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Original Article

Haematological and Biochemical status of Adolescent and Young Adults with Transfusion dependent thalassaemia – A study of 40 cases

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ABSTRACT: Transfusion dependent thalassaemia patients requires lifelong regular blood transfusion which is essential to address the consequence of genetic defect, mainly the severe anaemia and the bone marrow hyperactivity and later on iron chelation therapy. This study is carried out in an attempt to find out different clinicopathological parameters of transfusion dependent adolescent and young adult (AYA) patients. Data were collected from 40 transfusion dependant thalassemia patients aged 18 years or more receiving regular blood. The clinical data and laboratory results were subsequently compiled and analyzed. Of the 40 thalassaemic patients, 37 were HbE/ *b*-thalassemia and 3 were *b*-thalassaemia major. 39 (97.50%) of the patients were under transfused (mean Hb <10 gm/dl) and 35 (87.5%) of the patients were taking some form of chelation therapy but out of them only 1 (2.5%) were adequately chelated (S. ferritin <1000 ng/ml).

Keywords: Transfusion dependent thalassaemia, CBC-Complete Blood Count, Hb conc-Haemoglobin concentration, Chelation therapy, Iron overload

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INTRODUCTION

Thalassaemia major is a hereditary haemolytic disorder which is treated with repeated blood transfusions. About 240 million beta thalassemia carriers are present all over the world. Every year about 100,000 children are born with the disease of thalassemia[1]. It is also a major health problem in Bangladesh. Thalassaemic patients experience various problems if the transfusion is inadequate but at the same time repeated blood transfusions are associated with hazards like iron overload and risk of acquiring transfusion-transmitted infections (TTIs). Iron overload can lead to endocrinal dysfunction in the form of growth retardation and diabetes mellitus. Thus, chronic blood transfusion in thalassaemic patients is a double-edged sword. Ultimately thalassaemic patients die either due to transfusions or due to lack of it resulting the normal life upto 25 years.

The basic defect in β-thalassaemia is a reduced or absent production of β-globin chains with relative excess of α-chains. The direct consequences are a net decrease of the haemoglobin production and an imbalance of the globin chain synthesis. Ineffective erythropoiesis is the hallmark of β-thalassaemia[2].

In iron overload resulting from repeated blood transfusions or long-term increased iron absorption, iron that is not bound to naturally occurring molecules such as transferrin, or ferritin or to therapeutic iron chelators, generates a variety of reactive oxygen species (ROS), most notably hydroxyl radicals. This occurs in cells where labile plasma iron is taken up and accumulated as storage iron (ferritin and haemosiderin)[3].

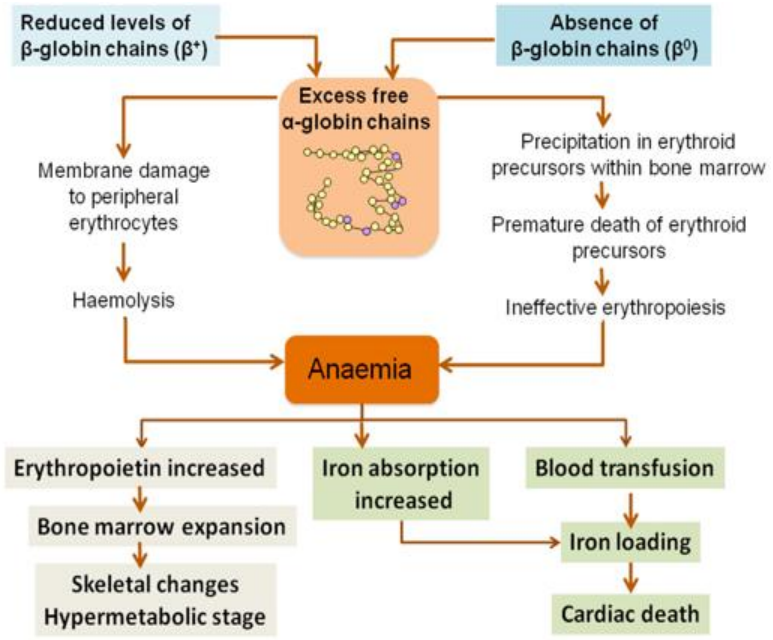

Figure 1. Effects of excess production of free α-globin chains in β-thalassaemia[2].

