IJPRP

Indian Journal of Pathology: Research and Practice
Volume 9 Number 2 (Part II), May – August 2020
DOI: http://dx.doi.org/10.21088/ijprp.2278.148X.9220.32

Original Research Article

Study of Hematological and Biochemical Parameters in Transfusion Dependent Thalassemia Patients

Prashant Kumar[1], Prakash M Patil[2], Mallanagauda M Patil[3]

[1]Junior Resident, [2]Associate Professor, Department of Pathology, [3]Professor, Department of Pediatrics, Shri B M Patil Medical College, BLDE (Deemed to be University), Vijayapura, Karnataka 586103, India.

Corresponding Author:

Prashant Kumar, Junior Resident, Department of Pathology, Shri B M Patil Medical College, BLDE (Deemed to be University), Vijayapura, Karnataka 586103, India.

E-mail: prashantrajput23@gmail.com

How to cite this article:

Prashant Kumar, Prakash M Patil, Mallanagauda M Patil. Study of Hematological and Biochemical Parameters in Transfusion Dependent Thalassemia Patients. Indian J Pathol Res Pract 2020;9 (2 Part II):207-214.

Abstract

Context: Thalassemia is one of the most common hereditary disorders in Asia and most parts of the world and has drawn the attention of scientific research by many.It is accompanied with metabolic dysregulation, iron overload, chronic hypoxia and cell damage. All physiological changes result in ineffective erythropoiesis, hemolysis and anemia. Most patients are dependent on either blood transfusion or bone marrow transplantation for survival. Regular transfusion has improved the span and quality of their lives but has been known to cause alterations in the hematological and biochemical parameters.

Aims: The present study was conducted to assess the changes in hematological and biochemical parameters in thalassemia patients for timely correction of any deranged parameters, to prevent any severe complications and to improve quality of life.

Methods and Material: The study was done on 35 thalassemia patients who were admitted for treatment and blood transfusions. Thirty-five healthy individual controls were matched by sex and age. Total iron binding capacity (TIBC), Serum Iron, Serum Calcium, Serum creatinine, Serum phosphorus (P), Serum Alkaline phosphatase (ALP) and Serum Ferritin were estimated. Hemoglobin (Hb), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), Platelet count, Red Cell Distribution Width-Standard Deviation and Coefficient of Variation (RDW-SD, RDW-CV) were measured.

Statistical Analysis Used: Statistical analysis was performed using statistical package for the social sciences (Version 17). Results were compared using independent t-test and Mann Whitney U test.

Results: Hb, HCT, MCHC, TIBC, Serum Creatinine and Serum Phosphorous were significantly decreased ($p<0.005$) in thalassemia patients when compared with control group. RDW(SD), RDW(CV), Serum Iron and Serum Ferritin showed a statistically significant increase ($p<0.005$) in the thalassemia patients. MCV, MCH, Platelet count, Serum Calcium and Serum ALP showed no significant difference.

Conclusion: Repeated blood transfusions in thalassemia cause derangement of many hematological and biochemical parameters. Regular monitoring of these parameters is important for better management of patients.

Keywords: Thalassemia; Blood transfusion; Hematological; Biochemical Parameters.

Key Messages: Regular blood transfusion in thalassemia patients causes derangement in their hematological and biochemical parameters. It is important to monitor these parameters in all thalassemia patients undergoing repeated blood transfusions. Regular assessment will help in providing timely correction of any deranged parameters, prevent severe complications and improve the quality of life in these patients.

Introduction

In 1925 Cooley and Lee first described a form of severe anemia that occurs early in life and is associated with splenomegaly and bone changes. This anemia was first observed in the Mediterranean region; therefore it came to be known as "Cooley's anemia" or "Mediterranean anemia". The term "Thalassemia" was coined by Whipple.[1] "Thalassemia" is derived from Greek word "Thalassa" which means "the sea". Thalassemia is an autosomal recessive heterogeneous group of disorders. It is characterized by decreased production of one or more globin protein chains. Thalassemia is broadly divided into α and β subtypes. β thalassemia has emerged as a big public health problem in Asia and most parts of the world. It is one of the most common genetic disorder and has drawn the attention of scientific research by many.

