Study of Serological Parameters in Thalassemic Patients of GMC Jammu

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Abstract
Objectives:
- *To study serological parameters in thalassemic patients.*
- *To correlate various parameters in these patients.*
- *To study the effect of regular safe blood transfusion & adequate iron chelation on these parameters.*
- *To identify parameters of prognostic significance in these patients.*

Material and Methods:
The present study was conducted in the Post-Graduate Department of Pathology in collaboration with department of biochemistry, Government Medical College, Jammu. This institution is a tertiary care centre of the entire Jammu province of Jammu and Kashmir state where in addition to our own state, patients from adjoining areas of Punjab and Himachal Pradesh come to seek medical attention. Proper consent was obtained from the patients after explaining the patients and their parents the nature and type of study.

I. Introduction

The thalassemias are a group of congenital anemias that have in common deficient (reduced or absent) synthesis of one or more of the globin subunits of the normal human hemoglobins .This results in excess production of the other chain which damages the red cell membrane and begins with cascade that ends with significant morbidity and mortality. They are inherited autosomal recessive disorders. The thalassemias are the commonest monogenic diseases in man The condition forms a part of the spectrum of diseases known collectively as hemoglobinopathies, which can be classified broadly into two types. The first subdivision consists of conditions involving qualitative disorders of Hb such as sickle cell anemia, that result from an inherited structural alteration in one of the globin chains. The second major subdivision of the hemoglobinopathies constitute the thalassemias.

The primary defect is usually quantitative but there are mutations resulting in structural variants produced at reduced rate (i.e. HbE, Hb Lepore) and mutations producing hyperunstable hemoglobin variants with a thalassemia phenotype (thalassemic hemoglobinopathies). Therefore, a rigid differentiation from the qualitative changes of hemoglobin structure that characterize the hemoglobinopathies is no longer appropriate.

According to the chain whose synthesis is impaired, the thalassemias are called α-, β-, γ-, δ-, $\delta\beta$-, or $\epsilon\gamma\delta\beta$-thalassemias. These subgroups have in common an imbalanced globin synthesis, with the consequence that the globin produced in excess is responsible for ineffective erythropoeisis (intramedullary destruction of erythroid precursors) and hemolysis (peripheral destruction of red cells) . For a better understanding of this disease one must know the importance of hemoglobin. Human hemoglobin is heterogenous at all stages of development. Different hemoglobins are synthesised in the embryo, fetus and adult, each adapted to the particular oxygen requirement. In vertebrates, haemoglobin (HbA) is the iron containing oxygen transport protein that is found in red blood cells which carries oxygen from the lungs to the rest of the body and then brings the carbon dioxide back to the lungs to be dispensed. People who have thalassemia produce fewer healthy hemoglobin proteins and their bone marrow produces fewer healthy red blood cells. With too few normal red blood cells, not enough hemoglobin is available to help carry oxygen to the body. Hemoglobin exists in various forms differing in structure of globin chain only, whereas haem portion is same in all types of haemoglobin.

Haemoglobin A (HbA) consists of two globin α–chains and two globin β-chains which contains 141 and 146 amino acids respectively. They are assembled each with an associated heme group into a tetrameric molecule with the formula $\alpha_2\beta_2$. It is the predominant hemoglobin found in adults with a normal amount of over 95%.`Hemoglobin F (HbF) consists of two globin α-chains and two globin gamma–chains. This is found in foetuses and newborns. It is replaced by adult haemoglobin at about approximately 6 months of age. In adults it

is 0.2-1%. Hemoglobin A_2 (HbA$_2$) consists of two globin α-chains and two globin delta chains. It is a normally present in adults in small amounts (2-3.3%).

Hemoglobin is a conjugated protein of molecular weight 64,000 KD and consists of an iron containing portion, the haem, and a protein portion called globin. The α globin gene clusters are present on chromosome 16 while the β gene clusters are present on chromosome 11.

Thalassemia syndromes result from a large series of molecular defects, which alter the expression of one or more globin genes.They are classified into three types:

1. **thalassemia**It is the most common single gene disorder in our country They refer to that group of inherited hemoglobin disorder which are characterized by reduced synthesis (β$^+$ thalassemia) or absence (β°thalassemia) of β-globin chain production which causes anemia.
2. **thalassemia**-There is defect in the synthesis of α chains. This manifests in both foetal and adult lives. It has main three clinical forms- Hemoglobin Bart's hydrops foetalis syndrome, haemoglobin H disease and α thalassemia carrier state.
3. **Miscellaneous Thalassemic syndromes**- These occur because of multiple combinations of β, α gene with other structurally abnormal hemoglobins like Hb D, Hb S and Hb E.

Inclusion Criteria

The study was conducted on all diagnosed cases of thalassemia registered with J&K state thalassemia welfare society attending thalassemia day care center at SMGS Hospital, GMC Jammu.

Exclusion Criteria
• Patients coming irregularly for transfusion
• Patients with incomplete records
• Patients with inadequate sample

Patients were studied prospectively for one year that is from november 2014 to october 2015. A total of 273 patients are registered with J&K thalassemia welfare society but 100 patients could be taken up for study as some were excluded due to sample inadequacy, incomplete records and some were irregular in attending the thalassemia clinic. Clinical details and demographic data (age, sex, socioeconomic status) was gathered from the records available at the day care centre. Other relevant details were collected from the patients and their attendants. Clinical details included history regarding blood transfusions (number of transfusions/month, whether regular/irregular), chelation therapy (adequacy, nature of therapy, side effects), vaccination history, history of jaundice or any blood transfusion related complication.