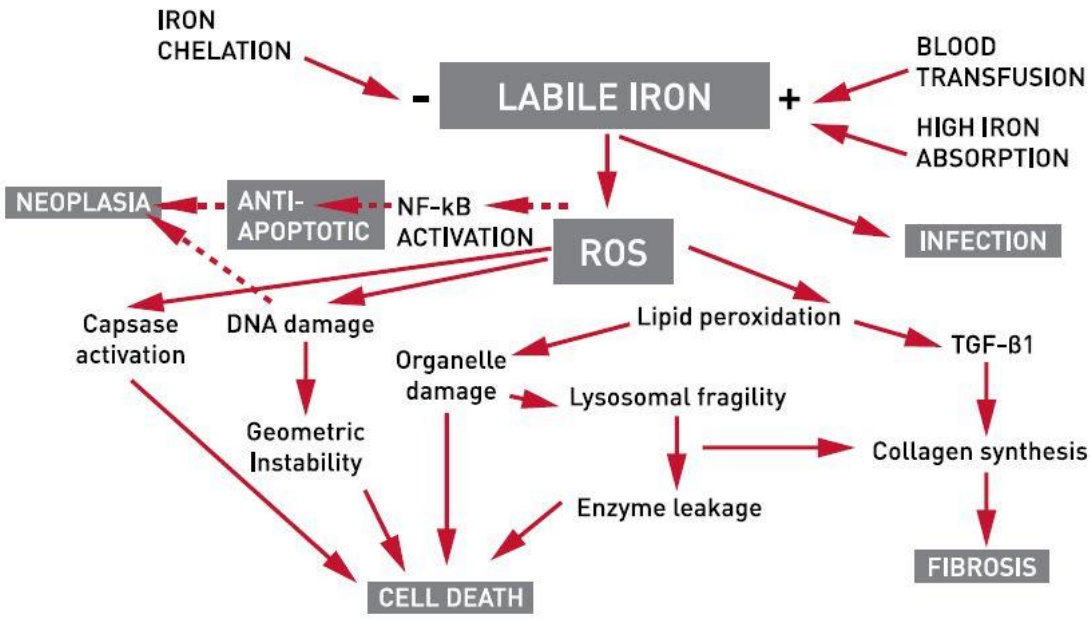

Figure 2. Pathophysiology and consequences of iron overload[3].

It is important to exclude the possible cause of iron deficiency anemia that remains common in several part of the world. Summary of diagnostic measures for patients with hypochromic and microcytosis and further with diagnostic features of common thalassaemia syndromes are shown in Figure 3. Patients with optimally treated thalassaemia can now enjoy a near-normal life and lifestyle, and experience regular physical and emotional development from childhood to adulthood. This is achieved by recognizing the limitations that the disease imposes but also the effect that the treatment regimens have on the patient's lifestyle, and the time that these treatments steal from normal living. Recognition of these needs comes with knowledge of all aspects of the disease, with experience, and through providing a holistic approach to patients. Beyond managing the physical condition, healthcare staff should be willing to listen to any queries that the patients may bring up and be able to advise on all lifestyle issues. Leading a "normal" life is an often-expressed priority for patients. This includes social integration, connecting and interacting with people and contributing to society, despite counter forces that the disease and its treatment bring, which can lead to isolation, and in some societies stigmatization. Marginalization will lead to depression and possibly increase health risks. The concepts of quality of life, social integration, living and experiencing life beyond

