ORIGINAL ARTICLE

Correlation between serum bilirubin and serum ferritin Level in thalassaemia patients

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ABSTRACT

Thalassaemia is the most common hereditary disorder in the world including Bangladesh. Beta thalassaemia major and Hb-E thalassaemia both are common in our country. Iron overload causes most of the mortality and morbidity associate with thalassaemia. To assess the iron over load and liver function a cross sectional comparative study was carried out in the Department of Biochemistry, Dhaka Medical College, Dhaka in collaboration with Thalassaemia Center and Department of Pathology, Dhaka Shishu Hospital, Dhaka during the period of July 2006 to June 2007. The study was carried out with the patients who visited regularly in Dhaka Shishu Hospital Thalassaemia Centre (DSHTC) and had multiple transfusions (more than five) and age more than 2 years. To compare the state of liver function with normal healthy individuals' normal healthy persons were also included. Total 70 subjects were included in this study. The study subjects were distributed into two groups, the group - A (cases, n=40) and group - B (healthy controls, n=30). According to the major types of thalassaemia present in our country, group -A again divided into two, group - AI β-thalassaemia major (n=12) and group - AII of Hemoglobin E β-thalassaemia (n=28). The mean of serum Bilirubin in group - A and group - B were (2.04 ±0.70) mg/dl and (0.67±0.15) mg/dl respectively. Group - A had higher serum bilirubin than group -B in p value <0.001.The mean level of serum bilirubin in group - AI was (1.70±0.70)mg/dl and the mean of bilirubin in group AII was (2.18±0.66) mg/dl. Group -AI had lower serum bilirubin than group AII in p value <0.05. Pearson's correlation between serum bilirubin and serum ferritin had r value -0.26 was statistically not significant. Mean level of serum ferritin in the total was (2729.40 ± 1935.87) ng/ml, minimum level was 304 ng/ml and maximum was 7256ng/ml. Mean level of serum ferritin in the patients of β - thalassaemia major was (4098.67 ± 1598.63) ng/ml, minimum level was 1212ng/ml and maximum was 7560ng/ml. Mean level of serum ferritin in the patients of HbE β - thalassaemia was (2232.57± 1598.63) ng/ml, minimum level was 304ng/ml and maximum was 630ng/ml. Group AI had higher level of serum ferritin than group AII in p value <0.05.

In conclusion, iron over load and jaundice are common finding of thalassaemia. In this present study serum ferritin and serum bilirubin parameter of iron over load and jaundice are correlated. But no statistical correlation was found between these two parameters.

Key words: thalassaemia, serum bilirubin, serum ferritin.

Introduction

Thalassaemia is the most common hereditary disorder in the world including Bangladesh. Thalassaemia is a major health problem all over the world but this is particularly in the developing countries where the resources are limited[1]. Thalassaemia is characterized by production of reduce normal hemoglobin due to absent or decrease synthesis of one or more type of polypeptide chain[2]. Hemoglobinopathies are characterized by the production of structurally defective hemoglobin due to abnormalities in the formation of globin moiety of the molecule[2]. There are two main classes of thalassaemia called alpha- and beta thalassaemia that result from defective alpha- and beta chain synthesis respectively. There is rare form in which both beta and delta chain production are reduced, delta-beta thalassaemia, or in which epsilon,

gamma, delta and beta chain production are defective, epsilon-gamma- delta-beta thalassaemia[3]. Alpha- thalassaemia is characterized by reduced or suppressed production of alpha-globin chain[4]. It is found most commonly in individuals with an ethnic background of Southeast Asia, Southern China, the Middle East, India, Africa and the Mediterranean. Clinically there are four alpha-thalassaemia syndromes. These occur because of inheritance of molecular mutations affecting the output of one, two, three or four of the -globin genes[5]. Beta thalassaemia is caused by decreased or suppress production of beta-globin chain. It is the most important type of thalassaemia, because it is common and usually produce severe anemia in homozygous and heterozygous status[2]. Clinically beta-thalassaemias are heterogeneous group of disorders and three types, beta-thalasaemia minor or beta-thalassaemia trait, beta-thalassaemia intermedia and beta-thalassaemia major. Beta thalassaemias occur widely in a broad belt, ranging from the Mediterranean and parts of north and West Africa through the Middle East and Indian subcontinent to South East Asia[6]. Hb-E beta-thalassaemia is the commonest severe form of thalassaemia in South East Asia and part of Indian subcontinent. It is the most common form of thalassaemia in Bangladesh. Hb-E is ineffectively synthesized and hence, when it inherited together with beta-thalassaemia there is marked deficiency of beta-chain production. The clinical and hematological changes are variable[6]. Clinically it is again divided into mild, moderate and severe form. Severe form of this type of thalassaemia shows clinical manifestations as beta-thalassaemia major. With the better treatment, survival of the patients with these disorders increase represents one of the most dramatic alteration in morbidity and mortality associated with a genetic disease in 19th century and the subjects are being constantly reviewed[7].

