ORIGINAL RESEARCH PAPER

Pathology

EVALUATING SERUM FERRITIN LEVELS IN THALASSEMIA PATIENTS ON MODERATE TRANSFUSION REGIME : A REPORT FROM TERTIARY CARE CENTRE IN JAMMU.

KEY WORDS: Ferritin, Thalassemia, Transfusion

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ABSTRACT

Background : Although regular blood transfusions is the mainstay treatment for thalassemia patients, it is associated with various complications. Of these, iron overload is present in almost all patients and results in various complications.
Aims and Objectives: Evaluation of iron overload by measuring serum ferritin levels in thalassemia patients.
Materials and methods: This study was carried out in 146 thalassemia major patients attending thalassemia day care center who received regular moderate transfusion regime in the Department of Transfusion Medicine and Immunohematology, Shri Maharaja Gulab Singh Hospital, Government medical college Jammu from November 2014 to October 2015. Serum ferritin levels were measured by using ELISA test.
Results: There were 146 thalassemia major patients in the study comprising of 57 females and 89 males, age ranged between 2 year to 32 years. It was observed that Serum ferritin levels increased with increasing age of the patients till 20yrs of age. Levels were also significantly high ($p<0.0001$) with increasing number of transfusions both at the beginning and end of study. Minimum level of Serum Ferritin at the start of study was 221ng/ml while maximum level was 6959 ng/ml with a mean of 1971.67(SD 1281.69) ng/ml. Minimum level of Serum Ferritin at the end of study was 200 ng/ml while maximum level was 5000 ng/ml with a mean level of 1805.6(SD1163.89) ng/ml. The difference of mean serum ferritin levels was significant (p value was 0.043 and t value was 2.04) implying a significant reduction in S. Ferritin levels by the end of the study.
Conclusion: In the present study, it was found that thalassemia patients suffer from iron overload owing to repeated blood transfusions. Thus meticulous monitoring of serum ferritin levels must be carried in these patients to minimize chances of associated complications along with moderate transfusion regime

Introduction

The Thalassemias are inherited hematologic disorders caused due to defects in synthesis of any of the hemoglobin chains. The primary pathology in thalassemia stems from the reduced quantity of globin chain production. The globin chains produced in excess can cause damage to the red cells or their precursors thus resulting in an overall deficit of hemoglobin tetramers in the red blood cells and as a result the red blood cell indices like the mean corpuscular volume and the mean corpuscular hemoglobin are decreased **(1).** Thalassemia is considered as the most common genetic disorder occurring throughout the world. It occurs with a high frequency in certain regions including the Mediterranean region, the Middle East particularly Iran, India and Southeast Asia.

The conventional treatment of beta thalassemia major depends upon regular blood transfusions initiated in the early childhood, which improves the hemoglobin levels and also reduces the skeletal deformities caused by excessive erythropoiesis **(2,3) .** Regular blood transfusions remain the mainstay of treatment for these children. The combination of transfusion therapy and chelation has resulted in a dramatic extension of the life expectancy of these patients. Although blood transfusion is a life saver for thalassemia patients, it is also associated with many complications such as iron overload causing organ damage, platelet and red blood cell alloimminization **(4,5).**

Transfusion related iron overload and iron toxicity:

Every unit of red blood cells that are transfused result in accumulation of some quantity of iron in our bodies. It has been estimated that each unit of packed red blood cells contain approximately 250 mg of iron **(6).** Our body on the other hand cannot excrete more than 1 mg of iron per day. Therefore any patient who receives 25 units per year, ends up accumulating at least 5 grams of iron per year if no chelation is given **(7).** These patients also show increased intestinal iron absorption which adds to the total iron being accumulated in their bodies. The underlying mechanism of organ dysfunction caused due to iron overload has not been fully elucidated as of now. However, high iron levels in the body lead to saturation of transferrin, and thus excess iron in the form of non transferring bound iron begins to circulate in the plasma. Unbound iron within cells or in plasma is labile and can undergo redox cycles thereby generating reactive oxygen species (ROS), causing lipid per-oxidation. Lipid per-oxidation under conditions of iron overload leads to the production of unsaturated and saturated aldehydes. These have been postulated to cause cellular dysfunction, cellular toxicity, and ultimately cell death **(8,9)**. There is a propensity in some tissues to be susceptible to excess iron incorporation when non transferring bound iron is present. Other than iron overload, some other factors which are responsible for causing organ damage include hypoxia caused by anemia which may lead to potentiation of the toxicity caused by iron deposition in the endocrine glands. The measurement of serum ferritin levels is the test that is most commonly used for evaluation of iron overload in Thalassaemia Major patients. A ferritin level not exceeding 1000 mg/l approximately is generally considered safe and is presently recommended standard level in the patients of thalassaemia major (10).