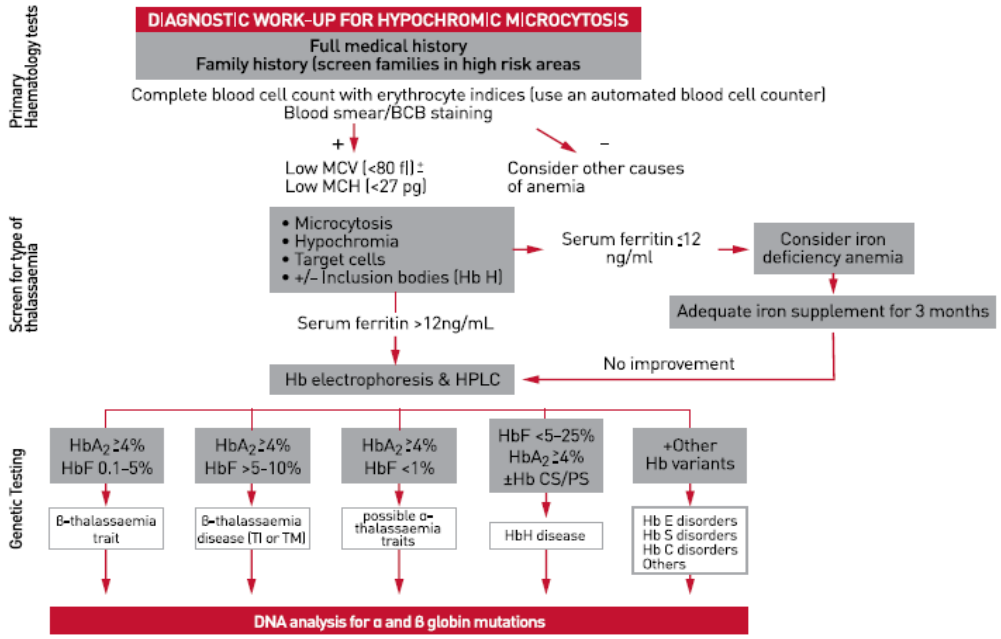

Figure 3. Diagnostic algorithm for individuals with hypochromic microcytosis [4].

health preservation, are interwoven. Psychosocial support is a necessary component of management, as is quality and organized holistic care.

Prevention efforts include pre-marital screening to make sure that the couple are not both carriers, provision on counseling and health education for the thalassaemics, their families and the public, provision of prenatal testing for thalassaemia and reduction of marriages between relatives.

OBJECTIVES OF THE STUDY

General objectives.

To study different haemato-biochemical and anthropometric parameters of the patients of transfusion dependent thalassaemia.

Specific objectives.

a. To see the effectiveness of transfusion in transfusion dependent thalassaemia.
b. To assess the quality of life and complication of transfusion in transfusion dependent thalassaemia patients.

MATERIALS AND METHODS

This was prospective cross sectional study conducted in Armed Forces Institute of Pathology (AFIP), and Combined Military Hospital (CMH), Dhaka cantonment, Dhaka during a period of 6 months from 1st July 2018 to 31th December 2018. Study population was 40 patients who were diagnosed as transfusion dependent thalassaemia. Total 40 adolescent and young adult patients who were getting regular blood transfusion diagnosed as transfusion dependent thalassaemia at CMH Dhaka, different CMHs, Kurmitola General Hospital (KGH) and other hospitals. Their most updated age, marital status, number of family members, height, weight, condition of spleen (palpability, splenectomy), duration of blood transfusion, duration and mode of iron chelation therapy, complete blood picture and serum ferritin level were collected from AFIP and history taking from the individual patient

Selection criteria

- Inclusion criteria.
  a. Patients diagnosed as thalassaemia.
  b. Patients who required blood transfusion.
  c. Patient who are adolescent and young adults.
  d. Patients who are getting treatment at CMH, Dhaka and KGH, Dhaka.

- Exclusion criteria
  a. Patients of Non transfusion dependent thalassaemia.
  b. Children and young patients who got few blood transfusion.
  c. Patients with any major disease other than thalassaemia and of its consequence.

Procedure of collecting data

Total 40 adolescent and young adult patients who were getting regular blood transfusion at CMH Dhaka and were diagnosed as transfusion dependent thalassaemia at CMH Dhaka, different Army hospital, Kurmitola General Hospital (KGH) and other hospitals earlier were selected. Their most updated age, marital status, number of family members, height, weight, condition of spleen (palpability, splenectomy), duration of blood transfusion, duration and mode of iron chelation therapy and complete blood picture, serum ferritin level were collected from AFIP and talking to the individual patients.

