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ORIGINAL ARTICLE

Assessment of Iron Status in Hemoglobin E and β Thalassemia Carriers

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Abstract

Background: *About 10-13% people of Bangladesh are carrier of HbE and β-thalassaemia. Many program have been taken by Government and NGOs for supplementation of iron to raise hemoglobin level of children which may not be beneficial or might be harmful to the carriers of this disease.*

Objectives: *of the study was to assess the iron status of Hemoglobin E and β thalassemia carriers thereby to develop iron supplementation strategy for these carriers.*

Methods: *This cross sectional analytic study was on 206 carriers of Hemoglobin E and β thalassemia and 54 healthy controls. Complete blood count with RBC indices, Hemoglobin (Hb) electrophoresis and serum ferritin, serum iron and TIBC were carried out for all subjects following standard protocol. Data were analyzed by Statistical Package for Social Science (SPSS) Version 12.*

Results*: Among 260 subjects 206 were carriers and 54 were control. Number of male was 137 and female was 123 and male to female ratio was 1.1:1. Age of the subjects ranges from 1 year to 59 years with a mean age (±SEM) of 23.07± 0.84 years. Mean age (±SEM) of cases was 22.78 ± 0.971 years and that of control was 24.17 ± 1.63 years. Hematological parameters such as mean (±SEM) Hb concentration, MCV, MCH, and MCHC of carriers were significantly low as compared to the control (p value < 0.01 in all comparison). Mean serum ferritin and iron level in carriers were higher than control however; statistical significance between the values were not found (p value >0.5). Out of 206 carriers 27 (13.1%) cases had IDA and it's frequency was similar among HbE (14.2%) and thalassemia carriers (12.2%) and prevalence of IDA among the carriers were high (37.5 %) in age group 1-5 years which rises to 52.5% in under nourished children.*

Conclusion: *Carriers of HbE and β thalassemia do have relatively higher iron profile as compared to control but not statistically significant. Iron deficiency anemia is not uncommon in carriers especially in children. So there is no contraindication of iron supplementation to the children in general.*

Keywords: *Iron status, hemoglobin E, β thalassemia carriers.*

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Introduction

Thalassemia and hemoglobinopathies are the most common inherited disorder in human and they represent a growing major public health problem in many countries of the world.[1,2] World Health Organization (WHO) estimates that 7% of world populations have hemoglobin disorders and each year 300000-400000 babies are born with severe disease.[1] Among the hemoglobinopathies, HbE is the most common abnormal hemoglobin in South East Asia reaching a carrier state of about 60%.[3,4] It is caused by substitution of glutamic acid by lysine at codon 26 of the â globin chain.[5] It is prevalent in Indian subcontinent including India, Pakistan, Bangladesh and Srilanka.[2,6] In heterozygous state â thalassemia and heterozygous state of HbE and homozygous HbE result in hypochromic microcytosis with minimal anemia.[7-9] The interaction between β thalassemia and HbE results in HbE- β thalassemia having thalassemic phenotype ranging from a condition indistinguisable from ß thalassemi major to a milder form of ß thalassemi intermedia.[10] HbE-â thalassemia is the most serious form of HbE syndromes affecting a million of people worldwide and it is found to be the commonest congenital hemolytic anemia in Bangladesh.[6,11]

In Bangladesh, two separate hospital based study done one in Dhaka Shishu Hospital on 100 samples and another in Pediatric Hematology and Oncology dept of Bangabandhu Sheikh Mujib Medical University on 300 sample showed HbE carrier status of 10-12% and β thalassemia carrier status of 1-2.3%.[12,13] The results indicate that large number of people is carrier of HbE and â thalassemia in our country. These people are having lower concentration of hemoglobin with an otherwise normal physical appearance.[14-16] Sometimes, early laboratory investigation like CBC and blood film shows indistinguishable report from iron deficiency anemia.[17,18] and these patients are regularly being prescribed with Iron supplements for correction of anemia, which might cause iatrogenic iron overload in these carriers. Moreover, many Government and NGO's are running programs for supplementing iron to raise hemoglobin routinely to the children and pregnant mothers which may not help these carriers rather might do harm for them.

