Research Article

# 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


## Abstract

Thalassemia is the name of a group of genetic, inherited disorders of the blood. More specifically, it is a disorder of the hemoglobin molecule inside the red blood cells. According to World health Organization (WHO), there are about 3% beta-thalassemia carrier and about 4% Hb E/beta-thalassemia carrier in Bangladesh. Our objective is to identify the prevalence of beta-thalassemia in our adolescent populations and to review risk factors that would most easily identify a subset of adolescent patients at greatest risk for the development of beta-thalassemia. We also made a study of clinical profile of 53 thalassemic patients, observing the relationship between the patients with their verity ages and sex. The cases are taken on the basis of their age (2-30 years), beta-thalassemia major, clinical jaundice with history of chronic blood transfusion. The cases excluded those who had jaundice due to viral hepatitis or hepatitis due to heavy metal poisoning (Arsenic) and those with spleenectomy. Liver function test has been evaluated in 53 patients. That were recorded with some relevant demographical data such as age, sex, blood group where median age was of 16 years and mean (±SD) age 15.4151 ± 7.90918. Among them were 21 (39.6%) female and 32 (60.4%) male. With an average 15.1% (8 in no.) beta-thalassemia, 7.5% (4 in no.) beta-thalassemia major and 77.4% (41 in no.) E-beta-thalassemia cases have been found in the study. Mean (±SD) TSB in total 53 subjects with age group 2-10 years and 21-30 years is significant. The study revealed that in thalassemic patients when the age is more, the disease progresses with their complication. Hepatic complication is mainly due to being hepatocellular in nature than that of obstructive one.



**Key words:**
*Beta-thalassemia, Chittagong, liver function test, spleenectomy, total serum billirubin, world health organization*


## Introduction

Thalassemia is the most common genetic blood disease in the world and varies in different population group in the world.[1] World Health Organization (WHO) estimates that at least 6.5% of the world populations are carries of different inherited disorders of hemoglobin.[2] Another WHO report estimates that 3% are carriers of beta-thalassemia and 4% are carriers of Hb E in Bangladesh. In Bangladesh, more than 7000 children are born with thalassemia each year.[3] Majority are born in countries with limited resources where priority tends to be given to tackling high rates of infant and child mortality from infection diseases and malnutrition.[4] The patients suffering from beta-thalassemia major and Hb E/beta-thalassemia do not survive for more than 5 years without blood transfusion.[5] Thalassemia (also known as Mediterranean anemia, Cooley's anemia, Beta-thalassemia or Alpha-thalassemia) is an inherited blood disorder affected by an abnormal form of hemoglobin blood disorder is the most common inherited single gene disorder in the world [Figure 1]. This specific type of blood disease results in excessive destruction of red blood cells which in turn leads to anemia.[6] For a better understanding of this disease one must know the importance of hemoglobin.[7] In vertebrates, hemoglobin 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


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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.[8] Thalassemia occurs more often among certain ethnicities, including people of Italian, Greek, Middle-eastern, Asian and African descent. Thalassemia is an inherited disorder which means they are passed from parents to their children.[8] a- thalassemia occurs commonly in the people of those from Southeast Asia, the Middle-east, China and those of African-American descent.[9] b- thalassemia occurs commonly in people of the Mediterranean region, Chinese, other Asians and African-Americans.[10]

In Bangladesh, a study carried out by the Dhaka Shishu Hospital Thalassemia Center in 2004 in school children of Bangladesh, showed that carrier status is higher and there is also regional variation. This study revealed that the overall prevalence of beta-thalassemia trait in Bangladesh was 4.1% and Hb E trait 6.3%.[11] A recent study showed that carrier status of Hb-E is 6.1% and as high as 40% in tribal children in Bangladesh.[12]

## Materials and Methods

The study was designed on the basis of prospective observational type of study. In the study the current data was obtaining form a specific diseased group with a progressive complication. The total procedure of the study was conducted in the Thalassemia Sheba Kendro, Jamalkhan, Chittagong and also in the Biochemistry Lab, Chattagram Maa-O-Shishu General Hospital (CMOSGH), Agrabad, Chittagong.