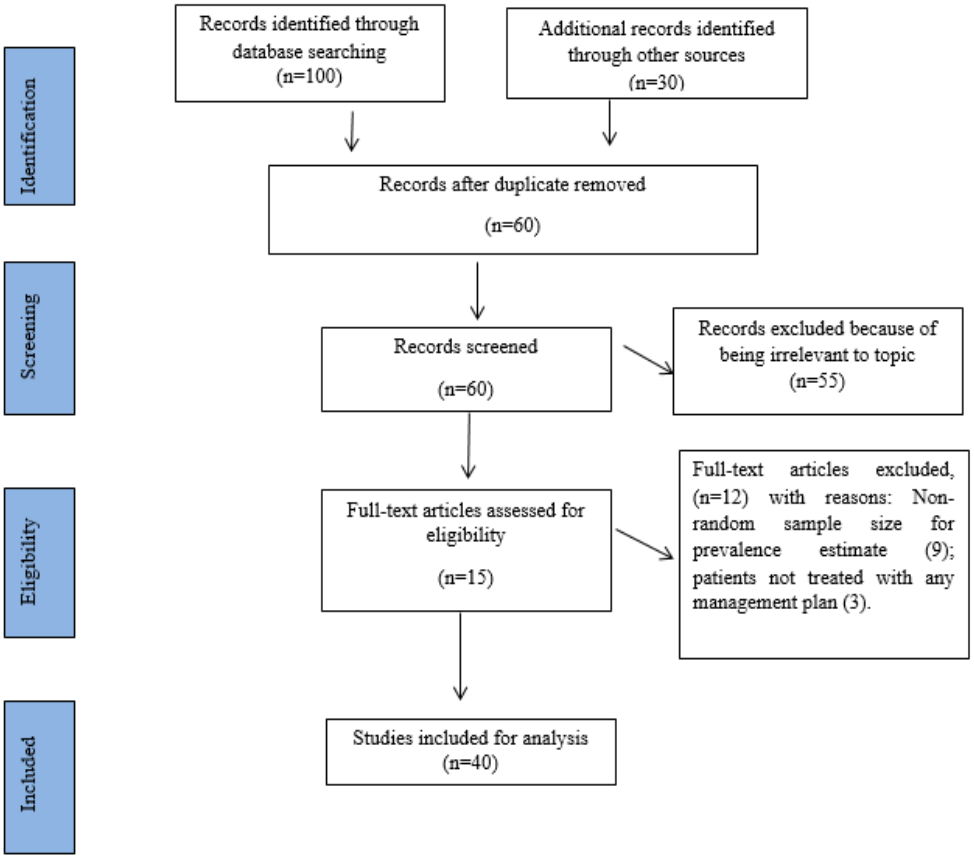

Figure 4. Study design

Ethical measures

a. Participation was volunteered.
b. Verbal consent was obtained after a brief of the study in bangla to all participants.
c. It was made clear to them that they were free to take part or refuse any part of the study.
d. All the investigation reports were kept confidential.
e. Every attempt was taken to conduct the interview privately.

Data analysis

Statistical analysis was done with the help of IBM SPSS statistics 20 software. After completion data were checked, verified, edited, and coded. For any analytical test the level of significance is 0.05 and p value <0.05 will be considered significant.

RESULTS

Relationship between age and blood transfusion

With the increase in age, the cumulative number of blood transfusions received will increase. That's why frequency of blood transfusions received per month also goes up. Such a relation is expected, as due to worsening of the disease with progression of age, the requirement of blood transfusions will increase. However majority of patients still received blood transfusions irregularly and looking at the pre-transfusion haemoglobin value, such a frequency of blood transfusions appears to be in adequate and needs to be reviewed along with consideration for their relevant factors. Table 1 showed that more than one third (40.0%) patients belonged to age 21-30 years. The mean age was 25.98±7.45 years with ranged from 18 to 40 years.

Figure-5 revealed that most of the patients aged less than 20 years received blood transfusions only once in a month. However, a major chunk of the patients aged more than 30 years got blood transfusions 2-3 months interval. Such frequency of blood transfusions appear to be inadequate.

