Original Article

Effect of Long-Term Transfusion Therapy on the Glycometabolic Status and Pancreatic Beta Cell Function in Patients with Beta Thalassemia Major

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Abstract

Background: Diabetes mellitus is a major complication of iron overload in patients with beta thalassemia major. **Design:** This is a descriptive study conducted in a Tertiary Care Teaching Hospital to analyze beta cell function and insulin resistance, and their relation to iron overload status in beta thalassemia major. Fasting glucose, two-hour post load glucose, fasting insulin, alanine amino transaminase (ALT), and ferritin were used as outcome measures. The homeostatic model assessment (HOMA model) was used to calculate the beta cell function and insulin resistance index. **Results:** Of the 30 cases, 20% had impaired fasting glucose, 3.3% had impaired glucose tolerance, and none had diabetes. Fasting glucose was not significant between the cases and controls ($P = 0.113$). Fasting insulin ($P = 0.001$), ferritin ($P = 0.001$), and ALT ($P = 0.001$) levels were significantly high in the cases. Insulin resistance index was significantly higher in the cases ($P = 0.001$) as also the beta cell function ($P = 0.001$). With increase in age and the number of units transfused there is a decline in beta cell function, fasting insulin, and insulin resistance after attaining the maximum level. This suggests that initial insulin resistance is followed by insulin depletion due to loss of beta cell function, leading to diabetes mellitus. **Conclusion:** Impaired glucose tolerance (IGT) and insulin resistance precede the onset of insulin-dependent diabetes and adequate chelation therapy is essential for delaying the onset or for prevention of diabetes.

Keywords: Children, diabetes mellitus, insulin resistance, thalassemia major

Introduction

β thalassemia major (TM) or Cooley's anemia[1] is widespread throughout the Mediterranean region, Africa, Middle East, Indian subcontinent, and South East Asia.[2] In India, every year 10,000 children are born with thalassemia, contributing to 10% of the global burden and the frequency of TM ranges between 3 and 15% in the general population.[3] Iron overload of tissue, if not adequately treated, is the most important complication of TM and is the major focus of management. Inadequate or absence of chelating therapy leads to progressive dysfunction of the heart, liver, and endocrine glands.[4] Over the years iron deposition may lead to pulmonary hypertension[5] and osteopenia.[6] Diabetes mellitus is one of the major endocrine problems and prevalence of impaired glucose tolerance and diabetes varies from 8.5 to 12.2% and 5.4 to 19.5%, respectively.[7-10] It has been observed that iron chelation therapy delays the onset of diabetes.[11]

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Materials and Methods

This study was done to find out the incidence of abnormal glucose tolerance and diabetes in TM patients, who were on long-term transfusion, and also to provide an insight into the possible mechanism of occurrence of diabetes. Various parameters of glycemic indices were correlated with the indicators of iron overload, such as ferritin, and the total number of units transfused.

Patients with TM undergoing regular transfusions were recruited from the Department of Pediatrics in a Tertiary Care Teaching Hospital in Mangalore, from February 2007 to August 2008. The sample size was 40, with 30 cases of TM and 10 age- and sex-matched controls. Those eligible for this study included TM diagnosed by hemoglobin electrophoresis

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and on regular transfusion, of age between five and seventeen years, and absence of any other known endocrinological problem. Patients with a family history of diabetes in first-degree relatives, on treatment with drugs causing diabetes, and presence of chronic illness, were excluded from the study. Ten normal children between five and seventeen years of age, with no family history of TM, and absence of diabetes in first-degree relatives were included as controls. Informed consent from a parent and approval from the Institutional Ethics Committee were obtained prior to the study. A detailed history was taken, such as, age at diagnosis, age at first transfusion, total number of transfusions, compliance with transfusion, and details of chelation therapy. Physical examination including pallor, icterus, skin changes, liver and spleen size, and a systemic examination were undertaken meticulously and recorded in a proforma. The investigations undertaken were Fasting Plasma Glucose (FPG), two-hour post load glucose, fasting insulin (FI), ferritin, alanine amino transferase (ALT), and hemoglobin.

Fasting Plasma Glucose was done from a blood sample collected after overnight fasting of eight hours. An oral glucose load of 1.75 g/kg was then administered and a repeat sample collected after two hours, for two-hour post-load glucose. Glucose estimation was done by the glucose oxidase–peroxidase method. The sample was collected for fasting insulin at the same time along with FPG. The test was done using RIAK-1 (radio immunoassay kit for insulin) supplied by the Baba Atomic Research Center, Mumbai.

