

Status of glucose metabolism including insulin resistance and beta cell function in overtly iron loaded Thalassemia patients

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ABSTRACT

BACKGROUND

Abnormality of glucose metabolism is a frequent complication in Thalassemia patients. Both insulin deficiency and insulin resistance has been proposed in its pathogenesis. Some form of abnormality in glucose metabolism is expected at an earlier age in these patients in developing countries like India and Nepal where iron overload is excessive due to lack of chelation therapy.

MATERIALS AND METHODS

Fasting serum glucose and fasting serum insulin (FSI) were measured in 40 beta-thalassemia major patients, 40 Ebeta- thalassemia patients and 40 controls, all aged between 5 and 12 years. 2 hours after an appropriate dose of oral glucose feed (Children ingested 1.75 g/kg body weight maximum 75 gram dissolved in 250 to 300 ml water) blood samples were drawn again to measure post prandial serum glucose. Iron overload was assessed by measuring liver size, spleen size, total amount of packed cells transfused and serum ferritin. Insulin resistance (IR), insulin sensitivity (%S) and beta cell functions (%B) were derived from the measured laboratory parameters using the latest version of Homeostatic Model Assessment (HOMA) calculator software.

RESULTS

No one had impaired glucose metabolism or diabetes mellitus beta-thalassemia major patients showed evidence of insulin resistance in the form of significantly higher fasting serum insulin (p value 0.002), IR (p value 0.003), %B (p value 0.017) and significantly lower %S (0.002) when compared with controls. FSI showed positive correlation with total amount of packed cells received ($r=0.372$, $p=0.018$), serum ferritin ($r=0.345$, $p=0.029$) and spleen size ($r=0.427$, $p=0.006$). Similarly, IR also showed positive correlation with total amount of packed cells received ($r=0.388$, $p=0.013$), serum ferritin ($r=0.336$, $p=0.034$) and spleen size ($r=0.425$, $p=0.005$). %S showed negative correlation with all these parameters. %B didn't show any statistically significant correlation with these parameters. Ebeta- thalassemia patients didn't have any statistically significant difference in FSI, IR, %S and %B than controls.

CONCLUSION

Insulin resistance develops as the earliest abnormality in glucose metabolism in overtly iron loaded beta thalassemia major patients at an early age. Ebeta- thalassemia patients with milder phenotype do not develop dysfunction of glucose metabolism at such an early age.

Key words : Glucose metabolism, Insulin resistance, Insulin sensitivity, Beta cell function, beta- thalassemia major, Ebeta- thalassemia.

INTRODUCTION

The thalassemias are a heterogeneous group of single-gene disorder characterized by reduced rate of production of normal haemoglobin due to absent or reduced synthesis of one or more types of globin polypeptide chains resulting in chronic haemolytic anaemia. Blood transfusion is the mainstay of care of individuals with thalassemia which improves anemia and suppresses ineffective erythropoiesis. Regular transfusions have prolonged and improved quality of life of patients with this disease.^{1,2} However, in absence of iron chelation therapy, blood transfusions increase iron overload. It is pronounced if transfusions are not optimum and regular due to GDF-15 (secreted from enormously expanded and active bone marrow) mediated increase in iron absorption. So, blood transfusions, whether adequate or inadequate, leads to iron overload in various organs like heart, liver, pituitary, thyroid, parathyroid, pancreas etc. Sixty percent of thalassemic patients have hypofunction of at least one endocrine gland.³ Disturbances of glucose metabolism are frequent in these patients. However, there is no consensus regarding etiology of abnormal glucose metabolism. Though insulin deficiency has been proposed as the causative factor, some studies report presence of insulin resistance and hyperinsulinemia. Evidence of insulin resistance has also been noted in thalassemics without any abnormality in serum glucose.⁴

In India and Nepal, chelation is suboptimal in most of the patients due to economic constraints. Even compliance to transfusion is often not optimal.⁵ Therefore, there is a possibility that our patients may develop some form of derangement in glucose metabolism at an earlier age. E beta thalassemia is a common variety of thalassemia found in Eastern part of India, besides beta- thalassemia major; but no study

is available regarding glucose metabolism status in them. Keeping these in mind, we planned our study to evaluate the status of glucose metabolism in terms of incidence of diabetes, impaired glucose tolerance and parameters related to insulin resistance in thalassemia patients (both beta thalassemia major and E beta thalassemia) who do not receive chelation therapy.