One of the major problems of ß thalassemia is iron overload, which causes morbidity and mortality due to dysfunction of organ and tissue because of transfusion, ineffective erythropoiesis and enhanced intestinal absorption. Morbidity from iron overload in non-transfused patients secondary to increased gastrointestinal absorption is also common.[14] So assessment of iron status is necessary for these carriers.

Iron absorption has been studied by different worker with variable result. Bannerman et al[19] found no difference in individual in regard to iron absorption in ß thalassemia trait on the other hand; Crossby et al[20] reported increased iron absorption in individual with heterozygous ß thalassemia. Vichinsky et al[6] also described about increased iron absorption in heterozygous HbE. Variable degree of increased iron absorption is expected in ß thalassemia trait because ineffective erythropoiesis is one of the factors that stimulate iron absorption from gastrointestinal tract.[21] Variable iron statuses in ß thalassemia trait are reported by many workers in different ethnic groups and in different settinngs.[22,23] In our country frequency of coincident of iron deficiency and ß thalassemia trait has been assessed in a small sample however assessment in regard to HbE has not been done.[16] So, this study has been designed to see the iron status of the carriers of HbE and ß thalassemia with a lager sample.

Materials and Methods

This **c**ross sectional analytic study has been carried out in department of Pediatric Hematology and Oncology, Bangabandhu Sheikh Mujib Medical University from May 2009 to June 2010. A total 300 cases were recruited from volunteers who wanted to know their ß thalassemia/Hb E carrier status, siblings, parents and relatives of diagnosed thalassemia syndrome of aged 1 year and more. Patients of known thalassemia syndrome, one who received blood transfusion within three months due to any cause or taking iron containing drugs or suffering from acute or chronic diseases and pregnant women were excluded from the study.

After getting informed written/verbal consent a short history regarding age, sex, family history, blood transfusion, food habit, iron therapy or any acute and chronic illness were taken. A thorough physical survey including measurement of height and weight was also made before primary inclusion. Findings of the history and physical survey were recoded in pretest questionnaire. From each case 4 ml blood

was drawn from ante cubital vein in 5ml syringe. Two ml sample was kept in apendorfs containing 100 µl EDTA (ethylene di amino tetra acetic acid) and 2 ml was kept in plain test tube without anticoagulant from which serum was separated after centrifugation and stored at -80^0 C for serum ferritin, serum iron and TIBC assay. From first sample hematological analysis was done on the day of collection and rest of the sample was then stored at 4^{0}C for Hb electrophoresis. Peripheral blood film was prepared during collection of the sample.

Hematologic analysis was done by Hematology analyzer (XT 800i, Fluorescence Flow cytometry 5 part differential, 40 parameters analyzer, Manufacturer Sysmex Corporation, Country of origin Japan). The reports were checked manually by observing Leishman stained peripheral blood smears.

Hemoglobin electrophoresis was done on each sample to see the variants of hemoglobin within 7 days of collection by Beckman Coulter (made in USA) Paragon^R Hemoglobin (Hb) electrophoresis system KIT (P/N441780) by Hydra Gel (agar gel) electrophoresis in alkaline media following manufacturer's instruction. Serum ferritin was assayed by Abbott A$_X$SYM system analyzer using Microparticle Enzyme Immunoassay (MEIA) technology and serum iron and TIBC were assayed by Dade behring Clinical chemistry analyzer from Biochemistry department of BSMMU, Dhaka.

HbE carrier and disease were considered with concentration of HbA_2+E >17% and <50% and HbA2+E >85% respectively irrespective of RBC indices[24] b thalassemia carrier was considered when HbA_2 level ³3.5% and <6.8%[24,25] irrespective of RBC indices and Hb concentration. Iron deficiency anemia (IDA) was considered when serum ferritin level <12 µg/L.[26]

Data were entered and edited and analyzed by Statistical Package for Social Science (SPSS) Version 12. Mean value with standard deviation of all carriers and control of hemoglobin MCV, MCH, MCHC and RDW; serum ferritin, serum iron, TIBC were calculated. Statistical significance was determined by Student's t test. A p value of <.05 was considered as minimal level of significance.