Imbalanced globin protein synthesis is the key factor in determining the severity of the disease in thalassemia syndromes.[1] Thalassemia occurs because of the presence of the homozygous state of one of the thalassemia genes or hemoglobin (Hb) Lepore genes during infancy and childhood. It is accompanied by metabolic dysregulation, iron overload, chronic hypoxia and cell damage.[1,2] All of these physiological changes result in ineffective erythropoiesis, hemolysis and anaemia.[2]

Thalassemia patients are mainly dependent on regular blood transfusions for management. Regular transfusion combined with chelation therapy has enhanced the quality and span of their lives.[3] These days many patients are undergoing bone marrow transplantation as well.

Repeated blood transfusions in thalassemia patients have been known to cause alterations in their hematological and biochemical parameters. Hence this study was done to find out these abnormalities in thalassemia patients in comparison with controls. Regular monitoring of hematological and biochemical parameters will help in providing timely interventions and prevent any complications that may arise due to changes in these parameters.

Subjects and Methods

The study was conducted on thalassemia patients who were admitted in the indoor of the Department of Pediatrics, for blood transfusion and treatment during the period of 1st December, 2017 to 30th June, 2019. Total thirty-five (n=35) thalassemia cases were taken. The control group also comprised of thirty-five (n=35) subjects.

Study subjects were diagnosed cases of thalassemia between 3 and 14 years of age. All patients received multiple units of blood transfusion. Each unit comprised of 15 milliliter of packed cell transfusion per kilogram of bodyweight approximately. The cases were selected at random whoever had fulfilled the above criteria.

Control subjects were chosen from out-patients who attended the OPD of the Department of Pediatrics for unrelated minor illnesses. They were between 4 to 13 years of age. None of the control subjects had any clinical symptoms or signs of anemia. The case and control groups were age and sex-matched. Controls were otherwise healthy and selected at random.

All known cases of thalassemia undergoing transfusions during the study period were included.

Exclusion Criteria

- iron, vitamin B12 or folic acid deficiency and other genetic disorders like
- hereditary spherocytosis, G6PD deficiency and other hemoglobinopathies
- Children who received a blood transfusion in past for any reason.
- Patients who received blood transfusion within last 15 days.
- Patients below 3 years or above 14 years.

The nature and purpose of the study were carefully explained to the parents of the cases and controls before obtaining their consent. After taking informed consent, under aseptic precautions, venous blood sample was collected from both cases and controls. Two ml of blood was taken in an EDTA vacutainer and immediately analyzed for complete blood count, that included Hemoglobin (Hb), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Platelet count, RDW-SD and RDW-CV levels using an automated 5-part differential hematologyanalyzer (SYSMEX XN-1000).

4 ml of blood sample was taken in a plain vacutainer and was centrifuged @ 1500 rpm. It was then analysed for Total iron binding capacity (TIBC), Serum Iron (Fe), Calcium (Ca), Serum creatinine, Serum phosphorus (P) and Serum

Alkaline phosphate (ALP) and Sr. Ferritin using Biochemistry analyzer (VITROS® 5,1 FS Chemistry System).

Results

Age of the patients in the study group (n=35) was within the range of 3-14 years. In the control group(n=35) age was in the range of 4-13 years. Maximum number of cases in both groups was between 6-10 years. In study group, maximum number of patients were males (23 cases) while in control group majority cases were females (22 cases). (Table 1)

Majority of the thalassemia patients had blood group B positive (45.71%), followed by A positive (17.14%). (Fig. 1)

On comparison of the hematological parameters between study and control groups, it was observed that the study group had decreased Hb and HCT in comparison to the control group. This decrease was statistically significant ($p<0.001$). There was a statistically significant decrease ($p<0.001$) in MCHC in the study group when compared with the control group. In the study group, both RDW-SD and RDW-CV were increased in comparison to the control group with a statistical difference of $p<0.001$ for both parameters. MCV ($p=0.4790$), MCH ($p=0.083$) and platelet count ($p=0.090$) showed no significant

Table 1: Age and Sex Incidence in Study and Control Groups.