The American Diabetes Association (ADA) criterion 2007, was used for diagnosis of diabetes.[11]

- Fasting plasma glucose (FPG) < 100 mg/dl (5.6 mmol/l) = normal fasting glucose
- FPG 100-125 mg/dl (5.6-6.9 mmol/l) = impaired fasting glucose (IFG)
- FPG ≥ 126 mg/dl (7.0 mmol/l) = provisional diagnosis of diabetes

The corresponding categories when the oral glucose tolerance test (OGTT) is used are the following:

- Two-hour post-load glucose < 140 mg/dl (7.8 mmol/l) = normal glucose tolerance
- Two-hour post-load glucose 140-199 mg/dl (7.8-11.1 mmol/l) = impaired glucose tolerance (IGT)
- Two-hour post-load glucose ≥ 200 mg/dl (11.1 mmol/l) = provisional diagnosis of diabetes

Patients with IFG and/or IGT are now referred to as having 'pre-diabetes' indicating the relatively high risk for development of diabetes in these patients.

All cases and controls were evaluated for Beta cell function index and Insulin resistance index using Homeostasis Model Assessment (HOMA).[12]

$$\text{Beta cell function index} = \frac{20 \times \text{fasting plasma insulin (uU/mL)}}{\text{Fasting plasma glucose (mmol/L)} - 3.5}$$

$$\text{Insulin resistance index (IRI)} = \frac{\text{Fasting plasma insulin} \times \text{fasting plasma glucose}}{22.5}$$

All data obtained were analyzed statistically. Test of significance was done by applying The Students unpaired t-test between the cases and controls. A value of < 0.05 was considered significant. Pearson's correlation co-efficient (r) was employed to assess the strength of association as well as linear relation between the variables.

Results

All our cases were well-matched in age and sex with the controls. The mean age among the cases was 10.16 years and that of the controls was 10.10 years. Among the cases 46.7% were males and 53.3% were females. Among the controls both were 50% each. Age at first transfusion varied between three and eighteen months with the mean at 9.9 months. The mean number of units transfused was 133.4 units. All children were on chelation with deferiprone. Deferoxamine was not taken routinely, due to the higher cost involved; 73.3% had poor compliance with chelation therapy. Left ventricular dysfunction was found in one child who had stopped chelation therapy; 53.3% of the cases had characteristic clay-colored skin. The mean liver span was 12 cm and the mean splenic size was 4.3 cm. Two cases were splenectomized due to hypersplenism [Table 1].

Impaired fasting glucose was seen in six cases (20%). IGT was seen in one case (3.3%) according to the ADA criteria. None had diabetes. There was no significant difference in fasting glucose between the cases and controls ($P = 0.113$) and the mean glucose value was within normal limits in both groups.

A statistically significant difference was observed in fasting insulin ($P = 0.001$), beta cell function ($P = 0.001$), IRI ($P = 0.001$), ALT ($P = 0.001$), and ferritin ($P = 0.001$) when compared with the controls. Fasting glucose had a good correlation with the other glycemic indices, such as, fasting insulin ($r = 0.434$, $P = 0.017$), insulin resistance index ($r = 0.544$, $P = 0.002$), and beta cell function index ($r = 0.389$, $P = 0.034$). Fasting insulin was

Table 1: Patient characteristics (Mean values)

Parameter (units)	Cases	Controls
Liver span (cm)	12.067	
Spleen size (cm)	4.267	
Age at first transfusion (months)	9.9	
Number of units transfused	133.4	
Fasting insulin (uU/mL)	13.4813±6.5822	5.6150±2.2030
Fasting glucose (mg/dl)	90.8667±9.4318	87.10±6.190
ALT (U/L)	110.2±71.08	17.60±4.993
Ferritin (ng/mL)	5340.33±1685.786	70.973±17.3929
Beta cell function index	178.61±76.889	84.514±44.3024
Insulin resistance index	3.128±1.669	1.1246±0.4772

significantly higher in the cases compared to the controls (P = 0.001). It correlated well with other glycemic indices like fasting glucose (r = 0.434, P = 0.017), beta cell function (r = 0.614, P = 0.001), and IRI (r = 0.944, P = 0.001).

The insulin resistance index was higher in cases compared to controls and the difference was highly significant (P = 0.001). It correlated well with other glycemic indices like fasting glucose (r = 0.544, P = 0.002), fasting insulin (r = 0.944, P = 0.001), and beta cell function (r = 0.430, P = 0.018).

The beta cell function index was also significantly higher in the cases compared to the controls (P = 0.001). It correlated negatively with the fasting glucose. The beta cell function also correlated with other glycemic indices like fasting glucose (r = –0.389, P = 0.034), fasting insulin (r = 0.614, P = 0.001), and insulin resistance index (r = 0.0.430, P = 0.018). However, the glycemic indices such as fasting glucose, fasting insulin, beta cell function, and IRI did not correlate with the indicators of iron overload, such as, ferritin and number of transfusions. There was also no correlation between the glycemic indices and age. The number of transfusions received in the cases correlated with the age (r = 0.687, P = 0.001) and serum ferritin (r = 0.385, P = 0.036). No correlation was seen with the other parameters. Similarly ferritin, although significantly high in the cases (P = 0.001) did not correlate with the parameters of the glycemic indices [Tables 2 and 3].