Results

Among 300 subjects recruited initially, after automated analysis of blood and electrophoresis, 40 cases were excluded and finally 260 cases were analyzed. Out of excluded 40 subjects, 21 had microcytic red cell with normal or low HbA2; 13 subjects had abnormal hemoglobin other than HbE (HbD carrier- 4, HbD disease- 1, Fast moving band-2, Hereditary Persistence of Fetal Hemoglobin (HPFH)- 4) and 5 subjects had microcytic red cell with borderline raised HbA_2 >0.3%.

Table I shows age distribution of subjects in which 18 % was in 1-5 yrs age group, 13 % was in 6-18 years group and 69 % was in the age group of more than 18 years. Age of the subjects ranges from 1 year to 59 years with a mean (±SEM) age of 23.07 ± 0. 84 years. Mean (±SEM) age of cases was 22.78± 0. 971 years and that of control was 24.17 ± 1.63 years. There is no statistical significant difference between the mean ages of the two groups (p value 0.46).

Table I
Age distribution of cases and control (N=260)

Age group (year)	Number	Percentage
1-5	46	17.6
6-18	35	13.4
>18	179	69.0

Table II shows nutritional status of the cases and control. Under nutrition was defined in age group 1-5 years by weight /age of <3rd centile of NCHS mean, in age group 6-18 years by BMI (Body Mass Index) of <5th centile for age and sex and in age group >18 years by BMI <18.5. Overall rate of under nutrition was 20%. In the age group of 1-5 years, frequency of under nutrition was very high (39.1%).

Table II
Nutritional status of the cases and control

Cases and control	Under nourished		Well nourished	
	Number	%	Number	%
All subjects (n=260)	52	20.0	208	80.0
All cases (n=206)	48	23.3	158	76.7
1-5 yrs (n=46)	18	39.1	28	60.9
6-18 years (n=35)	7	20.0	28	80.0
>18 years (n=179)	27	15.0	152	85.0

Among 260 subjects, 137 (52.7%) were male and 123 (47.3%) were female with a male to female ratio of 1:11 (Fig. 1). Most of the cases (41%) came from Dhaka division followed by 27% from Chittagang, 13% from Barishal, 11% from Rajshahi division, 6% from Khulna, and 2% from Sylhet division (Fig. 2).