Materials and methods

This study was carried out from November 2014 to October 2015 in the department of Transfusion medicine and Immunohematology, SMGS Hospital, associated with GMC Jammu which is a tertiary care hospital. This hospital has a dedicated Thalassemia day care centre in the department of Paediatrics. The subjects were patients of known thalassemia major with clinical manifestations, confirmed by hemoglobin electrophoresis and who are receiving regular transfusions in the department of Transfusion medicine, SMGS hospital. An informed consent was obtained from all the participants, their parents or legal guardians. Other types of congenital hemolytic anemias were not included in this study. The clinical details of all patients including name, age, sex, number of transfusions and any other relevant details were recorded. Serum ferritin levels were done both at the start and at the end of the study in all patients. A clean venepuncture was made to collect about 3 ml of patient's blood sample for the purpose of Serum ferritin measurement. The blood so collected was then allowed to clot. Serum was separated and it was stored at -20°C. Serum Ferritin levels were performed by using indirect enzyme linked immunosorbent assay (ELISA) kit.

Results

There were a total number of 146 Thalassemia major patients included in our study. This included 57(39%) females and 89(61%) male patients, who were put on regular moderate transfusion regime during the one year period of our study. The female: male ratio was 1:1.56.

Table 1: Distribution of serum ferritin with respect to Age

AGE	No. Of patients	Mean S.Ferritin level (SD) at the start (ng/ml)	Mean S.Ferritin level (SD) at the end (ng/ml)
0-5 years	31(21.23%)	852.0(468.4)	857.45(502)
6-10 years	51(34.93%)	1505.26(1099.1)	1493.17(909.4)
11-15 years	30(20.54%)	2532.68(986.9)	2218.63(946.8)
16-20 years	19(13.01%)	3232.67(1091.4)	2537.36(899.2)
21-25 years	10(6.84%)	2982.77(804.3)	3099(1749.1)
>25 years	5(3.42%)	3488.9(1042.7)	3030(1067.1)

The patients included in the study had ages ranging between 2 year to 32 years. Mean age was 11.02 years whereas median age was 10 year. All the patients were divided according to their age into 6 subgroups of class interval 5 ie 0-5 years ,6-10 years, 11-15 years, 16-20 years, 21-25 years, >25 years. Serum ferritin levels were observed to increase with increasing age of the patients till 20 years of age after which the serum ferritin levels plateaus.

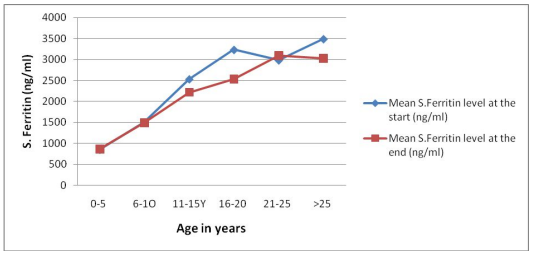


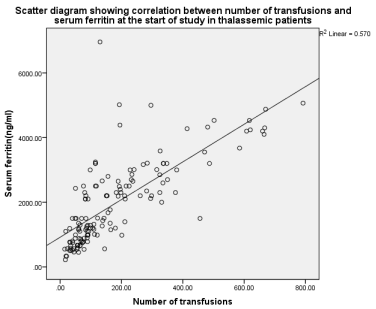
Figure 1: Relationship of serum ferritin with age of patients.

