*Original Articles*

# Insulin Resistance and Beta Cell Function in Chronically Transfused Patients of Thalassemia Major

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***Objective:*** *To assess the glycometabolic function in chronically transfused patients of beta-thalassemia major in terms of glucose tolerance, insulin secretion, insulin resistance index, and beta cell function index and to determine their relationship with clinical and biochemical profile.* ***Methods:*** *30 homozygous thalassemia major children (aged 8-15 years) receiving regular blood transfusion and 10 age and sex matched normal children attending a tertiary level hospital were subjected to glucose tolerance test, estimation of fasting plasma insulin level, insulin resistance index and beta cell function index. Liver enzymes, liver size and indicators of iron overload (serum ferritin, total units of blood transfused, splenic size) were recorded.* ***Results:*** *There was no diabetes mellitus or impaired glucose tolerance test in either the cases or the controls. Fasting plasma insulin levels were significantly higher in cases than controls (P = 0.004), and correlated well with indicators of iron overload like total units of blood transfused (r = 0.41, P = 0.03), serum ferritin (r = 0.38, P = 0.038) and splenic size (r = 0.43, P = 0.03). Insulin resistance was higher in cases compared to controls (P = 0.01). It correlated well with age (r = 0.56, P = 0.006), fasting blood glucose (r = 0.8, P = 0.003), fasting plasma insulin (r = 0.95, P = 0.00001), total units of blood transfused (r = 0.52, P = 0.005), serum ferritin (r = 0.4, P = 0.02) and splenomegaly (r = 0.51, P = 0.004). Insulin resistance was higher in patients not on chelation therapy compared with those on chelation therapy (P = 0.003). The beta cell function index was higher in cases compared to the controls, but not of statistic significance (P = 0.077). It did not correlate well with total amount of blood transfused (r = –0.32, P = 0.08), serum ferritin (r = –0.138, P = 0.46), spleen size (r = 0.16, P = 0.36), or chelation therapy (P = 0.98).* ***Conclusions:*** *Diabetes mellitus or impaired glucose was not seen in chronically transfused patients of thalassemia major (between 8 and 15 years of age), in our study. Insulin resistance, compensated by hyperinsulinemia, sets in early even before the onset of frank diabetes mellitus and correlated well with age, chelation therapy and indicators of iron overload like total units of blood transfused, splenomegaly and serum ferritin.*

**Key words:** *Beta cell function, Glucose intolerance, Insulin resistance.*

THE use of regular, frequent blood transfusions in thalassemia major has improved the span and quality of life of the patients. But it leads to chronic iron overload which frequently causes endocrine problems especially diabetes mellitus. The reported incidence of impaired glucose tolerance in thalassemia major is 4-24%(1-5,7), and that of

diabetes mellitus is 0-26%(1-9). Although insulin deficiency secondary to pancreatic islet iron deposition has been assumed to be the principle cause, reports of hyper-insulinema with abnormal glucose tolerance testing may suggest a role of insulin resistance(5,10). Moreover an increase in insulin resistance has been reported in thalassemics even without overt impaired glucose tolerance or diabetes, suggesting the advent of insulin resistance before the onset of glucose intolerance or diabetes(5,11). A retrospective study of 1861 patients of thalassemia major in 1994 has shown that over the years the prevalence of diabetes has decreased(6). Another study, in a series of 273 thalassemia major patients from 1954 to 1998, showed the decrease in the incidence of glucose metabolism abnormalities over the years and increase in the mean age of diagnosis of diabetes(12). Early initiation of chelation therapy has been postulated as one of the causes for the same(6). The present study was performed in chronically transfused thalassemia major children on regular blood transfusion therapy. They were evaluated for the incidence of diabetes mellitus, glucose intolerance, insulin resistance and beta cell function. These indices were further evaluated for correlation with biochemical and clinical parameters.