An interesting observation was found when internal grouping was done with the total number of transfusions < 100, 100-150, and >150 units. There was a progressive increase in beta cell function, fasting insulin, and IRI with an increase in the number of units transfused, and age followed with a decline, as shown in the graphs. A similar relationship was obtained with increasing age when compared with the beta cell function, fasting insulin, and insulin resistance index [Figures 1 and 2].

Table 2: Correlation among fasting glucose, fasting insulin (FI), beta cell function, and insulin resistance index (IRI)

		FI	Beta cell function	IRI
f. glucose	r	0.434	–0.389	0.544
	p	0.017	0.034	0.002
f. insulin	r		0.614	0.944
	p		0.001	0.001
Beta-cell function	r			0.430
	p			0.018

Table 3: Correlation of the number of units transfused with age and ferritin

		Age	Ferritin
No. of transfusions	r	0.687	0.385
	P	0.001	0.036

Discussion

The mean age of thalassemic children in our study was 10.1 ± 2.38 years, with the age distribution ranging between five and seventeen years. Our study population had a relatively younger age group when compared to other studies, where the mean age among the cases were 14.8 ± 6.9 and 21.7 ± 1.2 years, respectively.[7,14] However, the mean age almost matched that of the study from India, which was 10.89 years.[15]

The size of the liver and spleen did not correlate with any of the parameters like age, ferritin, number of transfusions or glycemic indices, like fasting glucose, fasting insulin, beta cell function, and IRI. There was no significant difference in fasting glucose between the cases and controls of our study. However, a

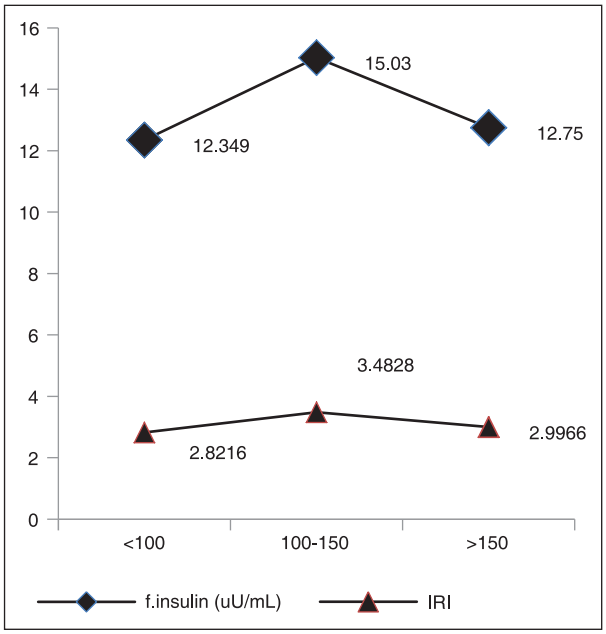

Figure 1: Number of units transfused with fasting insulin and IRI

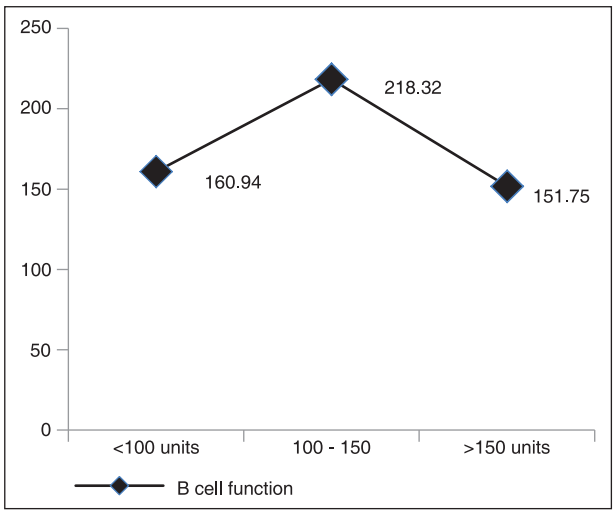

Figure 2: Number of units transfused with beta cell function

significant difference in the fasting glucose levels in thalassemic children ($P < 0.05$) was observed by others,[8] and this might be due to the inclusion of only post pubertal subjects, more than 18 years of age. They also demonstrated a progressive reduction of circulating insulin levels, which could lead to glucose intolerance. Our study population had a relatively younger age group similar to the study by Suvarna *et al*.[15] ALT was also significantly high in the cases ($P = 0.001$). Only three children had normal ALT. The mean ALT value among the cases was 110.20 ± 71.08. Features of chronic active hepatitis occurred with ALT values of 52.5 ± 19.7 and features of cirrhosis occurred with values of 106.7 ± 50.3, which needed confirmation by liver biopsy.[16] This liver dysfunction due to iron overload was one of the important pathogenetic mechanisms for abnormal glucose tolerance and diabetes. We did not evaluate the raised ALT with other tests, and liver biopsy was not done, as it was beyond the scope of the study.