Table 1. Distribution of the study patients according to age (n=40)

Age (in years)	Number	Percentage
≤20	14	35.0
21-30	16	40.0
31-40	10	25.0
Mean±SD	25.98±7.45	
Range(min/max)	18/40	

Table 2. Distribution of the study patients according to family member (n=40)

No of family member	Number	Percentage
≤5	33	82.5
6-10	7	17.5
Mean±SD	4.67	±1.75
Range(min,max)	2	10

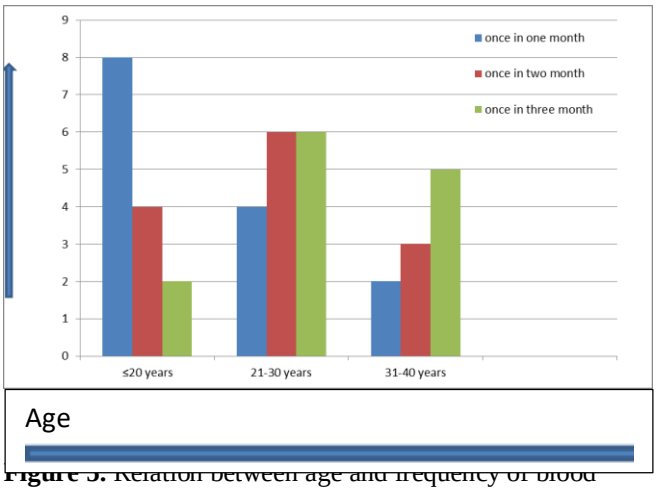

Figure 5. Relation between age and frequency of blood transfusion per month.

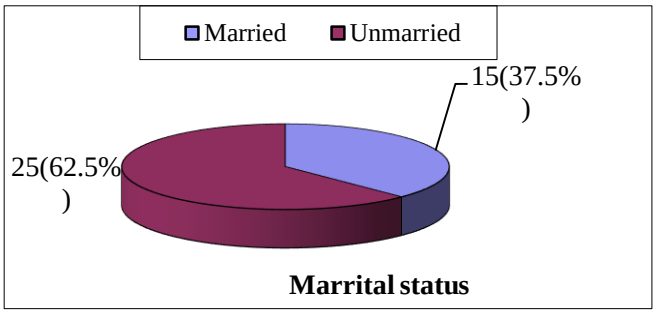

Figure 6. Pie chart showing marital status of the study patients (n=40)

Table 3. Distribution of the study patients according to type of the patient (n=40)

Type of patient	Number	Percentage
HbE/ *b*-thalassemia	37	92.5
b-thalassemia major	3	7.5

Table 4. Distribution of the study patients according to anthropometric data (n=40)

Anthropometric data	Mean±SD	Range (min,max)
Weight (kg)	43.78±6.27	32,61
Height (cm)	160.15±7.13	136,172

Table 5. Distribution of the study patients according to BMI (n=40)

BMI (kg/m^2)	Number	Percentage
Underweight (<18.5)	40	100.0
Mean±SD	16.18±	1.01
Range(min,max)	13.32	/18.29

Table 6. Distribution of the study patients according to systemic examination (n=40)

Systemic examination	Number	Percentage
Palpable Spleen	31	77.5
Splenectomy	9	22.5

Marriage and Reproductive Life

Getting married is widely accepted as a key goal in one's life, and thalassaemia patients have a good record in forming relationships. The role of the treatment in this respect is to ensure that from an early age that patients are seen by an endocrinologist to avoid hypogonadism as much as possible and to initiate treatment early, so that any delay or absence in sexual development is avoided provide general support and encouragement. Figure-6 revealed that two third (62.5%) patients were unmarried and 15(37.5%) are married. Table 2 showing out of majority (82.5%) patients belonged to family member ≤5. The mean no of family member was 4.67±1.75 with ranged from 2 to 10.

Thalassaemia Syndrome

The b-thalassaemias include four clinical syndromes of increasing severity: two conditions are generally asymptomatic, the silent carrier state and b thalassemia trait, and usually result from the inheritance of one mutant b globin gene, and two require medical management, thalassemia intermedia and thalassemia major. The more severe forms most often result from homozygosity or compound heterozygosity for a mutant b-globin allele and, occasionally, from heterozygosity for dominant mutations. Homozygous or compound heterozygous b-thalassemia usually presents no diagnostic problems. The early onset of anemia, characteristic blood changes, and elevated fetal hemoglobin concentrations are found in no other condition. Table 3 showed that 92.5% patients are of HbE/ *b*-thalassemia and 7.5% patients are of *b*-thalassaemia major.