## Subjects and Methods

This study was conducted in Department of Pediatrics of a tertiary care centre in Mumbai over a period of 1 year 2 months, from March 2001 to April 2002 after obtaining the requisite permission of the Ethics Committee of the hospital. The study included 30 homozygous thalassemia major patients, diagnosed at the mean age of 10 months with range between 3.5 months to 30 months, receiving regular blood transfusion in the hospital. Ten normal children following up in the school clinic were taken as controls. Written valid informed consent of parents of both subjects as well as control group was taken prior to the study. Inclusion criteria for cases were: (1) Thalassemia major diagnosed by clinical features and Hemoglobin electrophoresis. (2) Age between 8 and 15 years. (3) Absence of any disease, infection. (4) Not on medication known to cause glucose intolerance. (5) No family history of diabetes mellitus in 1st degree relatives. Inclusion criteria for controls: (1) Absence of cardiac, hepatic, renal or any other disease. (2) Age between 8 and 15 years. (3) No family history of thalassemia major. (4) No history of diabetes mellitus in 1st degree relatives. The cases and controls were comparable in age and sex. Of the 30 patients studied, 21 patients were on iron chelation therapy. Only one patient was on a combination of desferrioxamine and oral deferiprone, while the other 20 patients were on oral deferiprone (30-50 mg/kg). The duration of the chelation therapy ranged from 0.5 year to 2.5 years, with a mean of 1.5 years. A detailed history and physical examination was done. Liver size and spleen size recorded. Serum ferritin, liver enzymes (alanine amino-transferase-ALT, aspartate aminotransferase-AST), total units of blood transfused, details of chelation therapy, hemogram, liver and renal function were recorded in cases and controls. Glycometabolic status was assessed by performing oral glucose tolerance test with estimation of fasting and post glucose (2 hr) plasma glucose (by Trinder's glucose oxidase), fasting plasma insulin levels (by chemiluminescent immunoassay).

*Oral glucose tolerance test:* Fasting plasma glucose was done after 8 hours of fasting at 8.00 am. Blood for fasting plasma Insulin was collected at the same time. After collection of the sample, a glucose load of 1.75g/kg of body weight was administered orally and plasma glucose was estimated 2 hours later.

Diagnosis of diabetes mellitus and glucose intolerance was made by WHO criteria(13, 14). Impaired fasting glucose (IFG) was diagnosed if fasting plasma glucose >110 mg/dL and less than 126 mg/dL (6.1-7.0 mmol/L). Impaired glucose tolerance test was diagnosed if the 2hr post glucose plasma glucose was >140 mg/dL and less than 200 mg/dL (7.8-11.1 mmol/L). Diabetes was diagnosed if the fasting plasma glucose was >126 mg/dL (7.0 mmol/L) and 2 hour post glucose plasma glucose >200 mg/dL (11.1 mmol/L). Non-diabetic patients and controls underwent evaluation of insulin resistance index and beta cell function index using the Homeostasis model assessment (HOMA) model (15-16).

$$\text{Insulin Resistance Index} = \frac{\text{Fasting plasma glucose (mmol/L)} \times \text{Fasting plasma insulin } (\mu u/mL)}{22.5}$$

$$\text{Beta cell Function Index} = \frac{20 \times \text{Fasting plasma insulin } (\mu u/ml)}{\text{Fasting plasma glucose (mmol/L)} - 3.5}$$

The coefficient of correlation '*r*' was employed to test the strength of an association (value of '*r*' towards 1 indicates very high correlation, it can be positive or negative), P value was used to measure the strength of a result of a test (any P value ≤0.05 is considered significant), Student's unpaired 't' test was done to compare variables of two groups for significance (any't' value >2 is considered significant).

## Results

The mean age among the cases was 10.89 years with a range of 8-15 years and among the controls was 10.63 years ranging from 8-13 years. Of the 30 cases 18 (60%) were males and 12 (40%) were females, and of the 10 controls 6 (60%) were males and 4 (40%) were females. The cases and controls were age matched (P > 0.2) and sex matched. None of our cases or controls showed diabetes mellitus or glucose intolerance or impaired fasting glucose (*Table I*).

*Fasting glucose*

There was no significant difference between the fasting plasma glucose (P = 0.66) and post glucose (2 hr) plasma glucose (P = 0.51) of cases and the controls. The fasting plasma glucose correlated well with insulin resistance, total units of blood transfused, or splenomegaly, and showed negative correlation with beta cell function. Although fasting plasma glucose correlated with serum ferritin, it failed to reach statistical significance and it showed no correlation with the liver size (*Table II* ).

*Fasting plasma insulin*

Fasting plasma insulin was observed to be significantly higher in cases as compared to the controls (P = 0.004). It correlated well with the insulin resistance index, and with indicators of iron overload (like the total amount of blood transfused, serum ferritin and splenomegaly). No correlation was seen with beta cell function and liver size (*Table II*). No significant difference in the levels of fasting plasma insulin was seen between the patients on chelation and the patients not on chelation therapy (P = 0.32).