Age (Years)		Study group		Control group	
	Sex	No. of subjects	Percentage	No. of subjects	Percentage
≤5	Male	04	11.42	02	5.71
	Female	03	8.57	03	8.57
6 - 10	Male	11	31.42	08	22.85
	Female	08	22.85	12	34.28
≥11	Male	08	22.85	03	8.57
	Female	01	2.85	07	20.0
Total		35	100.0	35	100.0

M = Male; F = Female

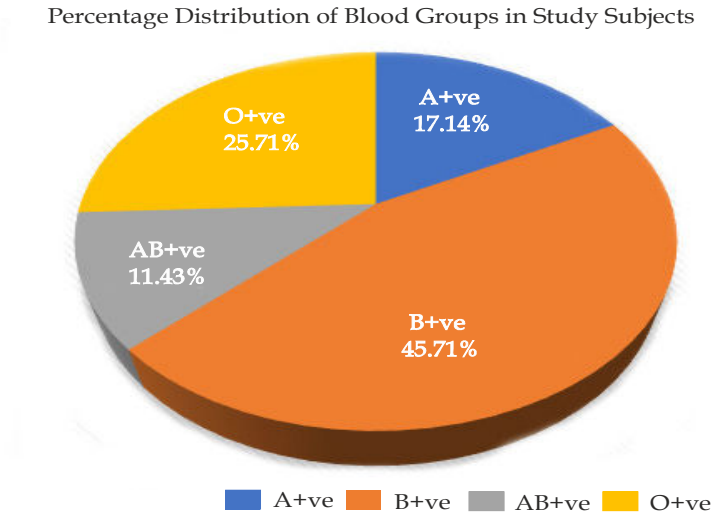

Fig. 1: Fig. Showing Abo-Blood Group Distribution in Thalassemia Patients.

Table 2: Comparison of Hematological Findings Between Study and Control Groups.

Variables	Descriptive statistics				Test Statistics		
	Group	N	Mean	± S.D.	Mann whitney U test/ Indepedent t-test	p value	Remarks
Hb (gm/dl)	Study Group	35	7.06	1.608	t=20.115	p<0.001*	HS
	Control Group	35	14.17	1.339			
HCT (%)	Study Group	35	22.51	4.985	U=35.000	p<0.001*	HS
	Control Group	35	41.20	7.045			
MCV (µm3)	Study Group	35	82.51	10.147	t=0.713	p=0.479	IS
	Control Group	35	84.02	7.261			
MCH (pg)	Study Group	35	26.97	2.695	t=1.719	p=0.083	IS
	Control Group	35	27.91	1.669			
MCHC (g/dl)	Study Group	35	31.60	1.459	U=103.500	p<0.001*	HS
	Control Group	35	35.09	1.961			
Platelets (lakh/µl)	Study Group	35	2.77	1.457	U=476.000	p=0.090	IS
	Control Group	35	3.03	.822			
RDW-SD (fl)	Study Group	35	58.06	19.934	U=1.500	p<0.001*	HS
	Control Group	35	37.11	2.709			
RDW-CV (%)	Study Group	35	19.46	3.641	t=6.702	p<0.001*	HS
	Control Group	35	14.86	2.487			

Hb = Hemoglobin; HCT = Hematocrit; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Hemoglobin; MCHC = Mean Corpuscular Hemoglobin Concentration; RDW-SD = Red cell Distribution Width (Standard Deviation); RDW (CV) = Red cell Distribution Width (Coefficient of Variation)

Note: * significant at 5% level of significance (p<0.05).

IS: Insignificant, HS: Highly significant

Table 3: Comparison of Biochemical Findings Between Study and Control Groups.