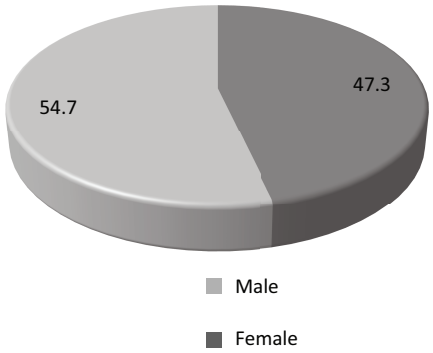

Fig 1 *Sex distribution of the subjects (n=260)*

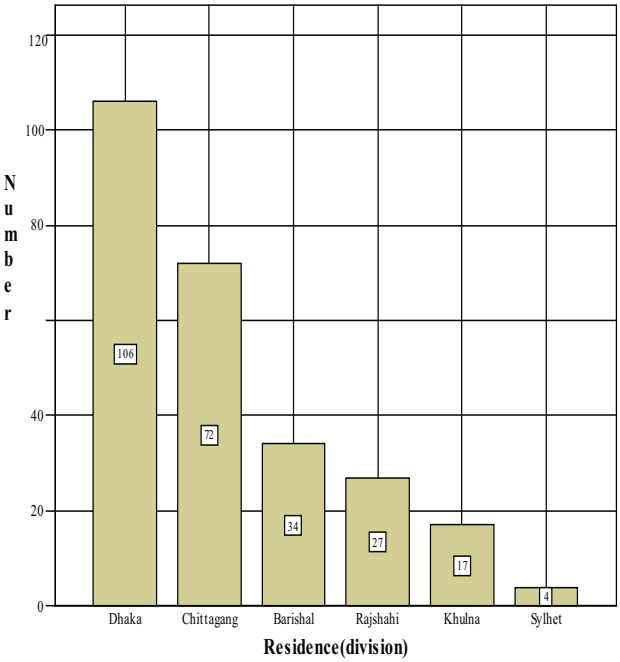

Fig 2 *Demographic distribution of subjects (n=260)*

Among 260 subjects, 206 were carriers of â thalassemia and HbE (HbE also included) and 54 were control. Out of 206 cases 114 cases were â thalassemia carriers of which 14 cases had IDA and 92 cases were HbE carriers and disease of which 13 cases had IDA (Fig. 3).

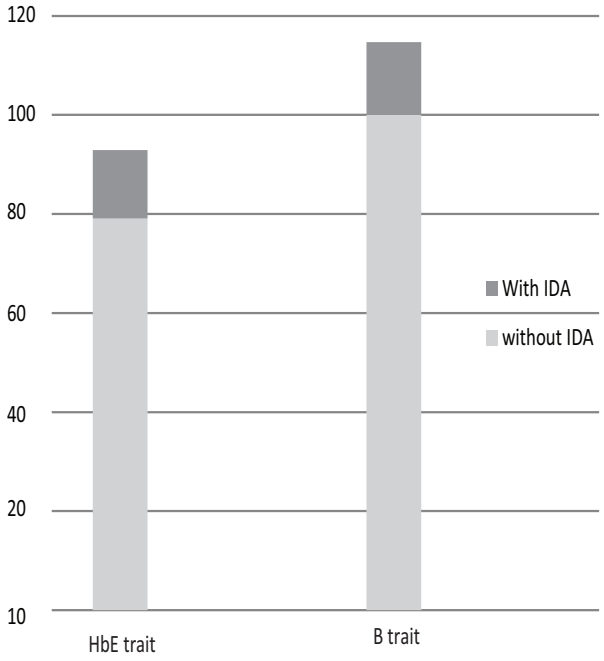

Fig 3 *Category of subjects according to case definition*

Mean (mean± SEM) Hb concentration of control was 12.93±0.23 gm/dl, in HbE carrier it was 10.73±3.49 gm /dl and in ß thalassemia carrier, it was 9.93±0.14 gm/dl. As compared to the control reduction of Hb level was statistically significant in carrier of both ß thalassemia and HbE (p value 0.00). Highest level of mean (mean± SEM) MCV was observed in control (86.6 ± 0.65 fl) and lowest level was found in ß thalassemia carrier (61.81± 0.52fl) and in HbE carrier it was 70.2±0.86 fl. Reduction of MCV observed both in HbE carrier and ß thalassemia carrier as compared to control was highly significant (p value 0.00 & 0.00). Mean value of MCH (mean±SEM) highest in 'Control' (27.13±0.33 pg) and lowest level was observed in ß thalassemia carrier (18.09±0.18 pg), level in Hb E carrier was in intermediate place (21.04±0.30 pg). MCH was also found significantly low both in HbE carrier and ß thalassemia carrier as compared to control (p value 0.001 & 0.00). Mean (mean±SEM) MCHC of HbE carrier was 29.64n±n0.21 gm/dl, 29.18±0.12 gm/dl in â thalassemia carrier, 31.05 ±0.23 gm/dl in control. The values found in carriers were significantly low as compared to the values of control ($p < 0.01$) (Table III).