### Subject collection and study procedure

Subjects were collected on every working day of the week from 10:00 am to 2:00 pm from the Thalassaemia Sheba Kendro and when needed from the Biochemistry Laboratory in the CMOSGH. This prospective study involved patients who admitted into the Sheba Kendro for the purpose of blood transfusion and for getting iron chelation therapy. Parents or guardians were informed of the purpose of the study. For each patient a detailed history was taken from mother or the attendant. After taking brief history preliminary selection was done, and the purpose to the study was explained in details to its subject. After taking consent from the parents, data was collected, which included sex, age at presentation, age at diagnosis and clinical symptoms at presentation. A thorough physical examination was done in each patient. Majority of the patients diagnoses were confirmed by Hb-electrophoresis. The patients who fulfilled the inclusion criteria were included for this study. All the planned information obtained and recorded in the data collecting sheet properly. A total of 53 subjects were included in this study. There was no specific preference for race, religion and socioeconomic status.

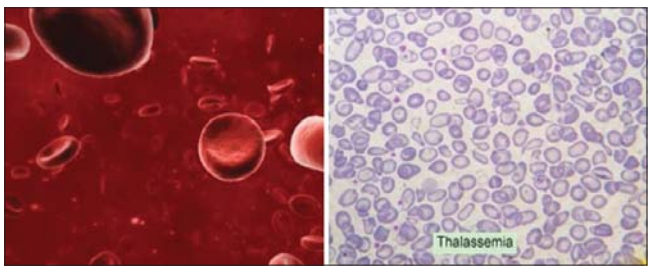


**Figure 1:** Blood film showing characteristics normal red blood cell and abnormal pale red blood cells

**Inclusion Criteria:** Patients with confirmed diagnosis of thalassemia were randomly selected as first as diagnosis up to the age 30.[13] The subjects on regular blood transfusions were enrolled. The inclusion criteria considered in data collection are: (1) Thalassemia major, (2) History of jaundice other than viral, alcohol or heavy metal induces jaundice, (3) Repeated blood transfusion, (4) History of taking iron chelating therapy and (5) Clinically diagnosed Hepatomegaly.

**Exclusion Criteria:** There is more problem to exclude subject only basis on clinical questionnaire and on the basis of some laboratory test.[14] The exclusion criteria are as follows: (1) Thalassemia trait or intermedia type, (2) History of jaundice due to viral, alcoholic or heavy metal induces, (3) History of spleenectomy, (4) Age more than 30 years.

Additional investigations done were:
- Observation of liver function test (LFT; Serum billirubin) in different thalassemic patients.
- Estimation of serum billirubin (Jendrassik and Grof method) by spectrophotometric/ filter photometric test.

### *Estimation of serum billirubin by spectrophotometric/ filter photometric test*

Conjugated (direct) billirubin in serum is coupled with diazotized sulfanillic acid to form red-colored compound. Ascorbic acid is used to stop the coupling reaction and to eliminate interference by hemoglobin. Caffeine benzoate solution is used to split the unconjugated billirubin protein complex releasing the billirubin so that it can react with diazotised sulfanillic acid.

The tartrate buffer makes the mixture alkaline and converts the red acid billirubin to a green-colored compound which shows peak absorbance at 607 nm. At this wavelength the absorbance due to hemoglobin or carotene is minimal.[15]

### Statistical analysis

Statistical analysis was carried out using SPSS statistical package (version 11.5). Analysis of variance (ANOVA) of the data was used to detect overall difference in group means. Differences among group means were assessed using least significance difference (LSD).

### Observation and results

Clinico-hematological study of thalassemia was done on 53 patients during the period of September 2011 to June 2012. Salient features observed in this study were:

### *Age*

Mean (±SD) age in total 53 patients between the age group 2 and 10 years was 6.444 ± 2.61719, between 11 and 20 years mean was 15.1875 ± 3.10309 and between age 21 and 30 years mean was 24.1053 ± 2.44710 were include in

the study [Table 1 and Figure 2].

***Sex***

Mean (±SD) of sex in 53 no. of patients in male sex 15.000 ± 7.95147 and females are 15.9524 ± 8.00922 [Table 2 and Figure 3].

***Type of thalassaemia***

Frequency of the type of thalassemia E-beta-thalassemia 41 in no. (77.4%), beta-thalassemia 8 in no. (15.1%) and beta major 4 in no. (7.5%).