MATERIALS & METHODS

Thalassemia patients attending Burdwan Medical College and Hospital and College of Medical Sciences Bharatpur for treatment were included in this study. They were divided into two groups – beta thalassemia major and E beta thalassemia; 40 patients from each group were selected. Patients aged between 5 and 12 years, diagnosed by electrophoresis or High Performance Liquid Chromatography, were included in the study. Exclusion criteria were: patients receiving chelation therapy, patients infected with Hepatitis B and C viruses and patients whose 1st degree relatives (non-thalassemic) and who were suffering from diabetes. 40 beta- thalassemia major patients (group 1) and 40 E-beta thalassemia patients (group 2) who satisfied these criteria were selected for the study. We also involved 40 controls (group 3), who were selected randomly from healthy siblings of patients (non-thalassemic) attending Pediatric Out Patient Department for minor ailments. The study was conducted after obtaining informed consent from parents of cases and controls and permission from institutional ethical committee. Following an overnight fasting for 8 hours, a venous blood sample was collected next day to estimate fasting plasma glucose, fasting serum insulin and serum ferritin. Then the patients were given a feed of glucose solution using dose of

1.75 gm glucose per kg body weight. After 2 hours, another blood sample was collected for post prandial plasma glucose estimation. Blood glucose level was assayed by Glucose oxidase- Peroxidase (GOD-POD) method, serum insulin and serum ferritin by Non Competitive ELISA method. Insulin resistance (IR), insulin sensitivity (%S) and beta cell functions (%B) were derived from the measured laboratory parameters using the latest version of HOMA calculator software. In our study, we have used the HOMA Calculator 2.2.2 software developed by Oxford University. Finally, all the data were analysed using SPSS software version 17.0.

RESULTS

In our study no one from any group had qualified to be labeled as suffering from diabetes mellitus (DM) or having impaired glucose tolerance.

Fasting serum insulin, insulin resistance and beta cell function (%B) all were significantly higher in group 1, when compared to group 2 and group 3. Insulin sensitivity (%S) was significantly lower in group 1, compared to other groups. No significant difference was noted between groups 2 and 3 in any of these parameters [Table 1].

Table 1: Demographic, clinical and biochemical parameters among various groups

Parameters	Mean±SD			p values		
	Group 1 β thal. major	Group 2 Eβ thalassemia	Group 3 Control	p ₁₋₂	p ₁₋₃	p ₂₋₃
Liver Size(cm)	6.78±2.02	2.61±1.07		<0.001		
Spleen Size(cm)	10.60±3.81	5.64±1.27		<0.001		
FBS(mg/dl)	89.35±5.71	87.72±5.62	88.27±5.31	0.204	0.386	0.654
PPBS(mg/dl)	118.10±7.06	116.73±6.14	115.97±5.15	0.356	0.128	0.556
SFI(ng/ml)	9.25±1.64	8.09±0.71	8.24±1.07	<0.001	0.002	0.494
IR	1.19±0.22	1.05±0.09	1.07±0.16	<0.001	0.003	0.449
%S	86.02±13.84	96.58±8.29	95.69±12.89	<0.001	0.002	0.713
%B	109.61±14.57	104.88±13.64	101.32±15.95	0.137	0.017	0.287
Serum ferritin	4360.00±1705.01	2036.77±415.84	68.97±15.04	<0.001	<0.001	<0.001

p₁₋₂ is p value when groups 1 and 2 are compared for significance, p₁₋₃ between groups 1 and 3 and p₂₋₃ between groups 2 and 3.