About 5 ml of venous sample was withdrawn from the patients. under all aseptic precautions with informed consent and subjected to various investigations.

The serological investigations comprised of following tests:

▪ **Serum ferritin** was estimated by the architect ferritin assay which is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of ferritin in human serum and plasma. It is a two step immunoassay using CMIA technology with flexible assay protocols, reffered to as chemiflex.
▪ In the first step, sample and anti-ferritin coated paramagnetic microparticles are combined. Ferritin present in the sample binds to the anti-ferritin coated microparticles. After washing, anti-ferritin acridinium labelled conjugate is added in the second step. Pre-trigger solutions {containing 1.32% (w/v) hydrogen peroxide} and trigger solutions (containing 0.35 N sodium hydroxide) are then added to the reaction mixture; the resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of ferritn in the sample and the RLUs detected by the architect i optical system.
▪ Specimens with a ferritin value exceeding 2000 ng/ml are flagged with the code ">2000" and may be diluted with either the automated dilution protocol or the manual dilution procedure.
▪ By using the automated dilution protocol, the system performs a 1:20 dilution of the specimen. The system will use the dilution factor to automatically calculate the concentration of the sample before dilution which is the reported result.
▪ For manual dilutions the suggested dilution is 1:20. For a 1:20 dilution, add 20 µl of the patient's sample to 380 µl of architect i multi-assay manual diluent.
▪ The operator enters the dilution factor in the patient or control order screen. The system uses this dilution factor to automatically calculate the concentration of the sample before dilution which is the reported result. The dilution is performed so that the diluted result reads greater than 80 ng/ml. If the diluted result reads less than 80 ng/ml, the sample is retested undiluted.
▪ **Other iron studies** like serum iron and total iron binding capacity were also done using automated analyzer.

- ▪ The serum sample is automatically mixed with a ferric solution, which saturates all available iron binding sites of transferrin. Under non-acidic conditions (pH 8.6), only unbound, excess saturating iron is available to be reduced to ferrous iron by ascorbic acid and to form a blue complex with ferene. Subsequent addition of acid (4.5) releases the iron bound to transferrin; this additional iron is reduced to ferrous iron by ascorbic acid and forms an increased amount of blue complex with ferene. The increase in absorbance upon shifting of pH 8.6 to 4.5, measured using a bichromatic (600,700 nm) end point technique, is proportional to the concentration of transferrin bound iron, and thus to the iron binding capacity of the serum sample.
- ▪ **Liver function tests** especially SGPT, SGOT, γGT, serum alkaline phosphatase were done using automated analyzer. In addition serum bilirubin, serum total proteins, serum albumin estimation was also done.
- ▪ **Serum urea and serum creatinine** estimation was done on Architect system using enzymatic methods.
- ▪ Analyzers are highly automated to improve user safety from biohazards, and to diminish the risk of cross-contamination. A probe measures an aliquot of sample and places it into a reaction vessel. Reagents are added from an on-board refrigerated supply. The sample is mixed with the appropriate reagent to produce a reaction that results in a color. Incubation time is allowed and then photometric testing determines the concentration of analyte.The concentration of the analyte determines the strength of color produced. The photometer shines light of the appropriate wavelength at the sample and measures the amount of light absorbed, which is directly correlated to the concentration of the analyte in the sample. Results were displayed on screen or sent to a printer and the results were printed.

  For analyses of liver function tests and renal function tests samples were loaded into the machine and tests were programmed by the user.
- • **Blood sugar** estimation was done by automated analyser using hexokinase method. The hexokinase method is a reference method for determination of glucose concentration. The method is specific for D-glucose. Under the action of the hexokinase enzyme, D-glucose is phosphorylated with ATP molecule to form glucose -6-phosphate. By the action of glucose-6-phosphate dehydrogenase (G-6-PDH) in the presence of NADP, thus formed glucose-6-phosphate transforms to 6-phosphogluconate, whereby NADPH is formed. The absorbance of NADPH is measured in the UV region (334, 340 or 365 nm).The system measures the resulting absorbance readings, calculates the absorbance difference and then converts the absorbance difference into reported units.
- • **Blood grouping** that was done by ID gel card method.

Requirements:
1. Microtubes containing sephadex gel prepared in a buffer such as LISS (low ionic strength saline) or saline are available. In the cards used for blood grouping, red cell specific antisera and a preservative are also added to the gel at the time of manufacture. Each card contains six such microtubes. ID-Card "Diaclon ABO/D + reverse typing cards" containing monoclonal anti-A, anti-B and anti-D within the gel matrix. The microtube control is the negative control. Two microtubes with neutral gel serve for reverse grouping with A & B cells.
2. ID-Diluent 2(modified low ionic strength saline)
3. Test cell reagents- ID Diacell A & B, O in a 0.8% ±0.1% suspension. This is available in 10ml vials which are ready to use.
4. 1D-Dispenser
5. ID-Pipetor and tips
6. ID-Working Table
7. ID-Centrifuge

Sample
A 5% red cell suspension is prepared by adding 0.5ml of Diluent 2 to 50μl of whole blood and mixed gently.