Table III
Hematological and biochemical parameter of cases and control (N=260)

Parameter	Control	HbE carrier	β thalassemia carrier	t -test(df)	p value
Hb conc	12.93 ±0.23	10.74± 0.21	-	6.8(126.9)	0.00
(mean± SEM)(gm/dl)		-	9.93±0.14	10.88(93.14)	0.00
MCV	86.6±0.65	70.20 ±0.86	-	15.1(143.9)	.000*
(mean± SEM)(fl)		-	61.81±.52	28.0 (68)	.000*
MCH	27.13±0.33	21.04±0.30	-	13.4(27.7)	.001*
(mean± SEM)(pg)		-	18.09 ±0.18	23.59(80)	.000*
MCHC	31.05±0.23	29.64± 0.21	-	4.44(129.8)	0.00
(mean± SEM)		-	29.18± .12	7.1(86.6)	.000
RDW	13.2±.0.12	15.8±.0.29	-	8.0(117.48)	.000
(mean± SEM)		-	17.06±0.20	16.1(164.5)	.000
Serum ferritin	58.45±.6.91	61.41.6.13	-	0.32(124.3)	0.74
(mean± SEM)µg/L		-	67.13±.7.9	82.6(156)	.041
Serum iron	66.36±.2.78	67.49.2.57	-	29(27.7)	0.76
(mean± SEM) µg/dl		-	72.86±.2.89	1.61(149)	0.10
TIBC	373.40±.11.6	354.03±.8.6	-	1.34(143)	0.18
(mean± SEM)µg/dl		-	368±.11.79	.32(166)	0.74

Mean (mean±SEM) serum ferritin value of the cases was 64.58 ±5.15 µg/L (range 0.38- 614.08µg/L), of HbE carrier 61.41±6.13 µg/L, of thalassemia carrier 67.13±7.9 µg/L and of control 58.45±6.91µg/L. Statistical significant difference between the mean values observed in cases and control was not found (p value 0.74 , 0.41). Mean serum iron level in cases was 70.46±1.9 µg/dl (range 15-188 µg/dl) and in control 66.36±2.7 µg/dl (range 171-646 µg/dl) respectively, means TIBC level of cases and control were 361.84±7.6 µg/dl and 373.40±11.7 µg/dl respectively. No statistical significant difference observed for value of serum iron and TIBC among the cases and control (p >0.10 in each comparison) (Fig. 4).

Iron status of the subjects was evaluated further based on serum ferritin (Table IV). Out of 206 carriers 27 (13.1%) had serum ferritin level <12 µg/L and were diagnosed as IDA. So prevalence of IDA among the cases was 13.1%. One hundred seventy six (85.4%) of cases had normal serum ferritin and only 3 (1.5%) carriers showed iron excess but none of cases had serum ferritin >1000 µg/L. Out of 92 HbE carriers 13 (14.1%) had IDA and 14 (12.2%) cases among 114 ß thalassemia carriers. On the other hand only 2 of the 54 (3.7%) controls had low serum ferritin. In the age group 1-5 years, 37.5% cases were IDA on the other hand; only 10% and 6.6% cases with IDA were found in the age group of 6-18 years and more than 18 years respectively.

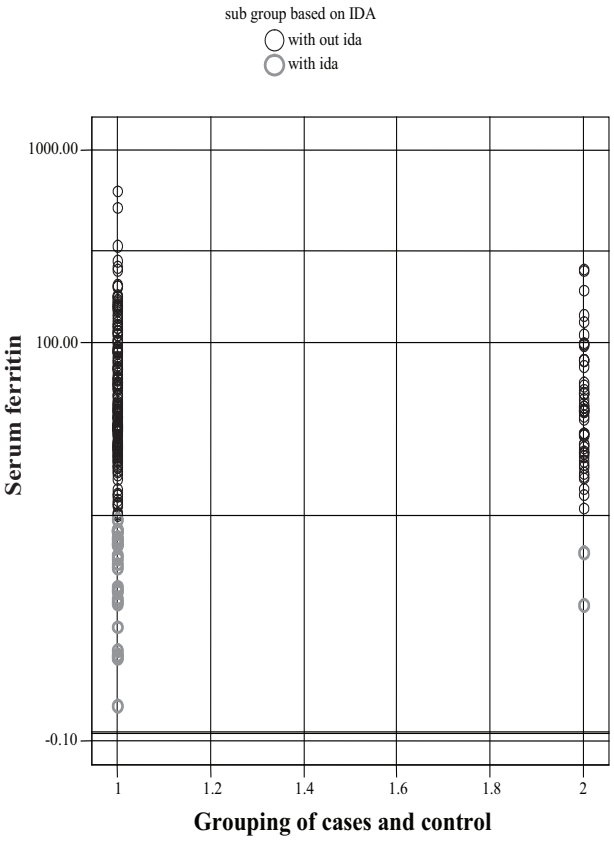

Fig 4 *Serum ferritin level of the study subjects (n=260)*

Table IV
IDA status of carriers in different age groups (N=206)