Beta thalassaemia major and Hb-E thalassaemia both are common in our country. A conservative world health report has estimated that three percent are carrier of beta-thalassaemia and four percent are carriers of Hb-E beta-thalassaemia[8]. A recent study on school children in different district of Bangladesh has shown overall prevalence of beta-thalassaemia trait was 4.1% and Hb-E beta-thalassaemia was 6.1%. Calculated value of the expected births of beta-thalassaemia major annually in our country is 1040 and Hb-E beta-thalassaemia is 64439. Due to availability of improved health service and diet the death of children from diarrhea, respiratory tract infection and malnutrition has been fallen, more children are now surviving and visiting hospital with hereditary disorders like thalassaemia. Proper management of thalassaemia patient can decrease morbidity and mortality and they can survive with a good quality of life up to third or fourth decade. Children who suffer from this disease need a lifelong repeated blood transfusion to maintain their hemoglobin level around 12g/dl, but unfortunately those will cause an accumulation of iron in various tissue accompanied by an increase serum iron level[9]. Iron over load occurs when iron intake is increased over a sustained period of time, either from the transfusion of red cells or because there is increased absorption of iron from digestive tract. Both of these occur in thalassaemia, blood transfusion being the major cause in thalassaemia major and increased iron absorption being more important cause in thalassaemia intermedia. Because there is no mechanism in human to excrete the excess iron, this has to be removed by chelating agent[10]. Iron overload causes most of the mortality and morbidity associate with thalassaemia. Iron deposition occurs in visceral organs mainly in heart, liver and endocrine glands causing tissue damage and ultimately gastrointestinal absorption are contributory. Paradoxically, excess gastrointestinal iron absorption persists despite massive increases in total body iron load. Hepcidin is a small peptide that inhibits iron absorption in the small bowel. Hepcidin levels normally increase when iron stores are elevated. Its levels were found to be inappropriately low in patients with thalassaemia intermedia and thalassaemia major[11].

The combination of iron overload and increase outpouring of catabolic iron from the reticuloendothelial system overwhelm the iron binding capacity of transferrin, resulting in the emergence of toxic non transferrin bound plasma iron (NTBI). NTBI promotes the formation of free hydroxyl radicals and accelerates the peroxidation of membrane lipids. Both lipid peroxidation and TGFbeta-1 expression resulted from iron over load may promote hepatic injury and fibrogenesis[12]. Furthermore beta-thalassaemia and Hb-E beta-thalassaemia patients treated with blood transfusion are also liable to infected with Hepatitis B and Hepatitis C if proper screening is not done which can cause hepatic fibrosis and cirrhosis[13]. So thalassaemia patients must be routinely checked for liver function.

In the condition of iron accumulation and chronic viral hepatitis, there is an increase of serum iron level, transferrin saturation and ferritin level. Ferritin is an intracellular storage iron, which is present mainly in the reticuloendothelial cells. Clinically significant concentration is found in serum and the level of serum ferritin reflects total body iron stores[14,15]. A serum ferritin concentration reflects iron storages in health and also in certain diseases[16]. Ferritin a high molecular weight iron containing protein, acts in human body as an iron storage depot. It is a major iron storage protein of liver, spleen, bone marrow and other tissue of the body. Its two major functions are to remove excess iron from cells converting it into a harmless soluble form, and to provide a mobilizable reserve of iron which can be drawn when needed. Most of the ferritin is intracellular but the measurement of circulating serum ferritin reflects the level of the body iron store[17,18].

To judge the extension of liver cell damage caused by iron accumulation, a very sensitive indicator for liver function, serum bilirubin was assessed. Hyperbilirubinaemia may be found in prehepatic (haemolytic), hepatic, and post hepatic (obstructive).

Thalassaemia patients if treated with proper blood transfusion and chelating therapy they can maintain apparently normal life but for the maintenance of healthy life they should be routinely checked for their liver function, cardiac function and endocrine function.