Procedure
1. The ID-Card with unique patient/donor number is identified.
2. Aluminum foil from first 4 microtubes is removed for ABO grouping by holding the ID card in upright position.
3. 10 or 12.5 μl of patient's red cell suspension is pipetted to microtubes 1-4 (A,B,D,ctrl)
4. Then it is incubated at room temperature for 10 min
5. The ID Cards are centrifuged for 10 mins in the ID Centrifuge.
6. Results were read and recorded.

Interpretation
Positive: agglutinated cells formed a red cell line on the surface of gel or agglutinates dispersed in gel

Negative: compact button of cells on the bottom of the microtube. Other investigation included routine urine examination.

Data was analysed using statistical software MS-Excel and SPSS for windows. Data reported as mean (SD) and proportions as deemed appropriate for quantitative and qualitative variables respectively.

Correlation between serum ferritin and SGPT and other parameters were evaluated using Pearson correlation coefficient (r). Point biserial values are reported wherever correlation with dichotomous variables (hepatitis and liver enzymes) are evaluated. A p-value of <0.05 is considered statistically significant. All p-values are 2-tailed.
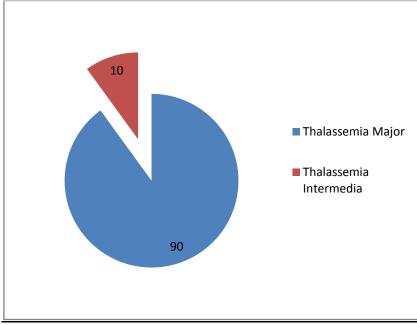
II.   Results

The present study was prospective in nature and was carried out in the Postgraduate Department of Pathology, Government Medical College, Jammu in collaboration with the department of biochemistry over a period of one year *w.e.f.* November 2014 to October 2015. This study was approved by IEC. Proper consent was obtained after explaining the patients and their parents the nature and type of study. 100 thalassemia patients were studied during the course of one year.

Following observations were made:

Table 1:
Distribution of thalassemia patients according to clinical severity

Type of thalassemia	No of patients	Percentage (%)
Thalassemia Major	90	90
Thalassemia Intermedia	10	10
Thalassemia minor	0	0

The present study comprised of 90 thalassemia major patients and 10 thalassemia intermedia patients.Though 6 thalassemia minor patients were registered with the thalassemia welfare society but were not included in the study as they were not attending the thalassemia clinic because of non requirement of transfusions.

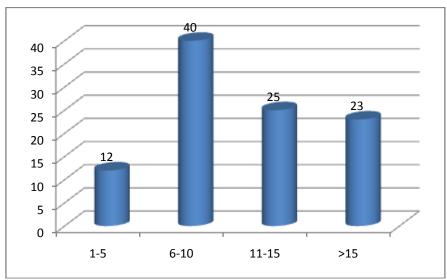**Graph 1:** Distribution of thalassemia patients according to clinical severity

Table 2:
Age wise distribution of thalassemia patients

Age group(years)	No of patients	Percentage(%)
1-5	12	12
6-10	40	40
11-15	25	25
>15	23	23
Total	100	100

In the present study majority of the patients (40%) were in the age group of 6-10 years followed by 25% in the age group of 11-15 years. 23% of the patients were above the age of 15 years. Only 12% of the patients were in the age group of 1-5 years. The youngest patient was 2 years old and oldest was 31 years old. The mean age was 11.85 ± 6.3 years.

Graph 2 Age wise distribution of thalassemia patients

Table 3: Sex distribution of thalassemia patients

Sex	No. of patients	Percentage(%)
Male	62	62
Female	38	38
Total	100	100

Males were more in number comprising of 62% of patients and females were 38%.

Graph 3: Sex distribution of thalassemia patients

Table 4: Spleen status of thalassemia patients

Spleen status	No. of patients	Percentage(%)
Enlarged	50	50

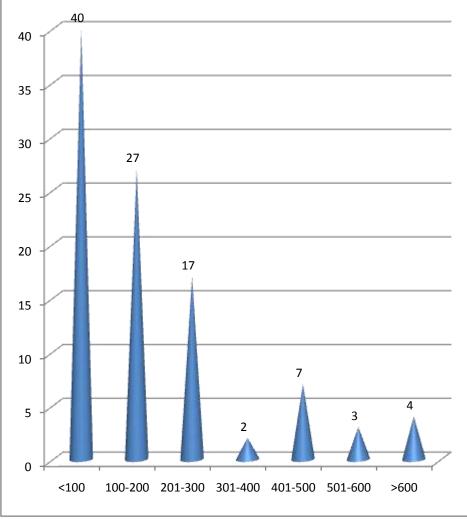| Normal | 41 | 41 |
| Splenectomy | 9 | 9 |
| Total | 100 | 100 |

Fifty patients had splenomegaly at the time of study. Out of these 50 patients, 14 had mild splenomegaly, 34 had moderate splenomegaly and 2 had massive splenomegaly. Nine patients had already undergone splenectomy at the time of study and rest 40 patients had normal spleen.