Age groups	HbE carriers		β thalassemia carriers	
	With IDA	Total numbers	With IDA	Total numbers
1-5 years	8	21	7	19
6-18 years	2	12	1	17
>18 years	3	59	6	78
Total	13	92	14	114

Carriers of HbE and ß thalassemia had significantly low mean Hb concentration, MCV, MCH, MCHC and serum iron and higher level of serum TIBC and RDW (p <0.001 for each comparison) (Table-V).

Table V
Comparison of mean hematological and biochemical parameters of carriers with and without IDA (N=260)

	Cases based on IDA	N	Mean	Std. Error Mean	t	df	p value
Hb concentration of cases	Without IDA	179	10.5417	.1259	5.422	204	.000
	With IDA	27	8.6226	.3663			
MCV of cases	Without IDA	179	66.3442	.5790	3.671	204	.002
	With IDA	27	60.3815	1.663			
MCH of cases	Without IDA	179	19.7975	.1944	5.309	204	.000
	With IDA	27	16.8704	.5972			
MCHC level of cases	With out IDA	179	29.6381	.1152	4.573	30.53	.000
	With IDA	27	27.7481	.3969			
RDW of cases of cases	With out IDA	178	16.138	.1609	-4.665	28.43	.000
	With IDA	26	19.138	.6228			
Serum ferritin level of cases	With out IDA	179	73.4044	5.6458	11.84	182.47	.000
	With IDA	27	6.1111	.64559			
Serum iron level of cases	With out IDA	179	73.4021	2.0785	4.392	37.15	.000
	With IDA	27	50.9900	4.6605			
TIBC of cases	With out IDA	178	348.6047	7.3847	3.753	30.42	.001
	With IDA	27	449.1333	25.745			

Frequency of IDA was 52.9 % in 1-5 years group, 33% in 6-18 years group and 8% in 18 years and more (Table VI).

Table VI
Frequency of IDA in under nourished carriers of different age groups (N=260)

Nutritional status			Sub group based on IDA		Total
			With out IDA	With IDA	
Normal	Age group	1 year - 5 yrs	17	6 (26%)	23
		6-17 yrs	22	1 (4%)	23
		≥18 yrs	105	7 (6.2%)	112
		Total	144	14(8.9%)	158
Under nourished	Age group	1 year -5yrs	8	9 (52.9%)	17
		6-17 yrs	4	2 (33.3%)	6
		≥18 yrs	23	2 (8%)	25
		Total	35	13 (27%)	48

Discussion

Iron status was assessed in carriers of HbE and â thalassemia and the result was compared with that of normal Control. Prevalence of iron deficiency and iron excess were also measured. There were 137 male and 123 were female and male to female ratio was 1.1:1. Ages of the subjects range from 1 year to 59 years with a mean age (±SEM) of 23.06±13.54 years. Mean age of the cases and control was 22.78±13.93 years and was comparable with that of control (24.17 yrs ±12.0) (p 0.46.) Participants were from all corners of the country, with the highest number from Dhaka division possibly due to the location of the study center in the middle of Dhaka division followed by Chittagang, Barishal, Rajshahi, Khulna and Sylhet division respectively.

