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Relationship of Thyroid Hormone Status with Iron Overload in the Beta-Thalassemic Tribal Children Receiving Multiple Blood Transfusions- A Cross-Sectional Descriptive Study

Suman Chatterjee[1], Phalguni Chakrabarti[2*], Pinaki Sarkar[3]

[1]Demonstrator, Department of Biochemistry, Purulia Govt. Medical College, Purulia, West Bengal, India
[2]Assistant Professor, Department of Biochemistry, B.S. Medical College, Bankura, West Bengal, India
[3]Professor and H.O.D., Department of Biochemistry, B.S. Medical College, Bankura, West Bengal, India

***Corresponding author:** Phalguni Chakrabarti
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Abstract | **Original Research Article**

Thalassemia, a major public health problem in India, especially in tribal populations, is an autosomal recessive inherited blood disorder in which blood transfusion is the mainstay of treatment which in turn may lead to iron-induced injury in the heart, liver, pancreas and endocrine system. The Paediatric tribal population receiving multiple blood transfusions for treatment of β-thalassemia had been studied for evaluating the relationship between iron overload with thyroid dysfunction. Information pertaining to demographics, blood transfusion and the nature of chelation therapy etc. was obtained on one hundred patients, attending Bankura Sammilani Medical College, Bankura, and receiving multiple blood transfusions. Blood samples were analyzed for assessment of serum Ferritin, TSH, fT3 and fT4 concentrations by ELISA and serum iron by auto-analyzer and compared with age and sex matched one hundred healthy comparison group. The study group was found to have higher average serum ferritin, iron and TSH levels but lower fT3 and fT4 levels compared to that of the comparison group but and the difference was statistically significant. There were positive correlations between serum TSH and serum ferritin while TSH had no relation with serum iron. Serum ferritin also possessed positive correlation with serum iron. Serum fT3 and fT4 had no relation with serum ferritin and iron. And it was also found that subclinical hypothyroidism was more than overt hypothyroidism among β-thalassemia major patients and female patients affected much in hypothyroidism than the males.

Keywords: Beta- thalassemia, blood transfusion, serum ferritin, iron, TSH, fT3, fT4.

INTRODUCTION

Thalassemia, one of the major public health problems in India, is an autosomal recessive in nature and also one of the most common monogenic disorders in the world [1]. In India, Hemoglobinopathies are commonest hereditary disorders and β-thalassemia is the commonest single-gene disorder [2].

Ten percent of the total world thalassemics are born in India every year [3]. Certain communities in India, like Sindhis, Gujratis, Punjabis, and Bengalis, are more commonly affected with β thalassemia, the incidence varying from 1 to 17% [4]. The tribal population of the country, as per 2011 census, is 10.43 crore, constituting 8.6% of the total population. 89.97% of them live in rural areas and 10.03% in urban areas. The decadal population growth of the tribal's from Census 2001 to 2011 has been 23.66% against the 17.69% of the entire population.

Literature review shows that thalassemia and other hemoglobinopathies are highly prevalent (0.028-18%) among the tribal communities in India making a public health problem among them [5].

The mainstay of treatment in β-thalassemia major is blood transfusion for maintaining the adequate levels of hemoglobin which ultimately causes iron overload resulting from multiple blood transfusions and increased iron absorption [6]. Excessive iron potentially catalyzes free-radicals generation and impairment in cellular function and integrity causing extensive iron-induced injury especially in the heart, liver, pancreas, and endocrine system [7]. Studies from different populations have reported evidence of various endocrine dysfunctions in β-thalassemia major patients with delayed puberty/ hypogonadism and short stature being the most frequent complications followed by hypoparathyroidism, hypothyroidism, and diabetes [8]. The reported thyroid dysfunction seen in patients with β-thalassemia major includes primary hypothyroidism,

subclinical hypothyroidism and also secondary hypothyroidism [9] and are mainly due to repeated blood transfusion and hypo activity of thyroid gland resulting from anemia and chronic hypo perfusion [10]. Primary hypothyroidism that may affect thalassemic patients from the second decade of life is mainly due to gland infiltration by iron overload. Autoimmune thyroiditis is generally absent [11]. Central hypothyroidism, caused by decreased secretion of TSH from the anterior pituitary gland or by decreased secretion of TRH from the hypothalamus, is less common. The thyroid gland appears to fail before the thyroid-pituitary axis, which is less sensitive than the gonadal axis to iron-induced damage [9]. A wide range of pathogenic mechanisms is involved causing thyroid dysfunctions. Tissue chronic hypoxia [12] and iron overload have a direct toxic effect on the thyroid gland. High concentrations of labile plasma iron and labile cell iron, responsible for the formation of free radicals and the production of reactive oxygen species (ROS), may lead to cell and organ damage [13].

