

Original Research Article

## Serum Ferritin Level in Sickle Cell Disease

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### ABSTRACT

The prevalence of sickle cell anaemia, a known genetic disorder of haemoglobin is approximately 10-12% in Chhattisgarh population. Iron (Fe) being the important constituent of haemoglobin, its level in sickle cell disease, plays a significant role in haemoglobin biosynthesis. Serum ferritin is one of the important indicators in the assessment of iron status in sickle cell disease. The objective of this study is to determine the level of ferritin in sickle cell disease patients, sickle cell traits and compare with normal population. The study subjects included blood transfused (SS-T) and never-transfused sickle cell disease patients (SS-NT), sickle cell traits (AS) and normal (AA) subjects. Both male and female subjects with the age range from 7-40 years were recruited for the study. The body mass index (BMI), haemoglobin and serum ferritin concentration of the SS-NT, SS-T, AS and AA subjects were determined. The standard deviation (SD) was calculated using MS Excel. All the results were analysed using Student's t test and SSPS-16 software. The mean haemoglobin level of SS-NT patients was very low. The serum ferritin concentrations of AS subjects were less than that of both SS-T and SS-NT subjects. Ferritin level of SS-T subjects with more with than 10 units of blood transfusion is significantly high compared to other study subjects. Serum ferritin significantly increased in transfused as well as sickle patients in crisis but marginally in non transfused steady state patients which indicates that steady state patients are also iron over loaded. Traits can be treated with iron supplements when deficiency is proven by laboratory investigation.

**Key words:** Ferritin, haemolytic anemia, transfusion, sickle cell disease.

### INTRODUCTION

Sickle cell disease (SCD) is an inherited disorder of haemoglobin synthesis characterised by abnormal, rigid and sickle shaped red blood cells that result in a risk of serious complications. The sickle haemoglobin (HbS) is a result of point mutation in which there is replacement of glutamic acid with valine at 6<sup>th</sup> position of  $\beta$ -globin chain of haemoglobin. In homozygous state of the disease, both the  $\beta$ -globin genes are mutated (HbSS) and the individuals suffer from life-long severe haemolytic anemia, attacks of pain crisis, chronic organ system damage and marked decrease in life expectancy. [1] In

heterozygous (HbAS) condition, both normal and mutated hemoglobins are produced. These individuals do not experience symptoms, are generally healthy and are said to be sickle cell traits.

Iron (Fe) being the important constituent of haemoglobin, its level in sickle cell disease play a significant role in haemoglobin biosynthesis. Iron deficiency cannot produce an adequate amount of haemoglobin to meet the oxygen requirement for cellular respiration. This would further lead to development of complications in sickle cell patients. On the other hand, iron-overload due to repeated transfusion is known to cause

haemochromatosis leading to serious damage of body tissues. Therefore, maintaining an appropriate level of iron in the body of sickle patients is inevitable.

A variety of physio-chemical strategies are being adapted to maintain the iron level in sickle cell disease. Gastrointestinal iron absorption is one such mechanism in sickle cell disease to cope with the iron loss due to associated haemolytic anemia. [2] The iron provided by red cell transfusion also provides sufficient iron. [3] Ferritin, the primary iron storage protein in tissues that releases iron into the body in a controlled fashion. This storage protein is found in the liver, spleen, skeletal muscles and bone marrow. The production of ferritin is mostly triggered by the presence of iron. [1] Ferritin is also an acute phase reactant. So elevated serum levels are found in the presence of inflammation, infection, and liver disorders. [2] Serum ferritin is also considered one of the most important tools in measurement of the state of iron balance in steady-state sickle cell disease. [4]

About 50% of the world populations of SCD cases are found in India. [5] Screening programme has revealed that the prevalence of sickle cell anaemia is approximately 10-12% in the population of Chhattisgarh. [6] In the present study, an attempt has been made to determine the serum ferritin level of SCD patients of different age groups of Chhattisgarh population. The ferritin level in heterozygous traits (AS) and in normal population (AA) has also been determined and compared with that of the homozygous patients (SS).

