Patients of Beta Thalassaemia Major" it was shown that mean serum ferritin levels was 3390 ± 135.6 ng/ml.

Cunningham et al., (2004) reported mean serum ferritin levels in beta thalassemia patients of North America to be 1696 ng/ml (26).

However, Choudhry VP et al in India reported mean serum ferritin levels to be 6723 ng/ml (27)

In a study conducted by Mishra K Amit et al (2013) in Bhopal Madhya Pradesh on "Iron Overload in Beta Thalassaemia Major and Intermedia Patients" it was shown that the mean serum ferritin level was 2767.52 ± 1849.1 ng/ml which is quite higher than normal.

Another study by Mohammed Saied Abdulzahra et al (2009) "on Study of the effect of iron overload on the function of endocrine glands in male thalassemia patients" it was shown that the mean concentration of serum ferritin was more than eight times higher than normal.

Claster S et al[15] in their study found that mean ferritin level in thalassemia and sickle cell disease patients were 3874 ± 4451 ng/ml and 2089 ± 1920 ng/ml respectively and it was found to be statistically significant in both the groups (p<0.02).

CONCLUSION

From the present study, it was observed that serum ferritin was significantly elevated in patients with haemoglobinopathies as compared to normal reference interval, however serum zinc was significantly decreased as compared to normal reference interval. Moreover, there was definite statistically negative correlation of serum ferritin to serum zinc. To conclude, measurement of zinc, ferritin in patients with haemoglobinopathies and replenishment of trace element in deficient states, by means of oral supplementation and removal of excess iron by chelation therapy could be a possible deterrent to the progression of disease. Regardless of the underlying etiology, these results suggest that all patients with thalassemia major and sickle cell disease who are repeatedly transfused should have periodic nutritional evaluation and supplementation as necessary.

However, as we are constrained by the limitation of time and relatively smaller sample size, it would probably be more predictive with larger sample size and longer period of study to explore more deep into this area for better management of the patients indisposed with haemoglobinopathies.

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