Materials and Methods

A cross sectional comparative study carried out in the department of Biochemistry, Dhaka Medical College, Dhaka in collaboration with Thalassaemia Center and department of Pathology, Dhaka Shishu Hospital, Dhaka during the period of July 2006 to June 2007. The study was carried out with the patients who visited regularly in Dhaka Shishu Hospital Thalassaemia Centre (DSHTC) and have multiple transfusions (more than five) and the age more than 2 years. To compare the state of liver function with normal healthy individuals' normal healthy persons were also included. Total 70 subjects were included in this study. The study subjects were distributed into two groups, the group - A (cases) and group - B (healthy controls). Group - A consisted of 40 thalassaemia patients and group - B consisted of 30 healthy individuals. According to the major types of thalassaemia present in our country, group -A again divided into two, group - AI patients of beta-thalassaemia major included 12 patients and group - AII patients of Hemoglobin E beta-thalassaemia included 28 patients. Inclusion and exclusion criteria were considered. The inclusion criteria were a. Diagnosed case of thalassaemia major and hemoglobin E beta-thalassaemia who have transfused more than five times, b. Age: two years and above, c. Sex: both sexes. Exclusion criteria were a. Below two years, b. Other hemolytic disorders, c. Acute systemic illness and d. Hepatitis B or Hepatitis C positive patients. The patients who fulfill the inclusion and exclusion criteria were selected for the study. After taking informed written consent of all the subjects, necessary information were recorded in a pre-designed pre-tested structured questionnaire. The questionnaire included name, age, sex, blood

group, economic status, Hb electrophoresis report, age of diagnosis, number of transfusion taken, state and type of chelating therapy, Hepatitis B and Hepatitis C status, Hepatitis B vaccination status and dietary habit of patients. After that 5ml of venous blood sample was drawn in a sterile test tube aseptically by venipuncture with sterile disposable syringe. Blood sample was taken in a plain sterile test tube, and kept for half an hour for clotting and then centrifuged. Supernatant clear serum was taken in two separate test tubes for the estimation of serum ferritin and serum bilirubin of the patients. For serum bilirubin estimation special precaution was taken to prevent oxidation. Data were entered in IBM PC and SPSS software (version 11.5) used to analyze data. Data were expressed as mean ± SD. Value of the serum bilirubin compares to see the case and control by using independent sample "t" test. Difference between serum bilirubin and ferritin in two groups of thalassaemia is also compared by using the Student's "t" test. P value less than 0.05 was taken as level of significance. Serum bilirubin was correlated with serum ferritin and correlation was done by using Pearson's correlation test using the same SSPS programme.

Results

Total forty subjects of thalassaemia of which, patients of beta-thalassaemia major (group AI) were 12 and patients of hemoglobin E beta-thalassaemia (group AII) were 28. The observation and finding about the patients age, sex, blood group, economic status, Hb electrophoresis report, age of diagnosis, number of transfusion taken, state and type of chelating therapy, Hepatitis B and Hepatitis C status, Hepatitis B vaccination status and dietary habit has been observed. Prothrombin time and serum ferritin were expressed as mean ± SD. Healthy individual (n=30) were considered as the same age group. Their serum bilirubin was also expressed as mean ± SD.

Age - mean age of the 40 patients was 8.32 ±5.17 years, minimum age was 2years and maximum age was 20 years. Mean age of the 30 normal healthy individuals was 7.97 ±4.62 years, minimum and maximum age was same as cases. Mean difference among case and control was 0.36, p value was 0.76 (table - I). Sex - among the 40 patients 26 (65%) were male and 14(35%) were female. Among the 30 control 19 (66.3%) were male and 11(37.3%) were female. Blood group -among 40 patients 10 (25%) had A positive, 14 had B positive, 12 (30%) had O positive and 4 (10%) had AB positive blood group. Hemoglobin electrophoresis report - among 40 patients 12 (30%) had beta-thalassaemia major and 28 (70%) had hemoglobin E beta-thalassaemia. Chelating therapy - among the 40 patients 26 (65%) had taken chelating therapy and 14(35%) had not taken chelating therapy. Hepatitis B and Hepatitis C status - all of the 40 patients (100%) were Hepatitis B and Hepatitis C negative. Hepatitis B vaccination status - among the 40 patients 36 (90%) were completely vaccinated 4 (10%) were not vaccinated. Dietary habit - among the 40 patients 39 (97.5%) had followed the diet chart which was advised by the physicians for consumption of low iron content only 1 patient (2.5%) had not followed the dietary advice. Table - II shows different general characters distribution of the thalassaemia patients.