***Total serum billirubin (TSB, mg/dl)***

Mean (±SD) TSB in total 53 patients with age 2-10 years and 11-20 years are 4.7056 ± 1.91694 and 5.8188 ± 2.24773, respectively [Table 3]. Statistically no significant billirubin difference was observed between these two groups. $P > 0.363$

Mean (±SD) TSB in total 53 patients with age group 11-20 years and age 21-30 years are 5.8188 ± 2.24773 and 7.3737 ± 2.01020, respectively [Table 4]. Statistically no significant billirubin difference was observed between these two groups. $P > 0.091$

Mean (±SD) TSB in total 53 patients with age group 21-30 years and 2-10 years are 7.3737 ± 2.01021 and 4.7056 ± 1.91694, respectively [Table 5]. Statistically significant billirubin difference was observed between these two groups. $P < 0.001$

Mean (±SD) TSB in total 53 patients with different sex group are 6.2714 + 2.35248 and 5.8188 ± 2.30181 for female and male, respectively [Table 6 and Figure 4]. Statistically no significant billirubin difference was observed between these two groups. $P > 0.491$

**Result from SPSS:** From the data it is easy to interpret that, there is a significant difference found in S. Billirubin with age group 2 to 10 years and 21 to 30 years of age [Tables 7-10].

## Discussion

Thalassemia a group of genetic disorder occur mainly due to defective formation of globin chain of the hemoglobin moiety of the RBC. This specific type of blood disease results in excessive destruction of red blood cells which in turn leads to anemia. In this disease RBC breakdown occur at an early stage due to abnormal globin chain unable to protect RBC in oxidative stress. Resulting destruction of RBC leads to produce billirubin production which ultimately metabolized in liver for excretion. In thalassemia the rate of destruction of RBC is so rapid that it exceeds the liver capacity to metabolize the excess billirubin.[16-18] Hb E is the most common variant hemoglobin with a mutation in beta-globin gene causing substitution of glutamic acid for lysine at position 26 in beta-globin chain. Hb E Disease presents in 3 forms namely heterozygous state (Genotype AE or Hb E trait), homozygous state (Genotype EE or Hb E disease) and compound heterozygous states [1. Hb E beta-thalassemia (E-beta-thalassemia) 2. Sickle Cell/Hb E Disease (SE Genotype)].[19-21]

Pathophysiology is complex which involves ineffective erythropoiesis, apoptosis, Oxidative damage and shortened red cell survival. Interaction between Hb E and beta-thalassemia alleles is main determinant in pathophysiology. Hb F level is strongest predictor of morbidity. Hb E Trait may be coinherited with either $\beta^0$-thalassemia or $\beta^+$-thalassemia. The compound heterozygous state is quite common in Thailand and occurs throughout a large part of Southeast Asia stretching from Indonesia to Sri Lanka, Northeast India and

**Table 1: Group statistics of LFT with age distribution**

| Subjects | No. of subject (%) |
|---|---|
| Age group 2-10 years | 34 |
| Age group 11-20 years | 30.2 |
| Age group 21-30 years | 35.8 |