Clinical diagnosis

Since the activity of the normal β gene on the allelic chromosome makes enough stable globin, under normal circumstances, β -thalassaemia trait has no important clinical effects. Clinical presentation of β -thalassaemia major usually occurs between 6 and 24 months with severe microcytic anaemia, mild jaundice, and hepatosplenomegaly. Affected infants fail to thrive and become progressively pale. Feeding problems, irritability, recurrent bouts of fever due to hypermetabolic state or inter-current infection, and progressive enlargement of the abdomen caused by spleen and liver enlargement may occur. In some resource-limited settings, the clinical picture in patients who are untreated or poorly transfused, is characterized by growth retardation, pallor, jaundice, poor musculature, genu valgus, hepatosplenomegaly, leg ulcers, development of masses from extra medullary haematopoiesis, and skeletal changes resulting from expansion of the bone marrow. Skeletal changes include deformities in the long bones of the legs and typical craniofacial changes: thalassaemic facie (bossing of the skull, prominent malar eminence, depression of the bridge of the nose and hypertrophy of

the maxillae, which tends to expose the upper teeth). If a chronic transfusion regimen is not started, patients with thalassaemia major usually die within the first few years of life.

Table 4 revealed that the mean weight was 43.78±6.27 kg with ranged from 32 to 61 kg. The mean height was 160.15±7.13 cm with ranged from 136 to 172 cm. Table- 5 showed that all (100.0%) patients belonged to BMI (<18.5 kg/m^2) as underweight. The mean BMI was 16.18±1.01 (kg/m^2) with ranged from 13.32 to 18.29 (kg/m^2). Table 6 showed that more than two third (77.5%) patients had palpable spleen and 9(22.5%) in splenectomy.

Transfusion and iron overload

Iron overload occurs when iron intake is increased over a sustained period of time, either as a result of red blood cell transfusions or increased absorption of iron through the gastrointestinal (GI) tract. Both of these occur in thalassaemias, with blood transfusion therapy being the major cause of iron overload in thalassaemia major and increased GI absorption being more important in non-transfusion dependent thalassaemia (NTDT). When thalassaemia major patients receive regular blood transfusion, iron overload is inevitable because the human body lacks a mechanism to excrete excess iron. Iron accumulation is toxic to many tissues, causing heart failure, cirrhosis, liver cancer, growth retardation and multiple endocrine abnormalities. Chelation therapy aims to balance the rate of iron accumulation from blood transfusion by increasing iron excretion in urine and or faces with chelators. If chelation has been delayed or has been inadequate, it will be necessary to excrete iron at a rate which exceed this. Because iron is also required for essential physiological purposes, a key challenge of chelation therapy is to balance the benefits of chelation therapy with the unwanted effects of excessive chelation. The second major challenge in chelation therapy is to achieve regular adherence to treatment regimens throughout a lifetime, as even short periods of interruption to treatment can have damaging effects. Table 7 revealed that the mean duration of blood transfusion and chelation therapy were 16.26±5.84 years and 5.01±4.47 years respectively. More than half (60%) patients had irregular and 16(40%) in regular transfusion. More than half (57.5%) patients had oral chelation therapy, 9(22.5%) in combined chelation therapy, 3(7.5%) in injectable chelation therapy and 5 (12.5) have not received yet any chelation therapy. Table 8 showed that the mean S. Ferritin was 4446±3890 (ng/ml) with ranged from 1012 to 17560 (ng/ml).

Monitoring of iron overload

The inevitable consequence of regular life-saving transfusions in thalassemia major is the accumulation of excess iron within tissues. Transfused iron is deposited first within the reticuoendothelial cells prior to parenchymal iron loading within the heart and liver.

Table 7. Distribution of the study patients according to H/O blood transfusion & chelation therapy (n=40)

H/O blood transfusion and chelation therapy	Mean±SD	Range (min,max)
Duration of blood transfusion (Years)	16.26±5.84	6,38
Duration of chelation therapy (Years)	5.01±4.47	0.5,20
Blood transfusion	*Number*	*Percentage*
Regular	16	40
Irregular	24	60
Mode of chelation therapy	*Number*	*Percentage*
Oral	23	57.5
Combined	9	22.5
Injectable	3	7.5
No chelation	5	12.5

Table 8. Distribution of the study patients according to S. Ferritin (ng/ml) (n=40)

	Mean±SD	
S. Ferritin (ng/ml)	4446±	3890
Range(min,max)	1020,	17560

However, as in primary iron overload, the majority of morbidity and mortality ultimately results from progressive heart and liver failure. Effective management of iron overload requires frequent evaluation of the body iron stores. There is, therefore, a need for quantitative, non-invasive methods for measuring body iron that are safe, accurate and readily available. The iron status of the body in overload conditions can be assessed by different methods. Serum ferritin measurement, although easy to perform frequently, offers variable results, still at present, no other serum test is a better predictor.