Variables	Descriptive statistics				Test Statistics		
	Group	N	Mean	± S.D.	Mann whitney U test/ Indepedent t test	P value	Remarks
Sr. Fe (µg/dl)	Study Group	35	185.74	66.42	t=2.12	P=0.04	S
	Control Group	35	163.31	23.61			
Sr. Ca (mg/dl)	Study Group	35	9.00	1.000	U=538.5	P=0.349	IS
	Control Group	35	9.60	1.557			
TIBC (µg/dl)	Study Group	35	197.97	62.442	U=65.0	P<0.001*	HS
	Control Group	35	332.37	43.759			
Sr. Creatinine (mg/dl)	Study Group	35	0.56	0.502	U=249.5	P<0.001*	HS
	Control Group	35	1.06	0.236			
Sr. Phosphorus (mg/dl)	Study Group	35	6.14	1.332	U=368.5	P=0.002	HS
	Control Group	35	6.66	0.802			
Sr. ALP (IU/L)	Study Group	35	157.80	52.169	t=1.183	P=0.242	IS
	Control Group	35	169.43	25.663			
Sr. Ferritin (ng/ml)	Study Group	35	1302.00	469.322	U=0.000	P<0.001*	HS
	Control Group	35	115.11	28.034			

Sr. Fe = Serum Iron; Sr. Ca = Serum Calcium; Sr. TIBC = Serum Total Iron Binding Capacity; Sr. ALP = Serum Alkaline Phosphate

Note: * significant at 5% level of significance (p<0.05).

IS: Insignificant, HS: Highly significant

Table 4: Hematological and Biochemical Parameters of Study Group Showing Significant Correlation with Sr. Ferritin.

Correlation between Sr. Ferritin and	Correlation coefficient (r)	P value	Conclusion
Hb	r=-0.492	0.003*	Moderate positive correlation. Statistically significant
HCT	r==0.574	<0.001*	Moderate positive correlation. Statistically significant
MCH	r=-0.357	0.035*	Moderate Negative correlation. Statistically significant
Platelets	r=-0.534	0.001*	Moderate Negative correlation. Statistically significant
TIBC	r=-0.347	0.041*	Moderate positive correlation. Statistically significant

Hb = Hemoglobin; HCT = Hematocrit; MCV = Mean Corpuscular Hemoglobin; Sr. TIBC = Serum Total Iron Binding Capacity
Note: * significant at 5% level of significance (p<0.05)

change in values, when compared between study and control groups. (Table 2)

On comparison of the biochemical parameters between study and control groups, it was observed that the study group had increased Sr. Fe and sr Ferritin in comparison to the control group. This increase was statistically significant with p=0.04 and p<0.001 respectively. There was a statistically significant decrease in TIBC (p<0.001), sr. creatinine (p<0.001) and sr. phosphorus (p=0.002) in the study group when compared with the control group. Sr. Ca (p=0.349) and sr. ALP (p=0.242) showed no significant change in values when compared between study and control groups. (Table 3)

Statistically significant moderate positive correlation was observed when Sr. ferritin value was compared with Hb (p=0.003), HCT (p<0.001) and Sr TIBC (p=0.041) in thalassemia patients. Statistically significant moderate negative correlation was noted when Sr. ferritin value was compared with MCH (p=0.035) and platelet count (p=0.001). (Table 4)

Discussion

Most patients with alpha thalassemia and beta-thalassemia trait are asymptomatic and need no or simple management while in case of beta-thalassemia major patients are managed with repeated blood transfusion.[4] Transfusion along with chelation therapy has dramatically improved the life expectancy of thalassemic children. However, frequent blood transfusions can lead to iron overload and derangement of many hematological and biochemical parameters. These deranged parameters, if not monitored regularly will lead to various complications that may decrease the quality of life and overall survival of thalassemia patients. In recent years, several authors have reported a high incidence of these complications, predominantly in patients diagnosed with thalassemia major.[5] Therefore monitoring of hematological and biochemical parameters in regularly transfused thalassemic patients is essential.

In our study, the mean age of the study subjects was 8.2 years. Most patients belonged to the age group of 6-10 years. Similar findings were noted in studies done by Suman R L et. al.[6] (8.80 years), Logothetis J et. al.[7](10.3 years) andDe A et. al.[8] (8.22 years).In studiesS done by Al-Kherbash H et. al.[9] and Joseph N. et. al.[10] maximum cases were in the range of 7-10 years and 5-10 years respectively. Since thalassemia is an inherited genetic disorder, most patients present early with symptoms of anemia leading to early diagnosis.[2] Majority of the patients in our study are males (65.7 %). This finding is correlating with studies done by Tyagi S et. al.[11] (64.5%), Patil S et. al.[12] (60.4%), Al-Kherbash H et. al.[9] (53.2%), and Joseph N. et. al.[10] (63.4%) who also reported similar gender distribution in thalassemia affected children.