A study conducted in Qatar by Soliman T. Ashraf showed that worsening of thyroid function was observed in 35% of the studied thalassemic patients by the age of 18 years. The lack of proper increase of TSH in response to the low circulating levels of fT4 in 76% of those patients indicated a relatively high incidence of defective pituitary thyrotrophic function in those patients [14]. A cross-sectional study conducted in Iran by Eshragi P *et al*. showed that 14.6% children of older than 10 years with thalassemia major received repeated blood transfusion had developed hypothyroidism [15]. A cross-sectional study done by Deniz T. *et al*. showed that all thyroid parameters in 90 patients were in the normal ranges compared with the controls except three of them which had high TSH levels but serum ferritin level in patients was significantly higher than in controls [16].

A study done by Habeb *et al*. in KSA reported that none of the patients had overt hypothyroidism, however, subclinical hypothyroidism was detected in 14.8% of 81cases [17].

The present study was undertaken to test the hypothesis that excessive iron overload is a precipitating factor for thyroid disorder in the β-thalassemia major tribal patients.

MATERIALS AND METHODS

Study area

This cross-sectional descriptive study was carried out in Department of Biochemistry and Thalassemia day care unit in the in-patient department of Dept. of Paediatric Medicine, B.S. Medical College, Bankura.

Study subject

Altogether 100 patients, admitted in the thalassemia day care unit in in-patient department of the Dept. of Paediatric Medicine, were considered in the study that was selected as per inclusion and exclusion criteria during the period of data collection. On an average 04 patients per week were selected. Equal number of age and sex matched subjects suffering from morbidity other than beta-thalassemia was also selected as comparison group from the Department of Biochemistry, B.S.Medical College, Bankura.

Inclusion criteria

- Age: 0-12 years,
- Sex: Both male & female patients according to convenience,
- Cast: Patients from tribal population of Bankura district,
- The patients of β-thalassemia major receiving repeated (at least 3 times) blood transfusions in which last blood transfusion was taken at least one month prior to blood collection

Exclusion criteria

- Diabetes Mellitus,
- Existing renal failure (serum creatinine >3 mg/dl),
- Any hematological or other malignancy,
- Jaundice,
- Known active infections,
- Severely malnourished individuals,
- Known chronic illness such as chronic kidney disease or chronic liver disease.

The test and the control subjects were screened using a detailed questionnaire, history and physical examination. They were physically examined and information pertaining to demographics, nature of illness was collected from them using a predesigned and pretested questionnaire. Duly signed informed consent was taken from parents. The study design was approved by institutional ethical committee.

Study Period

This study was carried out during the period from August, 2017 to January, 2018.

Laboratory investigations

The serum samples were collected from the 100 cases and 100 controls and stored at -20ºC before analyzing after doing centrifugation. Serum TSH, fT4, fT3 and ferritin levels were estimated by ELISA reader (Mindray MR 96A) with automated microplate washer (Mindray MW 12A) and serum iron levels were estimated by auto-analyzer (DS-302).

Statistical analysis

The data were compiled in MS excel and analyzed by different statistical methods. Data display was done by charts and tables. Data were described by proportion, mean, SD, range etc. Statistical tests like

independent "t" test, Spearman correlation coefficient (r) etc. were used to explore the relationship between variables. P value of <0.05 was considered significant to discard the null hypothesis at 5% precision and 95% confidence interval.

RESULTS

Out of 200 participants 132 were male and rest were female (Fig 1).

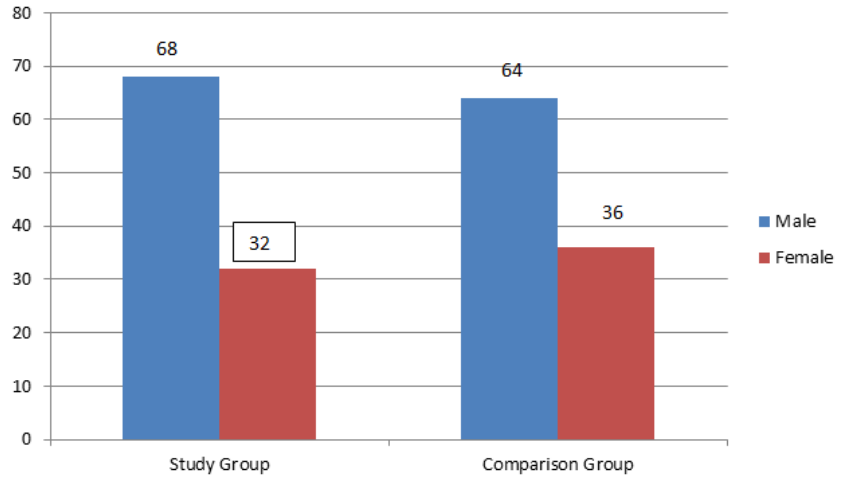

Fig-1: Distribution of study and comparison group according to sex

The members of 5-10 years age group were majority in both study group i.e. 38% and 42% in study group and comparison group respectively (table no-1).