**Table 2: Group statistics of LFT with sex distribution**

| Subject | No. of subject (%) |
|---|---|
| Male | 60.4 |
| Female | 39.6 |

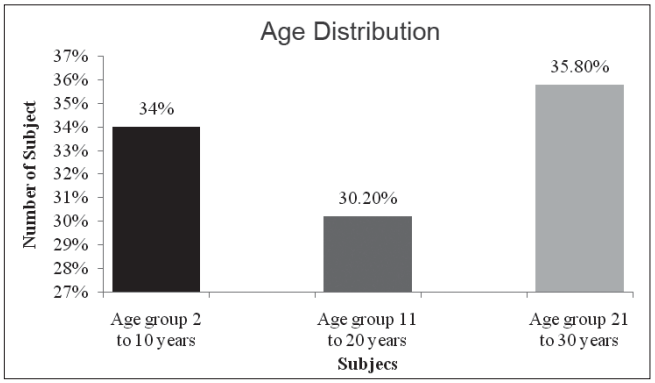


**Figure 2:** Colum diagram showing age distribution over LFT

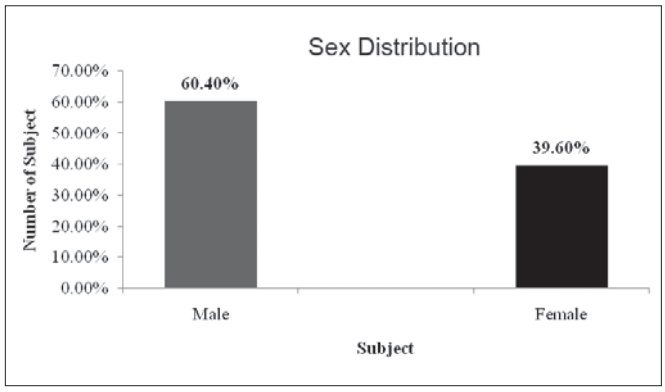


**Figure 3:** Colum diagram showing sex distribution over LFT

Bangladesh with prevalence rate of 30-40%, with very few. Pediatric cases being reported from India.[22-24]

In the study I enrolled LFT to observe the liver damage in thalassemic patient. I focus mainly the basic LFT such as S. Billirubin. S. billirubin is the first point that helps to recognized jaundice patient. I tried to find out the actual liver damage (hepatocellular/obstructive) in thalassaemic patient in different age group and different sex. My study is a prospective observational type of study done at Thalassaemia Sheba Kendra Chittagong with guidance from CMOSGH. In which study subjects are taken (Thalassemia patients) on different age and sex.

**Table 3: Group statistics for TSB (mg/dl) levels between age 2 and 10 and age 11 and 20 years**

| Subjects | No. of subjects |
|---|---|
| Age 2-10 years | 4.7056 ± 1.91694 |
| Age 11-20 years | 5.8188 ± 2.24773 |
| F/p | –1.111319/0.363 |

*P* is not significant

**Table 4: Group statistics for TSB (mg/dl) levels between age 11 and 20 and age 21 and 30 years**

| Subjects | No. of subjects |
|---|---|
| Age 11-20 years | 5.8188 ± 2.24773 |
| Age 21-30 years | 7.3737 ± 2.01021 |
| F/p | –1.55493/0.91 |

*P* is not significant

**Table 5: Group statistics for TSB (mg/dl) levels between age 2 and 10 and age 21 and 30 years**

| Subjects | No. of subjects |
|---|---|
| Age 2-10 years | 4.7056 ± 1.91694 |
| Age 21-30 years | 7.3737 ± 2.01021 |
| F/p | 2.66813/0.001 |

*P* is significant

**Table 6: Group statistics for TSB (mg/dl) levels between different sexes**

| Subjects | No. of subjects |
|---|---|
| Female | 6.2714 ± 2.35248 |
| Male | 5.8188 ± 2.30181 |
| t/p | –0.694/0.491 |

*P* is not significant

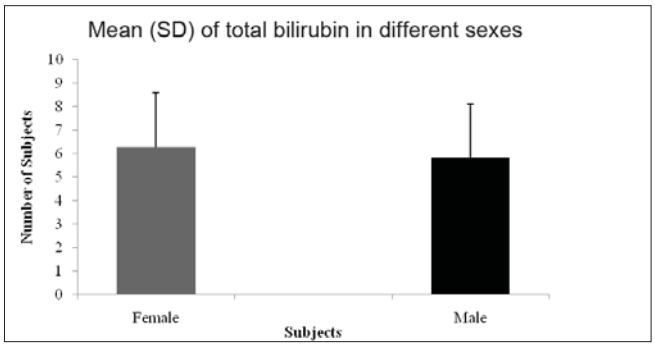


**Figure 4:** Colum diagram show mean (±SD) of total billirubin (mg/dl) in different sexes

In accordance with other studies the most common recognized abnormality was excess billirubin turnover with excess RBC damage in relation to liver damage (change in ALT level with age). Three principal clinical pictures have been recognized: a. acute intrahepatic cholestasis, b. hepatic crisis and c. lithiasis. (Curcio *et al*., BMC Gastroenterology 2010, 10:117). Cholelithiasis and choledocholithiasis are common complications in patients with sickle cell disease or thalassemia.[25] The altered shape of red blood cells favors intravascular hemolysis and thus occlusion of the liver vascular bed, leading ultimately to tissue injury. Furthermore, hemolysis induces deposition of billirubin causing intrahepatic cholestasis and cholelithiasis.[26]

## Conclusions

From the result of the study is that liver damage is augmented when age of the patients are increase. These are due to:

- increasing age with advancement of disease progression,
- repeated blood transfusion,
- Less use/intolerance of iron chelating agent,
- Decreased activity of hepatocyte to rescue them in such excess billirubin and iron flood. Secondary to hyperspleenism.