Serum Bilirubin : The mean of serum Bilirubin in group - A (case) and group - B (control) were (2.04 ±0.70) mg/dl and (0.67±0.15) mg/dl respectively. A statistically significant mean difference of bilirubin was found indicating group - A had higher bilirubin than group -B in p value <0.001 (table - III) the mean level of serum bilirubin in group - AI (beta - thalassaemia major) was (1.70±0.70)mg/dl and the mean of bilirubin in group AII (HbE beta - thalassaemia) was (2.18±0.66) mg/dl. A statistically significant mean difference of bilirubin was found indicating group -AI had lower bilirubin than group AII in p value <0.05 (table - IV). Peaeson's Correlation between serum bilirubin of patients in mg/dl and serum ferritin of patients in ng/ml had studied in two

ways as a whole correlation between bilirubin of all the patients in mg/dl and serum ferritin in ng/ml had r value -.26 was statistically insignificant at 0/05 level(table - V). on the other side correlation between serum bilirubin of the patient of beta - thalassaemia major in mg/dl and serum ferritin of same patients in ng/ml had r value -0.10 was statistically insignificant at the 0.05 level. And correlation between serum bilirubin of the patients of HbE beta - thalassaemia in mg/dl and serum ferritin of same patients in ng/ml had r value -0.16 was statistically insignificant at the level 0.05 (table - VI).

Serum Ferritin: Out of the 40 patients 10 patients (25%) had below 1001ng/ml serum ferritin, 6 patients (15%) had 1001 - 2000 ng/ml, 8 patients (20%) had 2001 - 3000ng/ml, 6 patients (15%) had 3001 - 4000ng/ml, and 10 patients (25%) had more than 4001 ng/ml serum ferritin (table - VII).Mean level of serum ferritin in the total was (2729.40 ± 1935.87) ng/ml, minimum level was 304ng/ml and maximum was 7256ng/ml. Mean level of serum ferritin in the patients of beta - thalassaemia major was (4098.67 ± 1598.63) ng/ml, minimum level was 1212ng/ml and maximum was 7560ng/ml. Mean level of serum ferritin in the patients of HbE beta - thalassaemia was (2232.57± 1598.63) ng/ml, minimum level was 304ng/ml and maximum was 630ng/ml. A statistically significant mean difference of ferritin was found indicating ferritin of group AI (patients of beta - thalassaemia major) had higher level of ferritin than group AII (patients of HbEbeta - thalassaemia) in p value < 0.05 (table - VIII).

Table-I: Mean age distribution of thalassaemia patients (case) and normal healthy individuals (control)

Variable	Group	N	Mean±SD	Mean difference	P
Age	Case	40	8.32±5.17	0.30	NS
	Control	30	7.97±4.62		0.76

*P value reached from independent sample stude*nt's "t" test. NS= not significant (p>0.05)

Table-II: Different general characters distribution of thalassaemia patients

Variables		N	%
Sex	Male	26	65
	Female	14	35
Blood group	A+ve	10	25
	B+ve	14	35
	O+ve	12	30
	AB+ve	04	10
Type of thalassaemias	β–thalassaemia major	12	30
	Hb E thalassaemia	28	70
State of chelation	Taken	26	65
	Not taken	14	35
Hepatitis B vaccination	Vaccinated	36	90
Dietary advice	Followed	39	97.5

Table-III: Level of serum Bilirubin in mg/dl in case and in control

Parameter	Group	N	Mean ±SD	Mean difference	t	p
Serum Bilirubin (mg/dl)	Case	40	2.04±0.70	1.36	10.46	<0.001
	Control	30	0.68±0.15			

P value reached from independent sample Student's "t" test. S= significant (p<0.001)

Table-IV: Level of serum Bilirubin in mg/dl in two types of thalassaemia patients

Parameter	Type of thalassaemia	N	Mean±SD	Mean difference	t	P
S Bilirubin (mg/dl)	β–thalassaemia major	12	1.7±0.70	0.48	2.08	0.04
	Hb-E β-thalassaemia	28	2.18±0.66			

P value reached from Independent sample student's "t" test. S = significant (p<0.05)

Table-V: Pearson's Correlation between serum Bilirubin of patient in mg/dl & serum Ferritin of patient in ng/ml

Correlation between	r	Interpretation
Serum Bilirubin in mg/dl & Serum Ferritin of patient in ng/ml	-.261	Weak negative correlation

r value -.26 was statistically insignificant at the 0.05 level

Table-VI: Showing Pearson's Correlation between serum Bilirubin in mg/dl & serum Ferritin of patient in ng/ml in β- thalassaemia major and Hb E β-thalassaemia