Table 2: Distribution of serum ferritin based on number of Transfusions

No. Of transfusions at the end of study	No. Of Patients	Mean S. Ferritin levels (SD) at the start (ng/dl)	Mean S. Ferritin levels (SD) at the end (ng/dl)
0-100	67(45.9%)	1055.48(579.4)	1104.79(728.0)
101-200	34(23.3%)	2249.46(1274.7)	1975.91(854.24)
201-300	18(12.3%)	2563.19(838.6)	2497.77(1368.0)
301-400	12(8.2%)	2828.75(463.7)	2888.33(1045.8)
>400	15(10.2%)	4038.21(847.7)	2854(1131.4)

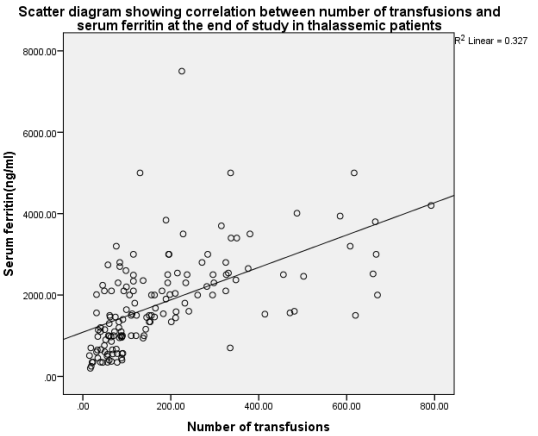
Total number of transfusions ranged from a minimum of 15 to a maximum of 792 in one patient. Mean no. of transfusions received were 180. Maximum patients (45%) had received less than 100 transfusions at the time of study. A total of 2950 transfusions were given during the study period. Mean no. of transfusions per patient per year was 20.20. Serum ferritin increased significantly ($p<0.0001$) with increasing number of transfusions both at the start and end of study except that serum ferritin levels plateaued after a total of 300 transfusions per patient at the end of study.

Figure 2(a)



As seen in figure 2(a) there is a positive linear relation between serum ferritin levels at the start of study and the number of transfusions and this correlation was found to be statistically highly significant (pearson's correlation coefficient r =0.755, $p<0.0001$). In other words, 57% of the variation in serum ferritin levels could be explained by the number of transfusions ($R_c = 0.570$).

Figure 2(b)



Similarly as seen in figure 2(b) there is a positive linear relation between serum ferritin levels at the end of study and the number of transfusions and this correlation was found to be statistically significant(pearson's correlation coefficient r =0.571, $p<0.0001$). In other words, 32.7% of the variation in serum ferritin levels could be explained by the number of transfusions ($R_c = 0.327$).

Table 3: Distribution based on S.Ferritin levels.

Serum Ferritin Levels(ng/ml)	No. of patients at the start of study	No. of patients at the end of study	%age change
0-1000	43	45	-4.65
1001-2000	35	44	-25.71
2001-3000	39	41	-5.12
3001-4000	14	10	+28.57
4001-5000	12	5	+58.33
>5000	3	1	+66.66

Serum Ferritin levels were measured both at the start and at the end of study. Minimum level at the start of study was 221 ng/ml in one patient and maximum level was 6959 ng/ml. Mean Ferritin level at the start of study was 1971.67(SD 1281.69) ng/ml. Minimum level of Serum Ferritin at the end of study was 200 ng/ml while maximum level was 5000 ng/ml. Mean ferritin level at the end of study was 1805.6(SD1163.89) ng/ml. The p value was 0.043 and t value was 2.04 implying a significant reduction in S. Ferritin levels by the end of the study. As we can clearly see in the table(3), percentage change in the serum ferritin levels between the start and end of study is showing a change from negative to positive direction i.e the number of patients with high serum ferritin levels is decreased at the end of study.

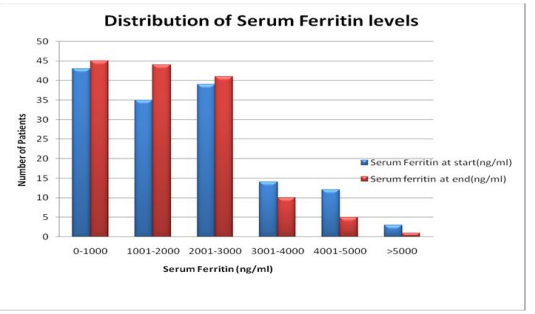


Figure 3: Distribution based on Serum Ferritin levels at the start and at the end of study.