Hematological parameters (Mean Hb concentration, MCV, MCH, MCHC) of the cases in the present series were lower than those of control and were statistically significant (p <0.001 for each comparison). Mean Hb concentrations of the cases were significantly lower than that of control. This finding was comparable with the findings of other authors.[19,27] Mean MCH was lowest in ß thalassemia trait and also low in HbE trait in the present series. Reduction of MCH found both in HbE and ß thalassemia traits was statistically significant as compared to that of Control (p <0.001 and <0.001). This finding was comparable with other observation.[6,28] Modell et al[29] described that MCH level in ß thalassemi carriers had become low because of defective production of globin chain and it usually had varied from 18-25pg in different thalassemic trait. Mannan et al[13] found lower level of MCH in ß thalassemia and Hb E traits, which was comparable to the finding of the present study. Mean MCV was found lowest in ß thalassemia trait and lower in HbE trait. Reduction of MCV level in both the traits was significant as compared to normal control (p <0.001 and <0.001). Lower levels of MCV reported by Katsanis et al[5], Vichinsky et al[6], Mannan et al[13] were comparable with our finding. Modell et al[29] described that the reduction of MCV in thalassemic red cells is due to reduced MCH that reduces intracellular oncotic pressure and fluid volume. Mean MCHC level observed both in HbE trait and ß thalassemi trait were also significantly low as compared to the level of the control (p <0.05). Other authors made different observation.[28,30] Mean RDW (±SEM) value of the cases (15.84±0.16%) were significantly high as compared to control (13.2±0.12%) and the level was much higher when associated with IDA (16.130.16% vs. 19.13±0.62 %).

Mean serum ferritin and serum iron of the cases were higher than the value observed in control but there was no statistical significant difference between the mean values (p >0.05). On the other hand, mean serum TIBC though lower than the value observed in the control, there was no statistical significance (p >0.05). So the results of the iron profile signify that iron statuses of the carriers were normal. Hussein et al[22] observed that patients with uncomplicated ß thalassemia trait in general have normal iron store. Bannerman et al[19] also stated that iron absorption was normal in ß thalassemia trait. White et al[31] and Mehta et al[23] made different observation that in ß thalassemia trait, ferritin concentrations was higher with lower incidence of iron deficiency anemia. Mehta et al[23] described that ß thalassemia traits had better iron nutrition suggesting these carriers had an advantage of maintaining good iron balance.

In the present series 13.1% of carriers showed iron deficiency anemia in addition to their existing carrier state. Prevalence was higher in the younger age group and gradual decreasing in older age group. In the age group 1-5 years the prevalence of IDA was much higher 37.51%. In the age group 5-18 years 10% of the carriers had IDA but only 6.6% had IDA in the age group of >18 yrs. When under nutrition has been taken in consideration, frequency of IDA was 52.9% among the 1-5 years age group, 33% among 6-18 years group and only 8 % in >18 years age group. Nutritional surveys conducted by Institute of Nutrition and Food Science of Dhaka University reported 65 -80% anemia in children <15 years which is higher than frequency found in our subjects.[32,33] As under nutrition affects mostly children that contributes the development of anemia in carriers also. So, advantage for protection to the development of anemia in the carriers of ß thalassemia and HbE may not be significant. Hinchliffe et al[34] had also similar observation. They had concluded 'clinical iron deficiency occurs with very high frequency in children under 5 years of age with ß thalassemia trait and advantage of iron supply is trivial in this context'. Ehrardt et al[35] also reported a figure of 48.5% prevalence of IDA in young children with ß thalassemia trait.

Only 3 (1.5%) of the carriers in this series had serum ferritin higher than reference value, however their level had not exceeded to the 1000 µg/L and none of them were in the children group and all of them were in well nourished group. Ineffective erythropoiesis with a variable spectrum observed in ß thalassemia trait might explain the possible role in increased iron absorption[22] however possibility of associated other disease of altered iron metabolism that was not evaluated here might be considered in this connection. Little data is available regarding iron status of HbE carriers in home and abroad to compare. However in our series HbE carriers behave like those of ß thalassemia carriers.

Conclusion

Carriers of HbE and â thalassemia do have relatively higher iron profile as compared to control though statistically not significant. A few carriers had high ferritin level than normal reference value but it had not crossed toxic level. Iron deficiency anemia is not uncommon in carriers especially in children and the prevalence is higher in children especially in those associated with under nourishment signifying that protection of iron deficiency by the carriers are not significant. So there is no contra indication of iron supplementation to the children in general.

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