In present study it was observed that majority of the patients in study group were having B +ve blood group. However in a study done by Sinha PAet. al.[13] majority of the patients were O +ve followed by B +ve cases.

Hb level of thalassemia group was decreased in the present study with an average value of 7.06 ±1.608 (Mean ±SD). The decrease in Hb in comparison to control subjects is also statistically significant with p<0.001. This finding is correlating with studies conducted by Karim F et. al.[1] (7.36 ±1.5), Ayyash H et. al.[14] (7.36 ±0.8), Verma S et. al.[15] (9.8 ±1.1), De A et. al.[8] (8.5 ±2.5) andFilizet. al.[16] (9.25 ±1.74) who also observed decreased Hb in thalassemia cases. Hb is usually low in cases of thalassemia because of defective Hb production and increased RBC destruction in the spleen leading to ineffective erythropoiesis.

In our study HCT level in thalassemia group was decreased with an average of 22.51 ±4.985 (Mean ±SD). This decrease in HCT in comparison to control subjects is statistically significant with

p<0.001. Similar findings were observed in studies conducted by Karim F et. al.[1] (21.5 ±5.3) and Filizet. al.[16] (27.07 ±4.65).

In the present study, no significant change in MCV values (p=0.479) was observed between study and control groups. This finding is in correlation with a study done by Filizet al.[16] who also noted no significant change in MCV in thalassemia patients. While Karim F et. al.[1] (p<0.05)observed a significant decrease in MCV in their study.

In the present study, no significant change in MCH values (p=0.083) was observed between study and control groups. This finding is in correlation with a study done by Filizet al.[16] who also noted no significant change in MCH in thalassemia patients. While Karim F et. al.[1] (p<0.05)observed a significant decrease in MCH in a study done by them.

MCHC level of thalassemia children was decreased in our study with an average of 31.60 ±1.459 (Mean ±SD). The decrease in MCHC in comparison to control subjects is also statistically significant with p<0.001. Similar finding was observed in studies conducted by Verma S et. al.[15] (30.2 ±2.35) and Jameel T et. al.[17] (30.2 ±2.35). However, Karim F et. al.[1] (34.1 ±2.8) did not find any significant decrease in MCHC in their study.

Platelet count in thalassemia children was within the normal range in present study with an average of 2.77 ±1.457 (Mean ±SD) and showing no significant difference with the control group. Similar finding was observed in studies conducted by Naithani R et. al.[22] (2.26 ±1.23), Bushra M et. al.[18], and Sultan S et. al.[19]

RDW-SD level in thalassemia children was increased with an average of 58.06±19.934 (Mean ±SD). The increase in RDW-SD in comparison to control subjects is also statistically significant with p<0.001. This finding is in correlation with studies done by Mahdi SL et. al.[20] (40.0 ±5.8) and Munir B et. al.[18]

In the present study, RDW-CV level in thalassemia children was also increased with an average of 19.46 ±3.641 (Mean ±SD). The increase in RDW-CV in comparison to control subjects is also statistically significant with p<0.001. This finding is in correlation with studies done by Jameel T et. al.[17] (16.5 ±1.8), Filizet al.[16] (15.19 ±2.86) and Rahman MU et. al.[21] (14.16 ±0.46).

Sr Iron level of thalassemia children was increased in our study with an average of 185.74 ±66.41 (Mean ±SD). The increase in Sr. Iron in comparison to control subjects is also statistically significant with p=0.04. Similar finding of Sr Iron in thalassemia children was found in studies done by Naithani R et. al.[22] (190.0 ±89.0), Livrea MA et. al.[23] (254.0 ±60.0), De A et. al.[8] (268.60), Ghone RA et. al.[24] (164.55 ±14.30) and Salma O S et. al.[25] (180 ±94.9). Increased Sr Iron in thalassemia patients is mainly because of transfusion-related iron overload. Also, the rate of iron absorption from G.I.T is approximately 3-4 times greater in β-thalassemic patients than normal. This may lead to iron accumulation about 2-5 gm per year.[6,26]

SrCa level of thalassemia children is within normal range in the present study with an average value of 9.00 ±1.00 (Mean ±SD). Similar finding was seen in studies done by Karim F et. al.[1] (7.9 ±0.6), Sultan S et. al.[19], Saboor M et. al.[27] (8.83 ±0.58) andSalama OS et. al.[25] (8.9 ±1.4), who found that Sr calcium values were within normal range in thalassemia patients.