The overall average was estimated to be 6.66 ± 3.20 (mean ± SD) years with median of 6 years and range of 11 years. Most subjects participated as case and control were belong to Santal community i.e. 94 out of 100 (94%) in case group and 86 out of 100 (86%) in control group.

Table-1: Distribution of subjects as per age groups (N=200)

Age group	Study group		Comparison group	
	Number.	Percentage	Number.	Percentage
Upto5 yrs	26	26.0	38	38.0
5-10 yrs	38	38.0	42	42.0
≥10 yrs	36	36.0	20	20.0
Total	100	100.0	100	100.0

The study group was found to have higher average serum ferritin, iron and TSH levels but lower fT3 and fT4 levels compared to that of the comparison group but and the difference was statistically robust (Table-2 & 3).

Table-2: Distribution of participants according to their serum thyroid parameters (N=200)

Parameter	Study group [n=100]		Comparison group [n=100]		Unpaired 't' test, p at df 98
	Mean	S D	Mean	S D	
Serum TSH	7.28	6.95	2.12	1.40	5.149,0.000
Serum fT3	1.43	1.00	1.99	0.44	3.671,0.000
Serum fT4	1.31	0.41	1.34	0.29	0.413, 0.681

Table-3: Distribution of participants according to their serum iron and ferritin status (N=200)

Parameter	Study group [n=100]		Comparison group [n=100]		Unpaired 't' test, p at df 98
	Mean	S D	Mean	S D	
Serum iron	181.78	29.81	79.58	16.42	21.232,0.000
Serum ferritin	472.97	271.20	75.19	36.03	10.281,0.000

Table-4: Distribution of participants of study group according to serum thyroid parameters and gender (n-100)

Parameter	Male		Female		Unpaired 't' test, p at df 98
	Mean	S D	Mean	S D	
Serum TSH	6.57	7.27	8.79	6.13	1.059,0.295
Serum fT3	1.60	1.05	1.06	0.81	1.805,0.077
Serum fT4	1.37	0.36	1.19	0.48	1.459, 0.151

There was no influence of gender on the serum level of thyroid profile among the participants belonged to the study group (Table 4).

The analysis reflected that gender had no role on the serum iron and ferritin.

Table-5: Distribution of participants in study group according to sex & serum iron status (n=100)

Parameter	Male		Female		Unpaired 't' test, p value at df 98
	Mean	S D	Mean	S D	
Serum iron	181.09	24.55	183.24	39.68	0.236, 0.814
Serum ferritin	471.17	261.84	476.82	299.01	0.068, 0.946

Table-6: Distribution of participants in study group of thyroid disorders according sex (n= 100)

Sex	Euthyroidism	Hypothyroidism		Hyperthyroidism	Total
		Subclinical	Overt		
Male	38	22(32.35%)	8(11.76%)	00	68
Female	12	8(25%)	12(37.50%)	00	32

Table no -6 showed that among 68 male beta-thalassemia patients 11.76% (8) were suffering from overt hypothyroidism while 32.35% (22) had subclinical hypothyroidism. But in female beta-thalassemics. 37.50% (12) were suffering from overt hypothyroidism while 25% (8) had subclinical hypothyroidism among 32 female patients. So, overall female beta-thalassemia patients (20 out of 32 patients) were affected much in hypothyroidism than the males (30 out of 68 patients). 20 patients out of 100 (20%) were suffering from overt hypothyroidism while 30 patients out of 100 (30%) had subclinical hypothyroidism.

Table-7: Correlation between variables in study group

Parameter	Significance (p value)
Serum ferritin Vs Serum iron	.000
Serum ferritin Vs Serum TSH	.015
Serum ferritin Vs Serum fT3	.875
Serum ferritin Vs Serum fT4	.450
Serum iron Vs Serum TSH	.057
Serum iron Vs Serum fT3	.615
Serum iron Vs Serum fT4	.275
Serum TSH Vs Serum fT3	.215
Serum TSH Vs Serum fT4	.030
Serum fT3 Vs Serum fT4	.002

Positive correlations between serum TSH and ferritin was established while no relation between TSH and serum iron was found. Positive correlations established between serum ferritin & serum iron. But,serum fT3 and fT4 had no relation with serum ferritin and iron (Table-7).