*Insulin Resistance Index*

Insulin Resistance Index was significantly higher in cases as compared to the controls (P = 0.01), correlating well with indicators of iron overload *i.e.*, total units of blood transfused, serum ferritin, splenomegaly, but not with liver span (*Table III*). Insulin resistance was found to be higher in patients not on chelation therapy as compared to those on chelation therapy (P = 0.003).

**TABLE I**–*Biochemical and Clinical Characteristics of Patients and Controls*

| Parameters | Mean values | |
|---|---|---|
| | Cases | Controls |
| Fasting plasma glucose (mg/dL) | 90.77 ± 9.73 | 88.60 ± 6.32 |
| Fasting (2hr) plasma glucose(mg/dL) | 127.07 ± 7.19 | 125.9 ± 6.94 |
| Fasting plasma insulin (mg/dL) | 9.60 ± 2.29 | 7.30 ± 1.31 |
| Insulin Resistance Index | 2.18 ± 0.645 | 1.60 ± 0.337 |
| Beta cell function Index | 135.45 ± 42.59 | 108.62 ± 32.49 |
| AST | 82.07 ± 37.36 | 27 ± 11.5 |
| ALT | 94.9 ± 47.76 | 25.9 ± 11.5 |
| Serum ferritin (ng/mL) | 7623 ± 2381 | |
| Total units of blood transfused | 135 ± 44 | |
| Splenic size (cm) | 7.00 ± 4.9 | |
| Liver span (cm) | 12.18 ± 3.51 | |

*Beta cell function Index*

Beta cell function Index was higher in cases as compared to controls but was not statistically significant (P = 0.077). It correlated better with total units of blood transfused than serum ferritin, but failed to reach statistical significance, and did not correlate with spleen size, liver span and chelation therapy (P = 0.98).

Marked derangement in the serum transaminases of cases *vs.* controls was seen, AST levels correlated well with the beta cell function index ($r$ = –0.43, P = 0.017), fasting plasma glucose ($r$ = 0.44, P = 0.01), and total units of blood transfused ($r$ = 0.51, P = 0.003), but not with the serum ferritin levels ($r$ = 0.04, P = 0.82) and Insulin resistance ($r$ = 0.27, P = 0.17). ALT did not correlate with Insulin Resistance ($r$ = 0.12, P = 0.52), beta cell function ($r$ = –0.24, P = 0.112), fasting plasma insulin ($r$ = 0.04, P = 0.83), fasting plasma glucose ($r$ = 0.25, P = 0.17), total units of blood transfused ($r$ = 0.33, $P$ = 0.07) or with serum ferritin ($r$ = 0.06, P = 0.74).

It was observed that Insulin resistance index ($r$ = 0.56, P=0.008), AST ($r$ = 0.44, P = 0.01), total units of blood transfused ($r$ = 0.91, P = 0.0.006) correlated well with age. Beta cell function was observed to decrease with increasing age, but not significantly ($r$ = –0.33, P = 0.08). Serum ferritin did not correlate with age ($r$ = 0.11, P = 0.57).

## Discussion

None of our patients showed frank diabetes mellitus or abnormal glucose tolerance test as opposed to the reported incidence of 0-26%(1-9) of diabetes mellitus and 4-24% (1-5,7) of impaired glucose tolerance test. A retrospective study done in 1861 patients of thalassemia major patients has shown 18.1 years as the mean age of onset of diabetes(6). Arrigo, *et al.* have demonstrated the age of onset of diabetes in thalassemics to be after 18 years(8). The low incidence could be due to the advancement in the age of occurrence of diabetes in thalassemics over the years(6,12), which can be attributed partly to iron chelation therapy(6,17). However, the non-diabetic

**TABLE II–***Correlation Between Fasting Plasma Glucose,Fasting Plasma Insulin Levels and Other Clinical and Biochemical Parameters*

| | | Insulin resistance | Beta cell function | Total units blood transfused | Spleno-megaly | Serum ferritin | Liver span |
|---|---|---|---|---|---|---|---|
| Fasting Blood sugar | r | 0.8 | –0.73 | 0.57 | 0.47 | 0.34 | 0.05 |
| | P | 0.003 | 0.0004 | 0.02 | 0.01 | 0.06 | 0.8 |
| Fasting Insulin | r | 0.95 | 0.04 | 0.41 | 0.43 | 0.38 | 0.27 |
| | P | 0.00001 | 0.84 | 0.03 | 0.03 | 0.038 | 0.18 |