In the present study a statistically significant decrease (p<0.001) was noted in the TIBC of thalassemia children with an average value of 197.97 ±67.442 (Mean ±SD). This finding is in correlation with studies done by Salama OS et. al.[25] (246.3 ±131.8), Livrea MA et. al.[23] (369 ±97), De A et. al.[8] (205.16)andGhone AR et. al.[24] (224.15 ±20.43) who also observed significantly decreased serum phosphorus levels in thalassemia patients.

Sr. Creatinine value of thalassemia children is decreased in the present study with an average value of 0.56 ±0.09 (Mean ±SD). The decrease in Sr. Creatinine in comparison to control subjects is also statistically significant with p<0.001. Similar finding was found in studies done by Karim F et. al.[1] (224.15 ±20.43),Bushra M et. al.[18] andAyyashet. al.[14] (0.59 ±0.3).

Serum Phosphorus of thalassemia children was increased with an average of 6.14 ±1.332 (Mean ±SD). The increase in Serum Phosphorus in comparison to control subjects is statistically significant with p=0.002. This finding is in correlation with the studies done by Salama OS et. al.[25] (5.3 ±1.0) and Costin G et. al.[28] (5.8 ± 0.2) who also found significantly higher serum phosphorus in thalassemia patients.

Sr. ALP did not demonstrate any significant difference (p=0.242) between patients and control groups. This finding is in agreement with studies done by Moulas A et. al.[29] (99 ±13), Solimanet. al.[30] (179 ±10), Salama OS et. al.[25] (194.1 ±37.1) and Asif M et. al.[31] (450.99 ±16.05) who had mentioned in their studies that iron overload leads to osteoblast poisoning leading to false results for ALP in thalassemia patients.

In general, the body iron stores have been found to correlate with serum ferritin levels. However, being an acute phase reactant, single values of serum ferritin are not always reliable. Despite serial measurement serum ferritin is a simple and reliable method to evaluate iron deposition and efficiency of chelation therapy. To evaluate clinical relevance, need for treatment, timing and monitoring of chelation therapy, iron status should be assessed accurately.

In the present study Sr. Ferritin was elevated in the study group with a value of 1302.0 ±469.322 (Mean±SD). The increase in Sr. Ferritin is statistically significant in comparison to the control group with p<0.001. This finding is correlating with studies conducted by Karim F et. al.,[1] (1249 ±59.2), Naithani R et. al.[22] (3709.0 ±1625), Livrea MA et. al.[23] (1866.0 ±996.0), De A et. al.[8] (1548.06), Ayyashet. al.[14] (7162.4 ±3297.3), Filizet. al.[16] (1300 ±477.14), Salma OS et. al.[25] (881.4 ±245.1) and Soliman A et. al.[30] (880 ±46).

Conclusion

Thalassemia patients undergoing regular blood transfusions show significant changes in their hematological and biochemical parameters in comparison to non-thalassemics. Amongst hematological parameters, Hb, HCT, MCV, MCH, MCHC and RDW values are usually deranged in thalassemia. Also, biochemical parameters like Sr. Iron, TIBC, Sr. Creatinine, Sr. Phosphorus, Sr. Calcium, Sr. ALP and Sr. Ferritin show significant changes. These deranged parameters may prove detrimental to the management of the patient. Therefore, along with Hb, the above-mentioned parameters should also be routinely evaluated in thalassemia patients to prevent associated complications. Better management of thalassemia can be provided by continuous monitoring of the hematological and biochemical parameters.

Legend to Tables/ Fig.