Graph 4: Spleen status of thalassemia patients

Table 5: Blood transfusions received by thalassemia patients

Blood transfusions	No. of patients	Percentage(%)
<100	40	40
100-200	27	27
201-300	17	17
301-400	2	2
401-500	7	7
501-600	3	3
>600	4	4
Total	100	100

Packed red blood cells were received by the patients. Minimum number of blood transfusions received by a patient was 10 and the maximum number of transfusions received was 979. 40% of the patients received less than 100 transfusions. 44% of the patients have received 100-300 transfusions. 16% of the patients have received above 300 transfusions. Only 25% of the patients were receiving regular blood transfusions (2-3/month). The mean and S.D. was calculated to be 193.4±176.6.
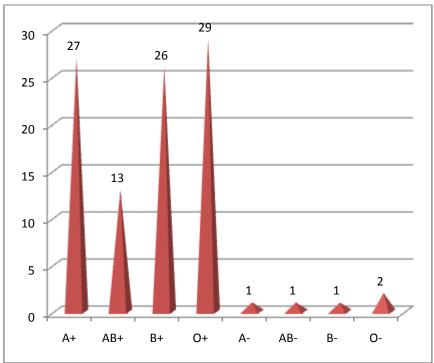
Graph 5: Blood transfusions received by thalassemia patients

Table 6: Distribution of ABO and Rh blood groups in thalassemia patients

Blood groups	No of patients	Percentage(%)
A+	27	27
AB+	13	13
B+	26	26
O+	29	29
A-	1	1
AB-	1	1
B-	1	1
O-	2	2
Total	100	100

Blood group O+ was found to be most frequent (29%) followed by A+ group (27%) and B+ group (26%). Rh negative blood groups were the least frequent comprising 5 % of the total thalassemic patients in the study of which 1 patient each was included in the A-, AB- and B- group. 2 patients were having O- blood group. In thalassemia intermedia patients blood group were arranged in descending order as A+>O+>B>AB+ comprising 4, 3, 2& 1 patient respectively None of the patient had Rh negative blood group. Among males most frequent blood group was O+ followed by A+, B+ and AB+ blood group comprising 21,19, 17 and 3 patients respectively. 2 patients were having O- blood group. Among females blood group were arranged in descending order as B+>A+>O+=AB+ comprising 10, 9, 8 & 8 patients respectively. Rh- blood group was foumd in 3 patients that were A-, B- and AB-.
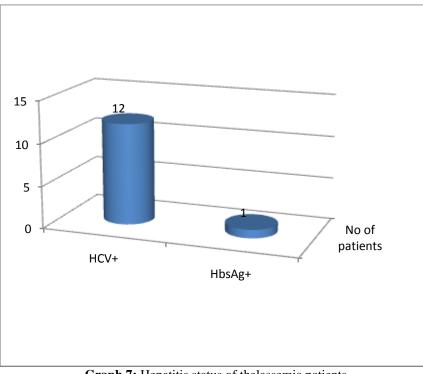
Graph 6: Distribution of ABO and Rh blood groups in thalassemia patients

Table 7: Hepatitis status of thalassemia patients

Hepatitis status	No of patients	Percentage(%)
HCV+	12	12
HBsAg+	1	1
Total	13	13

A total of 12 patients were positive for anti- hepatitis C antibodies and only one patient was found to be positive for hepatitis B surface antigen. In total 13% of the individuals were hepatitis positive.
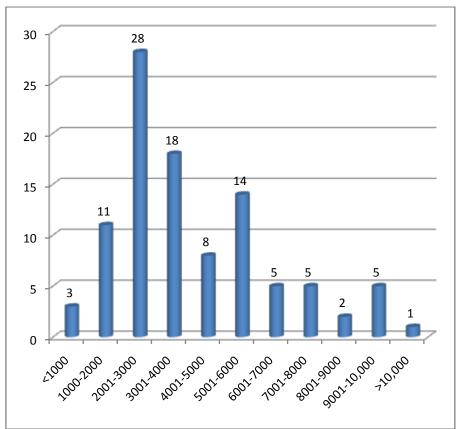

Graph 7: Hepatitis status of thalassemia patients

Table 8: Serum Ferritin of thalassemia patients

Serum ferritin(ng/ml)	No. of patients	Percentage(%)
<1000	3	3
1000-2000	11	11
2001-3000	28	28
3001-4000	18	18
4001-5000	8	8
5001-6000	14	14
6001-7000	5	5
7001-8000	5	5
8001-9000	2	2
9001-10,000	5	5
>10,000	1	1
Total	100	100

Majority (28%) of the patients had ferritin value between 2001ng/ml -3000ng/ml. The ferritin values ranged from 481.98 to 12,000 ng/ml. Only 1 patients had ferritin value greater than 10,000 that was 12,000 ng/ml. 3 patients had values less than 1000 ng/ml with values 481.98, 679.27 and 791.3 ng/ml respectively. The mean and S.D. was observed to be 4160 ± 2426 ng/ml.
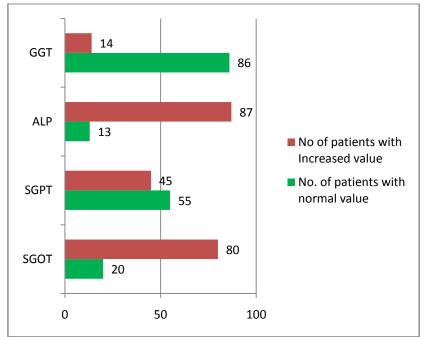

Graph 8: Serum Ferritin of thalassemia patients

Table 9: Liver Function Tests in thalassemia patients

	SGOT	SGPT	ALP	GGT
No. of patients with normal value	20	55	13	86
No of patients with Increased value	80	45	87	14