## **MATERIALS AND METHODS**

### **Place of study and characteristics of subjects**

The present study was carried out in department of biochemistry, Pt. J. N. M. Medical College, Raipur, Chhattisgarh and was approved by the ethical committee. Those subjects suffering from sickle cell disease, the sickle cell traits and the normal

population reporting to the department and the mobile camp for sickling test were included as the subjects for the present study. A total of 126 subjects were recruited for the study. Of these, 43 subjects consisting of 21 male and 22 females were SCD patients (SS) and 40 subjects consisting 18 male and 22 females were sickle cell traits. Out of the 43 SS subjects 24 subjects were never transfused (SS-NT) before the study where as rest 19 subjects had undergone blood transfusion more than ten 10 times before the study (SS-T). All patients were diagnosed by solubility test followed by cellulose acetate electrophoresis, the standard test followed for the diagnosis of sickle cell disease. Equal number of age and gender matched subjects that were tested negative for sickle cell disease was taken as controls (AA). All the subjects belonged to the age group of 7-40 years. The clinical and other details of the subjects were collected through structured questionnaire.

### **Measurement of physical parameters**

The height and weight of each participant were measured. The body mass index (BMI) was calculated using the formula weight in Kg/height in m<sup>2</sup>.

### **Collection and processing of samples**

About 4 ml of blood samples were collected from each subject by venipuncture. 1.0 ml of the blood was transferred into EDTA vials and was used for determination of haemoglobin content of the subjects. Remaining blood sample was allowed to clot in plain vial and then spun in a centrifuge at 2000 rpm for 5 min. The supernatant serum was transferred into a fresh eppendorf vial. Serum ferritin concentration was estimated on same day. Whenever it was not possible then the samples were stored at -80 °C till analysis.

### **Determination of haemoglobin**

Haemogram was done using automated 3 part haematology cell counter (Mindray, BC-3000 plus).

### **Determination of serum ferritin**

An enzyme-linked immunosorbent assay (ELISA) kit (Monobind Inc. Lake

forest, CA 9230, USA) was used for determination of ferritin. The kit had high specificity (with a cross reactivity of < 10%) and sensitivity of 0.75 µIU/ml at 95% confidence limit with cv of 0.9 %. The normal reference of the serum ferritin in males, females and new born is 16-220 ng/ml, 10-124 ng/ml and 220 ng/ml respectively.

**Statistical analysis**

The analysis of the results was done by Student’s t test. A ‘P’ value of 0.05 was considered significant. SPSS 16 (SPSS software Inc Chicago, USA) statistical software was used for analysis.

**RESULTS**

**Sample characteristics**

The study group consisted of 86 cases that include 43 sickle cell disease and 43 traits. The control group consisted of 40 individuals. The sample characteristics of the participants for this study including BMI, Hb concentration and ferritin have been presented in table I.

**Haemoglobin content**

The haemoglobin level of the SS subjects varied within the range of 6.1-9.7 g/dl where as in AA and AS subjects the range was 11.8-15.6 g/dl and 10.01-12.2 g/dl respectively. The mean haemoglobin content of AA, AS, SS-NT, SS-T subjects was 12.86 ±3.21, 10.25±1.73, 6.9 ±1.02 and 9.85±1.46. g/dl respectively. The difference in the level of haemoglobin between cases and controls was statistically significant (P<0.05).

**Serum ferritin concentration**

The mean serum ferritin concentrations in AA male and female subjects were observed as 121.21±17.74 ng/ml and 102.51±16.37 ng/ml respectively. Much variation was not found in the ferritin concentration in different age groups of control subjects. The mean serum ferritin concentration in AS subjects was measured as 45.22±8.12 ng/ml. The ferritin levels in male and female traits were 47.20±7.33 ng/ml and 41.47±6.29 ng/ml respectively. As in control subjects, the concentration of serum ferritin in different age groups of subjects belonging to the traits also did not show significant differences.

**Table I: Sample characteristics, haemoglobin and serum ferritin level of control, SS and AS subjects. Mean values with ±SD are presented. Asterisks indicate the significant difference at P<0.05 level by Student’s t test.**

Variables	Age (Years)	BMI (Kg/m <sup>2</sup> )	Hb (g/dl)	Ferritin concentration (ng/ml)	
AA	Male(n=18)	15.04 ±3.21	19.01 ±5.20	14.65 ±2.05	121.21 ±17.74
	Female (n=22)	13.06 ±2.24	18.36±4.15	12.25±1.95	102.51 ±16.37
AS	Male(n=20)	18.36 ±5.61	18.81±4.71	11.9±1.91	47.20*±7.33
	Female(n=23)	16.24 ±4.50	18.05±4.10	10.7±1.25	41.47*±6.29
SS-NT	Male(n=10)	13.54 ±6.12	16.68±4.16	7.12*±0.94	239.04*±15.48
	Female (n=14)	14.25 ±5.23	15.46±5.20	6.64*±1.27	190.16*±11.23
SS-T	Male(n=10)	12.6 ±4.02	17.12±7.68	9.28*±2.41	509.05*±21.46
	Female(n=9)	14.02 ±6.28	16.52±3.34	8.24*±1.87	492.36*±17.25