Haematologic diagnosis

Heterozygous carriers of β-thalassaemia, usually display a low mean cellular haemoglobin (MCH), low mean cell volume (MCV), and an increased level of HbA2, which may be associated with low normal or slightly subnormal haemoglobin levels. Peripheral blood smear shows less severe erythrocyte morphologic changes than affected individuals and erythroblasts are normally not seen. β-thalassaemia major is characterised by reduced haemoglobin level (<7g/dl), MCV >50 and <70 fl and MCH >12 and <20 pg. Affected individuals show microcytosis, hypochromia, anisocytosis, poikilocytosis (spiculated tear-drop and elongated cells), target cells and erythroblasts. The number of erythroblasts (nucleated red blood cell) is related to the degree of anaemia and is markedly increased after splenectomy. In general, these abnormal red blood cell morphology and features share among different types of thalassaemia syndromes even interactions with haemoglobin variants such as HbE/β-

thalassaemia. Table 9 showed that the mean Hb conc (gm/dl) was 7.13±1.95 with ranged from 4.3 to 11.7. The total RBC ($x10^{12}$/L) was 3.39±0.84 with ranged from 1.87 to 4.82. The mean HCT (%) was 22.81±5.37 with ranged from 13.9 to 34. The mean MCV (fl) was 68.41±9.52 with ranged from 48, to 89.7. The mean MCH (pg) was 21.17±2.94 with ranged from 14.36 to 26.54. The mean RDW (%) was 27.55±5.84 with ranged from 11 to 37.2. The mean total WBC ($x10^9$/L) was 9.22±3.68 with ranged from 3 to 20. The mean platelets ($x10^9$/L) were 361.13±326.83 with ranged from 42 to 1597.

DISCUSSION

Transfusion dependent thalassaemia is one of the major public health problems in Bangladesh. It is estimated that nearly 14,000 thalassaemic children are born every year here. World Health Organization (WHO) has estimated that 3% of our population carries β-thalassaemia[5].

In this present study 39 (97.5%) of total 40 patients had Hb level less than 10gm/dl and only 1 (2.5%) patient achieved the target Hb level which suggests the proper and more meticulous implementation of transfusion regimen. The correct transfusion regimen practice can ensure normal growth without excessive expansion of erythropoiesis and with effective prevention of iron overload.

In a similar study by Md. Fazlul Karim, Md. Ismail, AKM Mahbub Hasan and Hossain Uddin Shekhar from University of Dhaka, Bangladesh have found the Hb level is 7.2 ± 1.5 (g/dL)[6] which is consistent to the present study. A comparison between two studies is shown on Table 10.

A landmark study investigating role of desferrioxamine in prevention of complications of transfusional iron overload showed that survival to at least 25 years of age in poorly chelated β-thalassemia major patients was just one-third that of patients whose iron levels were well managed by deferoxamine[7]. Guidelines from the Thalassemia International Federation recommend that chelation therapy is initiated when serum ferritin levels reach approximately 1000 ng/mL, which usually occurs after the first 10 to 20 transfusions or around 2-3 years of age[5,8].

In the present study, we have found that only 1 out of 40 (2.5%) patients had S. ferritin <1000 ng/ml and 7 (17.5%) patients were not taking any kind of chelation therapy. Hence only 1 (2.5%) patients could be considered to be taking adequate chelation therapy. 33 (82.5%) patients were taking some form of chelation therapy. Out of these, 14 (35%) patients were taking regular chelation therapy and 19 (47.5%) were taking irregular chelation therapy. The mean serum ferritin level was 2767.52 (SD 1849.1) ng/ml.