SGOT, SGPT, ALP and GGT were increased in 80,45,87 and 14 patients respectively but significant increase was considered in patients having two times increase in the upper limit of the reference range. Significantly elevated levels of SGOT,SGPT and ALP were seen in 34, 19 and 9 patients respectively. None of these patients had significantly increased GGT values except one with GGT value of 166 IU/L.
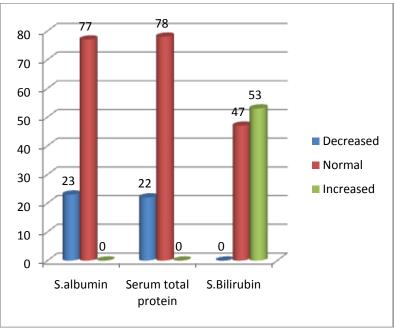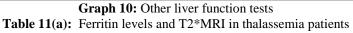
Graph 9: Liver Function Tests in thalassemia patients

Table 10: Other liver function tests

	S.albumin	Serum total protein	S.Bilirubin
Decreased	23	22	0
Normal	77	78	47
Increased	0	0	53

Serum albumin and serum total protein were found to be decreased in 23 and 22 patients respectively. 77 and 78 patients had normal serum albumin and serum total protein value respectively. Serum bilirubin was increased in 53 patients and normal in 47 patients. The mean and S.D. of serum albumin, serum total protein and serum bilirubin was observed to be 3.7 ± 0.53gm%, 7.06 ± 0.76gm% and 1.4 ± 1.1mg% respectively.
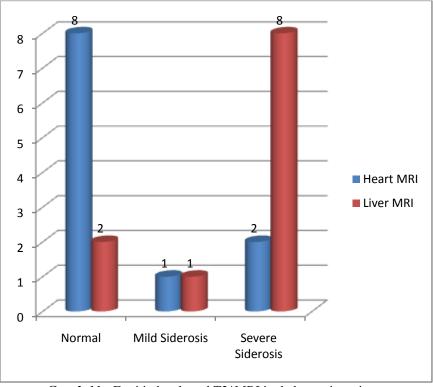

Graph 10: Other liver function tests
Table 11(a): Ferritin levels and T2*MRI in thalassemia patients

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	T2* MRI Heart	T2* MRI Liver	Ferritn levels (ng/ml)
Patient no1	Normal	Severe siderosis	5457.59
Patient no 2	Normal	Severe siderosis	9900.56
Patient no 3	Normal	Severe siderosis	2211.86
Patient no 4	Normal	Severe siderosis	4191.24
Patient no 5	Normal	Severe siderosis	5966.65
Patient no 6	Normal	Severe siderosis	1400.00
Patient no 7	Normal	Mild siderosis	3000.00
Patient no 8	Normal	Normal	2597.00
Patient no 9	Mild	Severe siderosis	2602.73
Patient no10	Severe siderosis	Normal	7251.86
Patient no 11	Severe siderosis	Severe siderosis	10,000

Table 11(b):

	Heart MRI	Liver MRI
Normal	8	2
Mild Siderosis	1	1
Severe Siderosis	2	8

T2* MRI was possible for 11 patients out of which 8 had severe liver siderosis and 2 had severe heart siderosis. Liver was normal in 2 patients and heart was normal in 8 of the patients. Mild liver and heart siderosis was seen in 1 patient each. While comparing ferritin levels it was observed that iron load in these two organs was not seen until ferritin levels cross 2200 ng/ml except one case where liver siderosis of severe degree was seen at 1400 ng/ml. Iron overload in heart was seen in 3 patients and their serum ferritin levels ranged from 2600-10,000 ng/ml. In them liver siderosis of severe degree was noted in 2 patients. So the table indicates that higher the ferritin level, more the chances of heart and liver siderosis but levels around 1500 ng/ml may also affect these organs in some patients. The picture would have been more clear had all patients registered for the study undergone T2*MRI scan. But an inference can be made that serum ferritin levels around 1500 ng/ml or less may not always indicate that liver and heart are not affected by iron overload.

Graph 11: Ferritin levels and T2*MRI in thalassemia patients

Table 12: Correlation between Serum Iron & TIBC

	TIBC	S.Fe
Normal	40	7
Increased	2	93
Decreased	58	0

58% of the patients had decreased TIBC. 40% had TIBC within the normal range. Only 2% patients had increased TIBC. 93% of the patients had increased serum iron and 7% of the patients had normal serum iron levels. The mean and S.D. of TIBC and serum iron was calculated to be $272.8\pm77.7\mu g\%$ and $223.47\pm64.2\mu g\%$ respectively.

Renal function tests in thalassemia patients

Serum urea estimated was in the range of 15-48 mg/dl out of which only 2 patients had urea levels above 40 mg/dl. Serum creatinine estimated was in the range of 0.4-0.8 mg% that is within normal limits. The mean and S.D. of serum urea and creatinine was calculated to be 24.4 ± 6.7mg% and 0.56 ± 0.12mg% respectively.

Blood sugar estimation in thalassemia patients

Blood sugar levels estimated in thalassemia patients were found to be increased in 3 patients out of which 2 had diabetes mellitus. The mean and S.D. of blood sugar was calculated to be 100.5 ± 41.9mg%. The ferritin levels of diabetic thalassemics were 10,000 ng/ml and 7251.86 ng/ml respectively.