Discussion:

The life span of thalassemia patients has increased dramatically over past few decades due to regular blood transfusions. Although early and regular blood transfusions have resulted in prolonged survival but it also causes a number of complications in these patients resulting in significant morbidity including hemosiderosis, chronic viral infections, alloimunization, endocrine dysfunction etc. **(11).** Despite the availability of newer chelation agents, hemosiderosis remains a significant challenge in patients recieving multiple transfusions. Frequent evaluation of the body iron stores is required for effective management of iron overload **(12).** An accurate assessment of body iron can be made by liver biopsy which by virtue of being an invasive and time consuming process may not be preferred. Therefore there is need for a quantitative, non-invasive method for measuring body iron stores that is safe, accurate and readily available. There are different methods to assess iron status of the body in overload conditions. Serum ferritin measurement is one of the most widely used method that is easy to perform and gives a fairly accurate idea about the body iron stores. Sometimes it may offer variable results, but still at present, there is no other serum test is a better predictor of body iron stores **(13).**

Serum Ferritin levels:

Serum ferritin levels were measured both at the start of study and at the end of the study. Serum Ferritin levels ranged from 200 ng/ml to 6959ng/ml. Minimum level at the start of study was 221 ng/m in one patient and maximum level was 6959 ng/ml. Mean Ferritin level at the start of study was 1971 ng/ml. Minimum level of Serum Ferritin at the end of study was 200 ng/ml while maximum level was 5000 ng/ml. Mean ferritin level at the end of study was 1805 ng/ml. Cunningham MJ et al (2004) reported Serum Ferritin levels ranging from 147 to 11010 ng/mL with a median of 1696 ng/mL **(10).** Ikram N et al (2004) reported Serum Ferritin levels in the range of 669 to 6325 ng/ml with mean of 3390 ng/ml**(14).** The high levels of serum Ferritin in our study at the start and a small decrease by the end indicate that Hemosiderosis remains the most important complication in thalassemia major patients. The minor but significant change in Serum Ferritin levels seen during this period could be due to combination of two oral drugs deferiprone and deferasirox introduced during this period. Moreover during the study period we followed moderate transfusion regime in order to maintain haemoglobin at 9 g/dl, thereby avoiding rise in serum ferritin levels by overtransfusion. Though details of Iron intake and chelation therapy were not studied in detail but despite newer chelation agents, hemosiderosis remains a significant challenge in this population. In our study there was a significant increase in serum ferritin levels with increasing age of the patients with mean serum ferritin levels of 857 ng/ml in age group of 0-5 years to 3099 ng/dl in age group of 20-25 years. In the study of Cario H et al (1999) 60% of patients in the first decade of life showed serum ferritin levels below 1800 ng/ml, 52% of patients more than ten years of age had serum ferritin levels above 2500 ng/ml **(15).** Bandyopadhyay et al., found that even younger patients had high serum ferritin levels. They found that among patients in 1-5 years age group, mean serum ferritin was 1750 ng/ml, and in patients of 11-15 years age group it was 3650 ng/ml **(16).** In the study of yin XL et al in 2011 The mean serum ferritin level was 3,143 ng/ml and levels increased with age **(17).** This increasing level of serum ferritin with advancing age is attributed to repeated transfusions recieved by patients. This fact was confirmed in our study when serum ferritin levels were compared with the number of transfusions recieved. The serum ferritin levels increased from 1104ng/dl in patients having recieved fewer than 100 transfusions to 2888 ng/dl in patients having recieved more than 300 transfusions and have plateaued thereafter. This plateau in the serum ferritin levels could be explained by the saturation of the ferritin receptors in the body by the increased serum ferritin. Increased levels of iron in body result in deposition of iron in vital organs like liver, heart and other endocrine organs resulting in various complications. Thus increasing number of transfusions with increasing age result in mean higher serum ferritin level in patients of thalassemia. There is therefore a need to monitor body iron levels and regular effective chelation therapy with the increasing age of thalassemia patients.

Conclusion

The increasing lifespan of thalassemia patients has also led to an increase in a number of comorbid conditions that are affecting these patients. A significant number of these comorbidities result from iron accumulation in the organs of the body leading to organ dysfunction. Hence to prevent these complications or to limit their extent proper chelation and monitoring of serum ferrittin levels to maintain serum ferritin in acceptable range needs to be practiced more vigorously.

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