From the overall study, we tried to correlate the liver damage in thalassemic patients with a hope to improve the liver damage in such patient with the goal of: (A) To prevent liver disease caused by viral hepatitis, iron overload, drug toxicity or hepatocellular carcinoma and (B) To monitor liver abnormalities routinely, and provide treatment for iron overload and any underlying liver disorder. The study revealed that in thalassemia patients when the age increases, the disease is progress with their complication. And in case of hepatic complication it is mainly due to hepatocellular in nature than that of obstructive one.

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**Table 7: Table shows descriptive type of analysis of age on LFT**

| Descriptives | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Age Limit | N | Mean | Std. deviation | Std. error | 95% confidence interval for mean | | Minimum | Maximum |
| | | | | | | Lower bound | Upper bound | | |
| Billirubin | 2-10 yrs. | 18 | 4.7056 | 1.91694 | 0.45183 | 3.7523 | 5.6588 | 2.40 | 8.70 |
| | 11-20 yrs. | 16 | 5.8188 | 2.24773 | 0.56193 | 4.6210 | 7.0165 | 3.00 | 10.80 |
| | 21-30 yrs. | 19 | 7.3737 | 2.01021 | 0.46117 | 6.4048 | 8.3426 | 3.20 | 10.80 |
| | Total | 53 | 5.9981 | 2.31022 | 0.31733 | 5.3613 | 6.6349 | 2.40 | 10.80 |

**Table 8: ANOVA table showing LFT on age group**

| ANOVA | | | | | | |
|---|---|---|---|---|---|---|
| | Groups | Sum of squares | df | Mean square | F | Sig. |
| Billirubin | Between Groups | 66.539 | 2 | 33.270 | 7.884 | 0.001 |
| | Within Groups | 210.991 | 50 | 4.220 | | |
| | Total | 277.530 | 52 | | | |

**Table 9: Table shows multiple comparisons (Bonferroni) on age group over LFT**

| Multiple comparisons (Bonferroni) | | | | | | | |
|---|---|---|---|---|---|---|---|
| Dependent Variable | (I) age group | (J) age group | Mean difference (I-J) | Std. error | Sig. | 95% confidence interval | |
| | | | | | | Lower bound | Upper bound |
| Billirubin | 2-10 yrs. | 11-20 yrs. | –1.11319 | 0.70581 | 0.363 | –2.8626 | 0.6352 |
| | | 21-30 yrs. | –2.66813 | 0.67567 | 0.001 | –4.3419 | –0.9944 |
| | 11-20 yrs. | 2-10 yrs. | 1.11319 | 0.70581 | 0.363 | –0.6352 | 2.8616 |
| | | 21-30 yrs. | –1.55493 | 0.69702 | 0.091 | –3.2816 | 0.1717 |
| | 21-30 yrs. | 2-10 yrs. | 2.66813 | 0.67567 | 0.001 | 0.9944 | 4.3419 |
| | | 11-20 yrs. | 1.55493 | 0.69702 | 0.091 | –0.1717 | 3.2816 |

*.The mean difference is significant at the 0.05 level

**Table 10: Table show Independent sample test (t-test) of LFT on different sex**

| | Independent sample test assumption= Equal variances assumed | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Levene's test for equality of variances | | t-test for equality of means | | | | | 95% confidence interval of the differences | |
| | F | Sig | t | df | Sig. (2 tailed) | Mean Differences | Std. Error Differences | Lower | Upper |
| Billirubin | 0.069 | 0.794 | –0.694 | 51 | 0.491 | –0.45268 | 0.65205 | –1.76172 | 0.85637 |


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