Table 13: Chelation therapy and ferritin levels

Type of chelation	No of patients	Ferritin levels(ng/ml)
Deferiprone+Hydoxyurea	1	1522.00
Deferoxamine+Deferiprone	5	2758.19
Deferiprone	62	3995.75
Deferasirox	20	4188.68
Deferiprone+Deferasirox	8	5446.13
Deferoxamine	4	6407.00

Majority (62%) of the patients are taking deferiprone as the chelating agent. Other drugs that are included in the treatment regime are deferasirox, deferoxamine as well as hydroxyurea. But most of them are not following the treatment regimes regularly. Only interpretation from the above table is that the chelating agents will have individualised effect. Some chelating agents may suit one group of patients and others may suit other group, and this needs further observation.

Although 100% compliance was not seen in most patients for regular blood transfusion and adequate iron chelation but individual patients who by and large could manage frequent blood transfusions and better iron chelation had higher hemoglobin levels, normal liver function tests and lower serum ferritin levels as compared to others. Overall quality of life was also observed to be better in these patients.

Higher hemoglobin level, serum ferritin <1000ng/ml and normal liver function tests are indicators of good prognosis as their growth, development and quality of life was observed to be better compare to patients having low hemoglobin level, abnormal liver function tests (due to either liver siderosis or hepatitis) and higher serum ferritin levels.

Clinically facial deformities-thalassemic facies, height and developmental abnormalities were observed to be minimum in patients who were better chelated and having regular blood transfusion as compare to those who were poorly chelated and irregularly transfused.

III. Discussion

Thalassemias are inherited disorders characterized by abnormal production of hemoglobin, associated with low hemoglobin production and excessive destruction of red blood cells. They are a heterogenous group of disorders and are considered as the most common monogenic disorder in the world. Present study was conducted to observe serological parameters in 100 thalassemia patients attending thalassemia clinic at SMGS hospital, Government Medical College, Jammu.

The present study comprised of 90% thalassemia major patients and 10% thalassemia intermedia patients.Though 6 thalassemia minor patients were registered with the thalassemia welfare society but were not included in the study as they were not attending the thalassemia clinic because of non requirement of transfusions. As thalassemia major patients require more transfusions, they are seen more in number than thalassemia intermedia.

In the study majority of the patients (40%) were in the age group of 6-10 years followed by 25% in the age group of 11-15 years. 23% of the patients were above the age of 15 years. Only 12% of the patients were in

the age group of 1-5 years. The youngest patient was 2 years old and oldest was 31 years old. The mean age was 11.85 ± 6.3 years. Without transfusions, majority of children with thalassemia major do not survive the first decade of life, but with the availability of blood transfusion services and setting up of thalassemia clinic, most of these children are living longer lives.

Males were more in number comprising 62% of the patients and females were 38%. This is comparable with most of the studies conducted where male predominance has been seen. However, increased male presentation can be partly explained by the deep-rooted gender bias among the parents of these chronically ill children who seek medical care and are ready to spend more for their male children only.

50% patients had splenomegaly at the time of study. Out of these 14(28%) had mild splenomegaly, 34(68%) had moderate splenomegaly and 2(4%) had massive splenomegaly. 9% had undergone splenectomy and rest 41 patients had normal spleen. The main symptom in beta thalassemia major is severe anemia due to ineffective erythropoeisis. Increased hemolysis instigates hyperplasia of the RES, erythroid hyperplasia and extramedullary hemopoeisis. Extramedullary hemopoeisis in the spleen initiates splenomegaly. Massive splenomegaly instigates pooling and increases sequestration, resulting in cytopenias. At this stage, to improve the anemia, splenectomy is performed.

40% of the patients have received less than 100 transfusions correlating with the fact that maximum number of patients were in the age group of 6-10 years. Thalassemia intermedia patients come for blood transfusion usually after 2-3 months except one who became transfusion dependent possibly secondary to hypersplenism. Majority of the thalassemia major patients come irregularly for transfusions due to the fact that they have to come from far flung areas and to avoid the burden of expenditure. Only 25% of the patients come regularly for transfusions that is 2-3 times per month. Some children miss their transfusion because of academic activities. Those who are regular come atleast twice a month. The groups of homozygous and heterozygous thalassemia intermedia required infrequent blood transfusions i.e. 1-2 unit/year. Four patients in both the groups required frequent blood transfusions. Three of these patients developed hypersplenism.

Blood group O+ was found to be most frequent (29%) followed by A+ group (27%) and B+ group (26%). Rh negative blood groups were the least frequent comprising 5 % of the total thalassemia patients in the study of which 1 patient each was included in the A-, AB- and B- group. 2 patients were having O- blood group.

12% of the patients were positive for anti- hepatitis C antibodies and only 1% of the patient was found to be positive for hepatitis B surface antigen. In total 13% of the individuals were hepatitis positive. None of the patients was positive for HIV which could be due to low prevalence of HIV in J&K. Professional donors and people at high risk are discouraged from donating blood in the blood bank and none of the thalassemic child has received blood from professional donors. Poly-transfusion in thalassemic patients is considered one of the major risk factors to acquire hepatitis. In this study, an association is found between the number of transfusions and hepatitis positive patients (r=0.288,p-value =0.004). A gradual increase was seen in the percentage of hepatitis positive thalassaemic patients with the increase in the number of transfusions. The decrease in percentage of hepatitis B virus infection as compare to hepatitis C infection may be explained by the increasing rate of vaccination due to public awareness about the disease and inclusion of hepatitis vaccine in expanded program on immunization (E.P.I) schedule. Moreover our centre is providing regular free prophylaxis against hepatitis B vaccination. Despite of receiving screened blood for transfusion, the residual risk of acquiring a TTI depends on the safety of the donor population, the sensitivity of the screening tests used, window-period donations and other reasons, such as mutant strains.