DISCUSSION

A big portion of population of Bankura, one of the big districts of West Bengal, is tribal. Study conducted by Mondal *et al.* at B.S.Medical College, West Bengal, taking 958 patients over 6 months of 2011 at the hospital clinic showed high prevalence of hemoglobinopathies (27.35%) where β-thalassaemia in heterozygous stated occurred more frequent than other hemoglobinopathies. Out of 958 patients, 72.65% were HbAA and 27.35% were hemoglobinopathies individuals where 17.64% β-thalassemia heterozygous, 2.92% β-thalassemia homozygous, 3.86% HbAE, 1.15% HbAS trait, 1.25% HbE-β thalassemia trait and 0.52% HbS- Beta thalassemia trait were found [18].

Iron overload in β-thalassemia major patients is secondary to multiple blood transfusions, the mainstay of treatment in β-thalassemia major, and increased iron absorption [6] and causes iron-induced injury develops in the heart, liver, pancreas, and endocrine system [7] by catalyzing free-radicals generations.

In the present study serum ferritin level was 472.97 ± 271.20 ng/ mL (mean ± SD) and serum iron level was 181.78 ± 29.81µg/ dL (mean ± SD) in the study group. In a study conducted by Joshi *et al*. [19] on the children with β-thalassemia major over the age of 4 years receiving regular blood transfusions, the serum ferritin level was much higher than our study. In Kurtoglu *et al*. [20], the serum ferritin level was also more than our study but the iron level was quite similar to our study. But in Abdul Hussein HK *et al*. [21], the serum ferritin level was 510.98±251.65 ng/ mL (mean ± SD) which was quite similar to our study. In our study, in cases TSH level was 7.28 ± 6.95 µIU / mL (mean ± SD), fT3 level was 1.42 ± 1.00 pg/ mL (mean ± SD) and fT4 level was 1.30 ± 0.41 ng/ dL (mean ± SD). In a study conducted by Abdul Hussein HK [21], the TSH level was 5.48±1.86 µIU/ mL (mean ± SD) and in Kurtoglu *et al*. [20], the TSH level was 4.73 ± 13.45µIU/ mL (mean ± SD) which was less than our TSH level in the children with β-thalassemia major. In the later study fT4 and fT3 levels were 0.67 ± 0.14 ng / dL (mean ± SD) and 2.86 ± 0.38 pg / mL (mean ± SD). In Najafipour *et al*. [22] TSH level was 19.58 ± 8.94 µIU/ mL (mean ± SD) which was more than our findings and fT4 level was 0.33 ± 0.96 ng / dL (mean ± SD).

By definition, when TSH value is in between 5.5 mIU/L and 10 mIU/L and fT4 within normal limit, the condition can be called subclinical hypothyroidism. When TSH is > 10 mIU/L and fT4 & fT3 are low i.e. fT4 is less than 0.8 ng/dL and fT3 is less than 1.4 pg/mL, condition can be called overt hypothyroidism. And when TSH is < 0.1 mIU/L or undetectable and fT4 is elevated, condition is called hyperthyroidism [23].

In the present study, it was observed that among 68 male beta-thalassemia patients 11.76% (8) were suffering from overt hypothyroidism while 32.35% (22) had subclinical hypothyroidism. But in female beta-thalassemia, it was reversed. 37.50% (12) were suffering from overt hypothyroidism while 25% (8) had subclinical hypothyroidism among 32 female patients. So, overall female beta-thalassemia patients (20 out of 32 patients) were affected much in hypothyroidism than the males (30 out of 68 patients). 20 patients out of 100 (20%) were suffering from overt hypothyroidism while 30 patients out of 100 (30%) had subclinical hypothyroidism. According to the study done by Kurtoglu *et al.* [20] on beta-thalassemia patients, nine (12.8%) had hypothyroidism out of 70 patients with thalassemia major. Habeb et al in KSA reported that none of the patients had overt hypothyroidism, however, subclinical hypothyroidism was detected in 14.8% of 81cases [17]. A study done by Gathwala G *et al*. [24] on beta-thalassemia patients of age group 3 – 13 years showed that 30% (15 out of 50 beta-thalassemia patients) were suffering from hypothyroidism among which 18% were compensated hypothyroid (9), 12% were uncompensated hypothyroid (6) and none was overt i.e. clinical hypothyroid.

CONCLUSION

Our study revealed that there were positive correlations between serum TSH and serum ferritin while TSH had no relation with serum iron. Serum ferritin also possessed positive correlation with serum iron. Serum fT3 and fT4 had no relation with serum ferritin, iron. And also revealed that subclinical hypothyroidism was more than overt hypothyroidism among β-thalassemia major patients and female patients affected much in hypothyroidism than the males. Thalassemia possesses a huge social and economic burden to the country. So, there should be a target for reducing this huge burden of the society.

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