1. Table 1 showing age and sex incidence in study and control groups. Average age of patients in the study group and control group was 8.20 ±3.376 years (Mean±SD) and 8.74 ±2.737 years (Mean±SD) respectively. In study group, maximum patients were males (23 cases), in control group maximum cases were females (22 cases).
2. Fig. 1 showing ABO blood group distribution in thalassemia patients. 45.71% of cases belonged to B positive blood group.
3. Table 2 showing comparison of hematological findings between study and control groups. Study group shows decreased Hb, HCT and MCHC (p<0.001) and increased RDW-SD and RDW-CV (p<0.001). MCV (p=0.4790), MCH (p=0.083) and platelet count (p=0.090) shows no significant change in values.
4. Table 3 showing comparison of biochemical findings between study and control groups. Study group shows increased Sr. Fe (p=0.04); sr Ferritin (p<0.001) and decreased TIBC (p<0.001); sr. creatinine (p<0.001); sr. phosphorus (p=0.002). Sr. Ca (p=0.349) and sr. ALP(p=0.242) showed no significant change in values.
5. Table 4 showing hematological and biochemical parameters of study group having significant correlation with sr. ferritin. Hb (p=0.003), HCT (p<0.001) and Sr TIBC (p=0.041) of thalassemia patients shows statistically significant moderate positive correlation when compared with Sr. ferritin value.

References

1. Karim MF, Ismail M, Hasan AM, Shekhar HU. Hematological and biochemical status of Beta-thalassemia major patients in Bangladesh: A comparative analysis. Int J HematolOncol Stem Cell Res 2016;10:7–12.
2. Weatherall DJ, Clegg JB. Thalassemia revised. Cell 1982;29:7–9.
3. Shams S, Ashtiani TMH, Monajemzadeh M, Koochakzadeh L, Irani H, Jafari F, Mohseni A. Evaluation of Serum Insulin, Glucose, Lipid Profile, and Liver Function in β-Thalassemia Major Patients and their correlation with iron overload. Laboratory Medicine 2010;41:486–9.
4. Bain B, Bates I, Laffan M. Dacie and Lewis Practical Haematology 12th ed. Elsevier.
5. Fauci A, Braunwald E, Kasper D, Hauser S, Longo D, Jameson J et. al. Harrison's Principles of Internal Medicine. 17th Edition. New York, USA: McGraw-Hill Professional Publishing; 2008.
6. Suman RL, Sanadhya A, Meena P, Goyal S. Correlation of liver enzymes with serum ferritin levels in β-thalassemia major. Int J Res Med Sci 2016;4:3271-4.