The ferritin values ranged from 481.98 to 12,000 ng/ml. The mean and S.D. of ferritin was calculated to be 4160.20 ± 2426.53 ng/ml. The ferritin levels in the patients of the present study are high as patients had undergone many transfusions but exhibit meagre compliance for chelation therapy.

TIBC is the ability of the plasma protein to bind iron and an indirect measure of transferrin levels. 58% patients in our study had decreased total iron binding capacity. 40% had TIBC within the normal range. Only 2 patients had increased TIBC. One of them had associated increased liver function enzymes and other had associated increased alkaline phosphatase. Majority of the patients had decreased TIBC because of the decreased capacity of the available transferrin levels to bind increased levels of iron. 93% of the patients had increased serum iron levels and 7% of the patients had normal serum iron levels. Increased iron levels reflects the transfusion load on the patients as well as inadequate chelation therapy. The mean and S.D. of TIBC and serum iron was calculated to be 272.8 ± 77.7 µg% and 223.47 ± 64.2 µg% respectively

SGOT,SGPT,ALP and GGT were increased in 80, 45, 87 and 14 patients respectively but significantly elevated (significant increase was considered in patients having two times increase in the upper limit of the reference range) levels of SGOT, SGPT and ALP were seen in 34, 19 and 9 patients respectively. None of these patients had significantly increased GGT values except one with GGT value of 166 IU/L. The mean and S.D. of SGOT, SGPT, ALP and GGT was estimated to be 85.3 ± 126.4 IU/L, 98.2 ± 146.4 IU/L, 179.2 ± 85.7 IU/L and 62.4 ± 22.5 IU/L respectively. Liver disease associated with chronic blood transfusions in thalassemic patients is caused by hepatotropic infections or hepatic siderosis. Both factors may act either synergistically or

independently in promoting chronic liver disease, inducing cellular damage through similar oxidative pathways. Through statistical analysis it was clear that a good correlation exists between hepatitis and SGOT (r=0.280,p=0.005) and, hepatitis and SGPT (r=0.313,p=0.002). Increased liver enzymes in the hepatitis negative patients could be attributed to iron overload on the liver due to chronic blood transfusions. Statistical analysis showed a good correlation between ferritin levels and ALP (r=0.200,p=0.046).

Serum albumin and serum total protein were found to be decreased in 23% and 22% of the patients respectively. Serum bilirubin was increased in 53 patients and normal in 47 patients. Increase in bilirubin in thalassemia may be related to hemolytic process and confirm the existing hepatic damage. Decreased serum total protein and albumin is due to secondarily decreased synthesis of protein by the liver. Elevation of liver protein enzymes in thalassemic patients further support this view. The mean values of serum albumin, serum total protein and serum bilirubin in the present study was found to be 3.7$\pm$0.53gm%, 7.06$\pm$0.76gm% and 1.4$\pm$1.1 mg% respectively.

Serum urea estimated was in the range of 15-48 mg/dl. Only two patients had value above 40 mg/dl and that were 47 and 48 mg/dl but their creatinine levels were within normal limits. The concentration of serum creatinine is the most widely used and commonly accepted measure of renal function in clinical medicine. Serum creatinine estimated was in the range of 0.4-0.8 mg%. The mean and S.D. of serum urea and serum creatinine was observed to be 24.4$\pm$6.7 (mg/dl) and 0.56$\pm$0.12 (mg%).

Blood sugar levels estimated in thalassemia patients and 2% of individuals were found to have diabetes mellitus. Their ferritin levels were 10,000 ng/ml and 7251.86 ng/ml respectively. The mean ferritin of non diabetic thalassemics was 4069.06 ng/ml. This is in accord with the fact that diabetes in these patients is usually due to iron deposition in the pancreas leading to beta cell destruction and consequent insulin deficiency.

T2* MRI was possible for 11 patients. We observed that higher the ferritin level, more the chances of heart and liver siderosis but levels around 1500 ng/ml may also affect these organs in some patients. The picture would have been more clear had all patients registered for study undergone T2* MRI scan. But an inference can be made that serum ferritin levels around 1500ng/ml or less may not always indicate that liver and heart are not affected by iron overload.

Majority (62%) of the patients are taking deferiprone as the chelating agent. Other drugs that are included in the treatment regime are deferasirox, deferoxamine as well as hydroxyurea. But most of them are not following the treatment regimes regularly. The main issue in assessing the affect of any chelating agent is irregular compliance that is regular intake of chelating agent as advised is not seen in most of these patients, the reason being side effects of any particular agent and affordability. Regarding deferasirox, most of the patients use it only when they stop deferiprone or deferoxamine due to side effects. So exact effect of these chelating agents on our patients can't be predicted and assessed. This aspect requires more study in a larger group of patients where compliance is 100%. Deferoxamine is the most effective chelating drug in iron overloaded multi-transfused thalassemic patients. In view of cost and unacceptability of daily deferoxamine injections, combination therapy is an effective method of chelation thus increasing the compliance and cost effectiveness.