Type of thalassaemia	r value	Interpretation
β - thalassaemia	-0.10	No correlation
Hb E β - thalassaemia	-0.16	No correlation

No correlation at the 0.05 level

Table-VII: Distribution of serum Ferritin in thalassaemia patients

	Frequency	Percentage	Ferritin level in ng/ml
	10	25	< 1000
	6	15	1000 – 2000
	8	20	2001 -3000
	6	15	3001 -4000
	10	25	> 4000
Total	= 40	100	

Table-VIII: Difference in serum Ferritin level between two types of thalassaemia

S Ferritin level ng/ml	Type of thalassaemia	N	Mean ± SD	Mean difference	t	P
	β-thalassaemia major	12	4098.67 ± 2086.83	1866.0	3.08	.004
	Hb-E β-thalassaemia major	28	2232.57 ± 1598.62			

P value reached from Independent sample Student's "t" test. Significant (p < 0.05)

Discussion

Beta thalassaemia major and Hb-E Beta thalassaemia is common in our country. The thalassaemia patient develops liver fibrosis as a result of iron overload due to excessive blood transfusion and also from excess intestinal absorption. In recognizing the hepatotoxic potentials of iron over load the cross sectional comparative study had been under taken to find the state of liver function in the thalassaemia patients. In addition serum ferritin level also had been determined in the patients to find the relation of serum ferritin with liver function test.

In this study 30% patients had beta - thalassaemia major and 70% patients had Hb-Ebeta - thalassaemia, this finding showed second group of patients were more than double of first one. This finding was not in accordance with Purnamawati SP et al and Daniel et al19. They had studied on more number of beta - thalassaemia major patients. Chelation therapy was taken 65% and 35% was not taken chelation therapy. This finding revealed that about two third of the patients were alert about their iron overload and were taking the proper management, this finding also is not consisted with Purnawati SP et al19. They were studied on irregularly chelated patients. Hepatitis B and Hepatitis C patients were 100% negative, actually during collection of sample from the patients 1 Hepatitis C positive patient was found he had excluded from the study in order to avoid possibility of undue influence on liver function. Regarding Hepatitis B vaccination 90% were completely vaccinated 10% were not vaccinated. This finding pointed that most of the patients were conscious that they might be infected through blood transfusion and had taken necessary action. The diet chart which was advised by the physicians for consumption of low iron content had followed 97.5% and had not followed only 2.5%. This finding again revealed that the patients and their parents were very much concern about their disease and as per direction of the physicians they have taken proper steps.

The mean level of serum bilirubin in patient of thalassaemia, and normal healthy children were (2.04 ±0.70) mg/dl and (0.67 ±0.15) mg/dl respectively. The total mean bilirubin was (1.46±0.86). a statistically signifi98cant mean difference of serum bilirubin was found indicating patient of thalassaemia had higher bilirubin than normal healthy children in p value < 0.001 level. Serum bilirubin may increase in thalassaemia due to haemolysis of red blood cells. The mean level of serum bilirubin in patient of beta - thalassaemia major was (1.70 ±0.70) mg/dl and the same level of bilirubin in patient of Hb-Ebeta thalassaemia was (2.18 ±0.66) mg/dl. A statistically significant mean difference of serum bilirubin was found indicating beta - thalassaemia major had lower bilirubin than patient of HbE beta - thalassaemia in p value <0.05 level. This finding pointed that haemolysis was occurring more in our study subjects of HbE beta - thalassaemia than beta - thalassaemia major. Peaeson's Correlation between serum bilirubin of patients in mg/dl and serum ferritin of patients in ng/ml had studied in two ways as a whole correlation between bilirubin of all the patients in mg/dl and serum ferritin in ng/ml had r value -.26 was statistically insignificant at 0/05 level. On the

other side correlation between serum bilirubin of the patient of beta - thalassaemia major in mg/dl and serum ferritin of same patients in ng/ml had r value -0.10 was statistically insignificant at the 0.05 level. And correlation between serum bilirubin of the patients of HbE beta - thalassaemia in mg/dl and serum ferritin of same patients in ng/ml had r value -0.16 was statistically insignificant at the level 0.05. this finding indicates there is no correlation at all between serum ferritin level and bilirubin in thalassaemia patients.

Iron over load and jaundice are common finding of thalassaemia. In this present study serum ferritin and serum bilirubin parameter of iron over load and jaundice are correlated. But no statistical correlation was found between these two parameters.

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