In SS-NT subjects, the serum ferritin concentration of both male and female subjects increased as compared to that of AA and AS subjects. The mean ferritin concentration of male and female SS-NT subjects was observed as 239.04±15.48 ng/ml and 190.16±11.23 ng/ml respectively. In SS-T subjects the ferritin concentration further increased significantly in both the sexes compared to that of the rest category of subjects including the SS-NT also. The mean ferritin concentration in SS-T male and female subjects was measured as

509.05±21.46 and 492.36±17.25 ng/ml respectively. The serum concentration varied remarkably among the subjects of different age groups. The differences in serum ferritin level between the SS-T subjects and AA and SS and AS were statistically significant (P<0.05).

The serum ferritin level in male and female subjects of all the categories and also in different age groups of these subjects has been shown in Fig. 1 and table I.

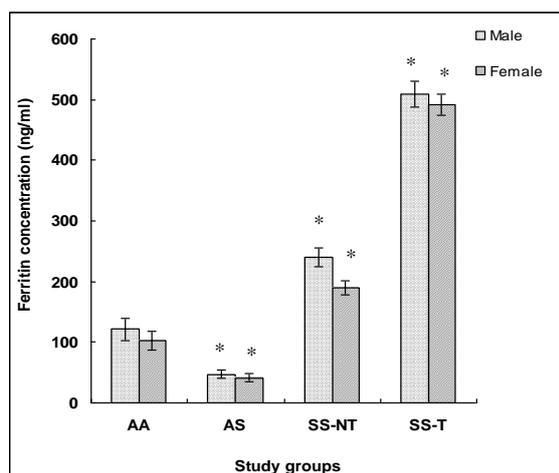


Fig. 1: Ferritin concentration of different subjects. AA, AS, SS-NT and SS-T represent control, sickle cell traits, sickle cell disease patients without and with blood transfusion and sickle cell disease patients with at least 10 units of blood transfusion respectively. Asterisks indicate the significant difference at  $P < 0.05$  level by Student's t test.

## DISCUSSION

Sickle cell anaemia is characterised by lifelong haemolytic anaemia, attacks of painful crisis and chronic organ damage. There is no evidence of iron overload per se in sickle cell anaemia patients. But serum iron, transferrin and ferritin remain normal or modestly elevated in steady state or never transfused patients. [7,8] A higher fold increase in ferritin level in transfused sickle cell patients has also been noticed in the present study. Further, the decline of ferritin level of sickle cell trait patients that has been observed indicates deficiency of iron among those patients. The mean haemoglobin level is also low in these patients (Table-I).

The mean haemoglobin levels are significantly decreased both in never transfused (SS-NT) and more than 10 times (SS-T) transfused sickle cell patients as compared to normal subjects. Though the serum ferritin in never transfused patients (SS-NT) is either normal or marginally raised, it remarkably increased in patients who have been transfused more than 10 times (SS-T). The marginal elevation of ferritin in never transfused patients or steady state patients may be due to increased red cell turn over. [7,9,10] The significant increase in ferritin in patients transfused more than 10 times (SS-T) may be due to repeated transfusions in addition to increased red

cells turn over. Chronic haemolysis also causes increased absorption of iron from gastrointestinal tract. [11,12] These factors together compound to increased ferritin level in the transfused patients. Ferritin is also an acute phase reactant protein. Patients who are transfused more than 10 times obviously have had attacks of crisis which is also a compounding factor for elevation in ferritin level. Sickle cell crisis may cause a rise in serum ferritin that may persist for 1-3 weeks. [13,14] The clinical impression that the degree of rise in ferritin is related to severity of crisis is not just a casual relation but can be a useful marker of extent of vaso-occlusion and tissue damage. [4]

## CONCLUSIONS

The serum ferritin was the most important marker in the above study and is a measure of available stores of body iron only if measured in steady state. It is significantly increased in transfused as well as patients in crisis but marginally in non transfused steady state patients. This indicates steady state patients are also iron over loaded and iron supplement should be deferred in them as well. Contrary to this in traits there is iron deficiency. Traits can be treated with iron supplements when deficiency is proven by laboratory investigation.

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