Impaired fasting glucose was seen in 20% of the cases and impaired glucose tolerance was seen in 3.3%. Diabetes was not diagnosed in any of them. This was higher than the reported incidence in the study conducted in 2001-2002, where none of the thalassemic children had abnormal glucose tolerance. Among 74 Greek cases of TM, the prevalence of IGT was 12.2% and that of diabetes 5.4%. However, all were aged above 10 years, with a mean age of 20.5 ± 5.2 years.[8] Other studies had the incidence of IGT varying from 12.5 to 24.1%.[17-19] The proposed risk factors for the development of glucose intolerance in TM were elevated ferritin levels, hepatitis C infection, age, family history of diabetes, and splenectomy.[8] The mean age of onset of diabetes, assessed in a large set of cases in Italy, was 18.1 years.[11] Another smaller study also reported the onset of diabetes to occur after 18 years of age.[20] Chelation therapy delayed the onset of diabetes[21] and reportedly the incidence, which was 19% in 1974 was 4.9% in 1995, and it also improved glucose tolerance. All the cases in this study were on chelation therapy, at least on oral deferiprone, but compliance was poor in a majority (73.3%), which was one of the reasons for the high incidence of IFG and IGT. One of the cases had left ventricular dysfunction due to complete cessation of the chelation therapy, but showed improvement following intensive chelation therapy. Although our patients did not have diabetes, the fasting insulin and insulin resistance indices were high, with normal fasting glucose, which was suggestive of the occurrence of insulin resistance before the onset of diabetes. Similar findings of the occurrence of insulin resistance before the onset of diabetes were reported by others.[14,17,18,22]

The occurrence of insulin resistance may be due to, (a) direct effect of iron overload or (b) hepatic dysfunction leading to reduced hepatic extraction of insulin, as a result of which the liver, which is the principal site of gluconeogenesis, continues to produce excess glucose into the bloodstream.[9] Insulin resistance was not seen in pre-pubertal patients, which made researchers conclude that insulin resistance was acquired rather than genetic in origin.[17,23] Likewise, these researchers found an increased insulin response to hyperglycemia in pubertal subjects and concluded that an elevated insulin level was due to the hypersecretion of insulin, as the C-peptide levels were also elevated.[23]

Insulin resistance increases with age and beta cell function reduces with age,[15] and the mechanism behind this has not been explained, probably because there has been no abnormal glucose tolerance in their study. However, the present study has no such correlation and supports the results and hypothesis by Merkel *et al*.[23] As the iron burden increases, sensitivity to insulin decreases, even in the face of chelation therapy. At this stage, a compensatory increase in insulin secretion serves to maintain the carbohydrate homeostasis near normal, implying that impairment of insulin action precedes the defects in insulin secretion. Ultimately, beta cell activity fails to keep pace and overt diabetes develops.

Statistical significance in beta cell function between cases and controls was observed in our study. Beta cell function is observed to deteriorate with age,[8] unlike in the present study, where we found that it increased with age followed by a decline. There was a linear relation with age and ferritin levels, showing an increasing trend with an increase in the number of units transfused. However, when compared with fasting insulin, there was an increasing trend with the number of units transfused, which was then followed by a decline with a further increase in the number of units transfused. A similar relation was observed with the beta cell function and the insulin resistance index also.

Our results showed a similar finding, where with increasing age and total number of transfusions, the insulin resistance increased, and to compensate, the beta cell activity also increased thereby producing more insulin up to a certain limit of maximum and then started declining when it could not keep up with the demand. Hence, persistent insulin resistance with progressive reduction in insulin secretion may lead to glucose intolerance and diabetes, which have a higher prevalence in thalassemia major.

In recent times, zinc deficiency has been thought to play a role in abnormal glucose metabolism. It leads to inability of the pancreas to secrete sufficient amounts of insulin in response to glucose stimulation.[24] Diabetic ketoacidosis might be the initial presentation of diabetes in 31% of thalassemia major patients.[7] Hence, all of them should be periodically screened, at least after 10 years of age, to prevent this life-threatening complication. After the diagnosis of abnormal glucose tolerance or diabetes, they should be given intensive chelation therapy, as it improves glucose tolerance and prevents the development of overt diabetes.

In conclusion, IGT and insulin resistance precede the onset of insulin-dependent diabetes mellitus, and hence, children with thalassemia major on long-term transfusion should be periodically monitored with glycemic indices and serum ferritin levels after the age of 10 years. Adequate chelation therapy is essential for delaying the onset of diabetes.

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