7. Logothetis J., Loewenson RB, Augoustaki O, Economidou J and Mathios C. Body growth in cooley'sanemia (homozygous beta-thalassemia) with a correlative study as to other aspects of the illness in 138 cases. Pediatrics 1972;50;92.
8. De A, Samaddar D, Sen D, Kumar Sen D. Comparative assessment of serum liver enzymes (AST and ALT) in thalassemia patients of Murshidabad and matched controls. IOSR Journal of Dental and Medical Sciences 2019;17:01-5.
9. Al-Kherbash H, Al-Awdi A, Hasan N. Pattern and clinical profile of thalassemia among pediatric patients attending the Yemeni Society Centers for Thalassemia and Genetic Blood Disorders in Yemen. The Scientific Journal of Al-Azhar Medical Faculty, Girls 2017;1:43.
10. Joseph N, Pai S, Sengupta S, Bharadwaj S, Dhawan S, Khare K. A clinico-epidemiological study of thalassemia cases in India. Journal of Natural Science, Biology and Medicine 2018;9:236.
11. Tyagi S, Kabra M, Tandon N, Saxena R, Patil HP, Choudhry VP. Clinico-Haematological Profile of Thalassemia Intermedia Patients, Int J Hum Genet 2003;3:251-8.
12. Palit S, Bhuiyan RH, Aklima J, Emran TB, Dash R. A study of the prevalence of thalassemia and its correlation with liver function test in different age and sex group in the Chittagong district of Bangladesh. J Basic ClinPharma 2012;3:352-7.
13. Sinha P A, Mulkutkar SH, Bhavani JB. Study of distribution of ABO blood groups in ß-thalassemia patients. Int J Res Med Sci 2017;5:3479-83.
14. Ayyash H, Sirdah M. Hematological and biochemical evaluation of β-thalassemia major (βTM) patients in Gaza Strip: A cross-sectional study. International Journal of Health Sciences 2018;12:18-24.
15. Verma S, Gupta R, Kudesia M, Mathur A, Krishan G, Singh S. Coexisting Iron Deficiency Anemia and Beta Thalassemia Trait: Effect of Iron Therapy on Red Cell Parameters and Hemoglobin Subtypes. ISRN Hematology 2014;2014:1-5.
16. Filiz ;ÖZTÜRK Ş. Oxidant and antioxidant status in beta thalassemia major patients Beta talasemi major hastalarindaoksidanveantioksidandüzeyl eri. Ankara Üniversitesi Tıp FakültesiMecmuası. 2005;58:1.
17. Jameel T, Baig M, Ahmed I, Hussain MB, Alkhamaly MD. Differentiation of beta thalassemia trait from iron deficiency anemia by hematological indices. Pak J Med Sci 2017;33:665-9.
18. Munir B, Iqbal T, Jamil A, Muhammad F. Effect of β-Thalassemia on Hematological and Biochemical Profiles of Female Patients. Pakistan Journal of Life and Social Sciences 2013;11:25-8.
19. Sultan S, Irfan S, Ahmed S. Biochemical Markers of Bone Turnover in Patients with β-Thalassemia Major: A Single Center Study from Southern Pakistan. Advances in Hematology 2016;2016:1-5.
20. Mahdi L, Faraj S and Ghali S. Significance of Red Blood Cell Indices in Beta-Thalassaemia Trait. Mustansiriya Medical Journal 2015;14:27-30.
21. Rahman M, Nayem M, Begum W, Begum F, Ahmed M, Sultana S. Pattern of Red Cell Count and Red Cell Distribution Width (RDW %) in Beta Thalassaemia Trait in Adults. Bangladesh Journal of Medical Biochemistry 2018;9:31-5.
22. Naithani R, Chandra J, Narayan S, Sharma S andVarinder Singh V. Thalassemia major - on the verge of bleeding or thrombosis?, Hematology2006;11:57-61.
23. Livrea L. Tesoriere A.M. Pintaudi A. Calabrese A. Maggio HJ, Freisleben D. D'Arpa, R. D'Anna, and A. Bongiorno. Oxidative Stress and Antioxidant Status in P-Thalassemia Major: Iron Overload and Depletion of Lipid-Soluble Antioxidants. Blood 1996;88:3608-14.
24. Ghone, R.A., Kumbar, K.M., Suryakar, A.N. et. al. Indian J ClinBiochem2008; 23:337.
25. Salama OS, Al-Tonbary YA, Shahin RA, Eldeen OA. Unbalanced bone turnover in children with beta-thalassemia. Hematology 2006;11:197-202.
26. Stefano MD, Chiabotto P, Roggia C, et. al. Bone mass and metabolism in thalassemic children and adolescents treated with different iron-chelating drugs. J Bone Mineral Metab 2004;22:53–57.
27. Saboor M. Levels of Calcium, Corrected Calcium, Alkaline Phosphatase and Inorganic Phosphorus in Patients' Serum with β-Thalassemia Major on Subcutaneous Deferoxamine. Journal of Hematologyand Thromboembolic Diseases 2014;02.
28. Costin, G. Endocrine Abnormalities in Thalassemia Major. American journal of diseases of children 1979;133:497.
29. Moulas A, Challa A, Chaliasos N, Lapatsanis P. Vitamin D metabolites (25-hydroxyvitamin D, 24,25-dihydroxyvitamin D and 1,25-dihydroxyvitamin D) and osteocalcin in β-thalassaemia. ActaPaediatrica 1997;86:594-9.
30. Soliman A, Banna N, Fattah M, ElZalabani M, Ansari B. Bone mineral density in prepubertal children with β-thalassemia: Correlation with growth and hormonal data. Metabolism 1998;47:541-8.
31. Asif M, Manzoor Z, Farooq M, Kanwal A, Shaheen U, Munawar S et. al. Correlation between serum ferritin level and liver function tests in thalassemic patients receiving multiple blood transfusions. International Journal of Research in Medical Sciences 2014;2:988.