Although 100% compliance was not seen in most patients for regular blood transfusion and adequate iron chelation but individual patients who by and large could manage frequent blood transfusion and better iron chelation had higher hemoglobin levels, normal liver function tests and lower serum ferritin levels as compared to others. Overall quality of life was also observed to be better in these patients. Higher hemoglobin level, serum ferritin <1000ng/ml and normal liver function tests are indicators of good prognosis as their growth, development and quality of life was observed to be better compare to patients having low hemoglobin level, abnormal liver function tests (due to either liver siderosis or hepatitis) and higher serum ferritin levels. Clinically facial deformities-thalassemic facies, height and developmental abnormalities were observed to be minimum in patients who were better chelated and having regular blood transfusion as compare to those who were poorly chelated and irregularly transfused.

IV.   Conclusion

- The registered patients of thalassemia in thalassemia day care centre are 273. Out of these 100 patients satisfied the inclusion criteria and were taken up for the study.
- There were 90 thalassemia major and 10 thalassemia intermedia patients between 2-31 years of age.
- 40 patients were in the age group of 6-10 years followed by 25 patients in the age group of 11-15 years. 23 patients were above the age of 15 years.
- The study comprised of 62 males and 38 females.
- Fifty one patients had splenomegaly at the time of study. Out of these 51 patients 14 had mild splenomegaly, 35 had moderate splenomegaly and 2 had massive splenomegaly. Nine patients had already undergone splenectomy at the time of study for various reasons especially cytopenia or hypersplenism.

- Blood group O+ was found to be the most frequent (29%) followed by A+ group (27%) and B+ group (26%). Rh negative blood groups were the least frequent comprising 5 % of the total thalassemic patients in the study.
- 12 patients were positive for anti- hepatitis C antibodies and only one patient was found to be positive for hepatitis B surface antigen. In this study, an association is found between the number of transfusions and hepatitis positive patients (r=0.288, p-value =0.004). None of the patient was HIV positive.
- A good correlation existed between hepatitis and abnormal liver function tests SGOT and SGPT.
- Regular blood transfusion is experienced in only about 25% of the patients and their pre transfusion hemoglobin level was between 9-10 gm/dl. Majority of the patients don't receive regular blood transfusion because of various factors like distance, hilly areas, non-availability of transport facilities, and socioeconomic status.
- The ferritin values ranged from 481.98 to 12,000 ng/ml. The mean ferritin was calculated to be 4160 ± 2426 ng/ml.
- SGOT, SGPT, ALP and GGT were increased in 80,45,87 and 14 patients respectively but significant increase was considered in patients having two times increase in the upper limit of the reference range. Significantly elevated levels of SGOT, SGPT and ALP were seen in 34, 19 and 9 patients respectively. None of these patients had significantly increased GGT values except one with GGT value of 166 IU/L.
- Increased liver enzymes in the hepatitis negative patients could be attributed to iron overload on the liver due to chronic blood transfusions and inadequate chelation. Statistical analysis showed a good correlation between ferritin levels and ALP (r=0.200,p=0.046).
- Blood sugar levels were estimated and two patients were found to have diabetes mellitus. Their ferritin levels were 10,000 ng/ml and 7251.86 ng/ml respectively. The mean ferritin of non diabetic thalassemics was 4069.06 ng/ml. This is in accord with the fact that diabetes in these patients is usually due to iron deposition in the pancreas leading to beta cell destruction and consequent insulin deficiency.
- T2* MRI was possible in only 11 patients. It didn't correlate well with serum ferritin levels. In other words serum ferritin levels may not indicate liver and heart siderosis in all the patients. Hence, the need of T2* MRI in these patients is must atleast once a year.
- Clinically facial deformities like thalassemic facies, height and developmental abnormalities were minimum in patients who were better chelated and having regular blood transfusion as compare to those who were poorly chelated and irregularly transfused.
- Majority of the patients are taking deferiprone as the chelating agent as it is provided free of cost by the thalassemia centre. Other drugs that are included in the treatment regime are deferasirox, deferoxamine as well as hydroxyurea, but most of them are not following the treatment regimes regularly. Compliance is not 100%.
- Higher hemoglobin level, serum ferritin <1000 ng/ml, normal liver function tests and, normal liver and cardiac status are indicators of good prognosis.

100 thalassemia patients out of 273 registered patients were included in the study comprising of 90 thalassemia major and 10 thalassemia intermedia patients. Regular and safe blood transfusion (pre transfusion hemoglobin level greater than 10 gm/dl), effective iron chelating agents and controlling ferritin levels are most important factors for preventing morbidity and mortality, and assuring a good quality and prolonged life in these patients. Serum ferritin may not always indicate cardiac and liver siderosis. Hence T2*MRI is indicated in these patients once a year. Prophylaxis against hepatotropic viruses are must to prevent hepatitis and other complications. Safe blood transfusion means proper facility of screening blood donors especially NAT testing to prevent transmission of hepatotropic viruses and HIV viruses during window period. In addition to these a regular follow up and assessment of endocrine functions is necessary in assuring normal pubertal development and complication free disease. Higher hemoglobin level, serum ferritin <1000ng/ml, normal liver function tests, normal liver and cardiac status are indicators of good prognosis.

Finally the most important message in this study is that proper awareness and education about the disease is minimal which is indicated by the fact that only 100 patients out of 273 attend the centre either once a month or once in two to three months. Rest of the patients were highly irregular or didn't come at all. Therefore health care personnel in general and society in particular need to do much hard work especially reaching to patients residence and convincing them about regular follow up at the thalassemia day care centre. At the same time screening camps at school level is recommended to identify cases especially thalassemia trait and further counselling as desired should be initiated. Civil society can also contribute in coming out openly and offer financial and other support of these patients as it is not possible only at government level.

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