**TABLE III–***Correlation Between Insulin Resistance Index, Beta Cell Function Index and Clinical and Biochemical Parameters*

| | | Total units blood transfused | Splenomegaly | Serum ferritin | Liver span |
|---|---|---|---|---|---|
| Insulin Resistance Index | r | 0.41 | 0.43 | 0.38 | 0.27 |
| | P | 0.03 | 0.03 | 0.038 | 0.18 |
| Beta cell Function Index | r | –0.32 | 0.16 | –0.138 | 0.19 |
| | P | 0.08 | 0.36 | 0.46 | 0.27 |

thalassemics in our study showed higher fasting plasma insulin levels with increased Insulin Resistance Index and normal plasma glucose, suggesting the presence of insulin resistance before the onset of frank impaired glucose tolerance test or diabetes. The high insulin level is probably in compensation for the insulin resistance in an attempt to maintain euglycemia. A similar increase in the insulin levels, before the advent of impaired glucose tolerance test and diabetes, was also demonstrated in a study by Flynn, *et al.*(4). An increase in the insulin levels in thalassemics has also been demonstrated after oral glucose administration(18), or to intravenous tolbutamide(11). The increase in insulin levels has been postulated due to reduced hepatic insulin extraction rather than an increase in the secretory response(11). An increase in insulin resistance in non-diabetic patients of thalassemia was also seen in studies by Cario, *et al.*(5) and Dmochowski, *et al.*(11). When 7 out of 10 thalassemia patients with decreased insulin sensitivity (interpreted as insulin resistance) were prospectively analyzed over 6 months to 1 year, Dmochowski, *et al.*(11) showed that the insulin sensitivity remained the same but the integrated insulin response decreased. Thus they postulated that persistent insulin resistance with a progressive reduction in the circulating insulin levels may lead to glucose intolerance and diabetes which have a higher prevalence in thalassemia major(11). In contrast Arrigo, *et al.* in their study demonstrated a decrease in insulin resistance and beta cell function index (by HOMA model) in non-diabetic thalassemia major adult patients(8).

In our study fasting plasma insulin co-

## Key Messages

- Insulin resistance with compensatory increase in the insulin levels to maintain euglycemia occurs early in thalassemics before the onset of impaired glucose tolerance and diabetes. Chelation therapy appears to postpone these changes.
- Glucose tolerance tests, insulin levels, insulin resistance index, beta cell function index should be an integral part of the long term follow up of thalassemia patients on an annual basis from the second decade onwards (especially the second half).
- Iron overload studies, liver function tests should be done regularly to correlate with the parameters of glucose metabolism.
- Intensive iron chelation therapy and prevention and treatment of hepatic infections are the most important means of preventing glucose homeostasis disturbances in thalassemics.

related well with serum ferritin levels, similar to other studies(11,19); and also co-related well with other indicators of iron overload like splenomegaly and total units of blood transfused. Insulin Resistance in our study correlated significantly with the age similar to other studies(5,10), and also with parameters of iron overload, *i.e.*, total units of blood transfused, splenomegaly and serum ferritin. Insulin resistance was found to be higher in our patients not on chelation therapy. All these suggest the role of iron overload in the pathogenesis of the insulin resistance.

The insulin resistance has been postulated to be at the level of the liver (due to iron deposition), where it may interfere with the insulin's ability to suppress hepatic glucose uptake, and also at the level of the muscle, where iron deposits may decrease the glucose uptake(7). With advancing age a persistent insulin resistance along with the decrease in the circulating insulin levels (due to declining beta cell function), leads to the onset of glucose intolerance and frank diabetes mellitus(7,11). However even in the face of adequate chelation a significant amount of carbohydrate metabolism dysfunction occurs (9,20), suggesting that the development of diabetes might be complicated by other factors(7). Pancreatic autoimmunity demonstrated by islet cell antibodies(21), liver abnormalities like cirrhosis(17,23), liver fibrosis(12), Hepatitis C Infection(23,25), genotype-IVS11nt 745(3), family history of diabetes(24) are some of the factors postulated.

Ketoacidosis has been reported to be the presenting manifestation of diabetes in 13.8% (26), 31.1%(7) of thalassemics. To prevent this life threatening complication active surveillance for the occurrence of impaired glucose tolerance and diabetes should be carried out in thalassemics especially in the second decade. Moreover, thalassemic patients with clinical diabetes are at a high risk for other complications like endocrine (especially thyroid dysfunctions) or cardiac or both, and should be strictly monitored for these(26). Assessment of iron overload in these patients and intensive iron chelation therapy may be required subsequently.

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