Table 9. Distribution of the study patients according to haemogram parameters (n=40)

haemogram parameters	Mean±SD	Range (min,max)
Hb (gm/dl)	7.13± 1.95	4.3, 11.7
Total RBC ($x10^{12}$/L)	3.39± 0.84	1.87, 4.82
HCT (%)	22.81± 5.37	13.9, 34
MCV (fl)	68.41± 9.52	48, 89.7
MCH (pg)	21.17± 2.94	14.36, 26.54
MCHC (gm/dl)	31.02± 2.07	26, 34.4
RDW (%)	27.55± 5.84	11, 37.2
Total WBC ($x10^9$/L)	9.22± 3.68	3, 20
Differential Neutrophils (%)	54.33± 12.23	23, 85
Platelets ($x10^9$/L)	361.13±326.83	42,1597

Table 10. Comparison of mean haemoglobin level according to age with similar study

Study	No of sample	Mean age (year)	Male patient	Female patient	Mean ± Hb level (g/dl)
Md. Fazlul Karim, Md. Ismail, AKM Mahbub Hasan, Hossain Uddin Shekhar[6]	54	7.8	27	27	7.2 ± 1.5 (g/dL)
Present study	40	25.98	28	12	7.13±1.9

Table 11. Comparison serum ferritin level with other similar studies

Study	No of sample	Male patient	Female patient	Mean Serum ferritin level
Choudhury VP et al.[8]	95	54	41	6723 ng/ml
Bandyopadhyay et al.[9]	101	61	40	3650 ng/ml
Amit Kumar Mishra and Archana Tiwari[11]	72	41	31	2767.52 ng/ml
Cunningham et al.[12]	130	73	57	1696 ng/ml
Present study	40	28	12	2767.52 ng/ml

In a study by Bandyopadhyay et al., patients of younger age group showed high serum ferritin levels. They found that average serum ferritin was 3650 ng/ml in 11-15 years older patients. The serum ferritin level could not be controlled well as only few patients fully complied with recommended regimen at home[9]. Similarly, in our study, the mean serum ferritin level was 2767.5 ng/ml, which is markedly higher than the normal recommended levels for normal individuals. Normal values of serum ferritin for men and women are 12-300 ng/mL and 12-150 ng/mL, respectively[10].

A similar study was conducted by Amit Kumar Mishra and Archana Tiwari from School of Biotechnology, Rajiv Gandhi Proudyogiki Vishwavidyalaya, Madhya Pradesh, India at School of Biotech-nology, Rajiv Gandhi Proudyogiki Vishwavidya-laya, Bhopal, India. In their study a total of 72 cases consisting of thalassaemia major and intermedia were examined, of which 56.9% were male and 43% female, with a male to female ratio of 1.32: 1. The mean age of males was 5.9 years whereas mean age of females was 6.2 years. The mean serum ferritin level was 2767.52 (SD 1849.1) ng/ml. Only nine patients (12.5%) had serum ferritin level less than 1000 ng/ml[11]. So their mean serum ferritin level was consistent to our study.

Cunningham et al in 2004 reported mean serum ferritin levels in beta thalassemia patients of North America to be 1696 ng/ml[12]. How-ever, Choudhury VP et al in India reported mean serum ferritin levels to be 6723 ng/ml[8] even higher than in our study. The following table showing comparison of serum ferritin level with other similar studies:

In this study it is found, all the patient have a BMI below normal. In a similar study by Shazia Ali, Sarwat Jahan also found BMI of 300 thalassaemic patient in Pakistan is below normal as per age and sex[13]. It is suggested that newer protocols of treatment, in addition to optimization of transfusion and chelation requirements, should increase the caloric intake of these patients and properly manage their pubertal delay-failure in order to improve their adult height[14].

CONCLUSION

Management of transfusion dependent thalassaemia has improved to a point where life expectancy will reach that of the normal population. It is time demanding to step up the transfusion guideline to achieve haemoglobin level of 10 gm% (as per the moderate transfusion regimen) and effective chelation with a view to keep serum ferritin level below 1000 ng/ml. Strict compliance to the guideline will go a long way in improving the quality of life (QoL) in patients of transfusion dependent thalassaemia. Despite of few limitations the findings reflected certain distinctions which will help to conduct further study in this issue.

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