In group 1, FSI and IR showed positive correlation with some parameters related to iron overload like total amount of packed cells received, serum ferritin and spleen size. %S showed negative correlation with these parameters. %B, though, was significantly higher among patients of group 1; it did not show any correlation with these parameters [Table 2].

Table 2: Correlation of FSI, IR, %S and %B with various clinical and biochemical parameters among patients of beta thalassemia major

		Total amount of packed cells received	liver size	spleen size	serum ferritin
FSI	r	0.372	0.237	0.427	0.345
	p	0.018	0.140	0.006	0.029
IR	r	0.388	0.248	0.425	0.336
	p	0.013	0.123	0.006	0.034
%S	r	-0.421	-0.287	-0.465	-0.392
	p	0.007	0.073	0.002	0.012
%B	r	-0.127	-0.019	-0.052	0.009
	p	0.434	0.909	0.751	0.955

DISCUSSION

Result of our study reflects the ground reality of condition of most of the thalassemic patients in developing countries including ours where iron chelation therapy is suboptimal or not done at all. Regular blood transfusion is also not done in some cases. This is reflected by very high serum ferritin levels in them.

In our study no one among the cases or controls had Diabetes Mellitus (DM) or Impaired Glucose Tolerance (IGT). The reported incidence of impaired glucose tolerance and diabetes mellitus vary from 0-19.5% (4-11) to 0-24% respectively in different studies.^{4-5, 7-11} Though diabetes has been observed as early as 5.5 years⁵, but mean age of onset of DM and IGT is late adolescence onwards, as found in different studies.^{9,12} Findings of various studies are given in Table

3. We worked with thalassemic patients with lower age group of 5 to 12 years. These patients, if followed up, might eventually turn out to have deranged glucose metabolism. Apart from iron overload, development of impaired glucose metabolism is also influenced by various other factors. Viral hepatitis affects glucose metabolism in these patients in various ways. Diabetes has been precipitated shortly after an episode of acute viral hepatitis in these patients.¹³ Chronic viral hepatitis has a diabetogenic effect.¹⁴ Some workers have found that iron induced hepatic damage is accentuated by HCV infection.¹⁵ Hepatic cirrhosis is another liver related condition implicated as causal factor.¹⁴ Besides these, other factors postulated include pancreatic autoimmunity demonstrated by islet cell antibodies,¹⁶ possible genetic factors⁹ and family history of diabetes.¹⁷ In this study, our aim was to ascertain the sole effect of

iron overload. So, we excluded these confounding factors as much as possible during selection of cases as per inclusion and exclusion criteria. This may be another reason why we did not find any case with impaired glucose metabolism. A study from India did not find any case with DM or IGT in the age group of 8 to 15 years.⁴This similarity in result may be due to common genetic factors in this region of world which needs further exploration. The conventional method of detecting glucose metabolism abnormalities is glucose tolerance test. Recently, Soliman AT et al¹⁸ used Continuous Glucose Monitoring System and found it to be more sensitive in detecting such abnormalities.

Though we could not detect DM or IGT among our patients, evidence of insulin resistance was detected in the form of higher FSI and IR in patients with beta thalassemia major, when compared with controls. Cario H et al¹⁹ found that patients with impaired glucose tolerance had hyperinsulinemia and delayed peak insulin during glucose tolerance test. Hafez M et al²⁰ did not mention on fasting plasma insulin levels but found mean postprandial insulin to be significantly higher ($p < 0.0001$) in cases with impaired glucose metabolism. Higher fasting plasma insulin levels were also detected in the study by Suverna J et al⁴ among beta thalassemia major patients having no derangement in glucose metabolism. Some other studies also demonstrated increased insulin level following oral glucose or intravenous tolbutamide administration. Dmochowski K et al²¹ progressively analysed seven thalassemic patients with decreased insulin sensitivity and found that the insulin sensitivity remained the same but the integrated insulin response decreased. So they postulated that insulin resistance persists but at the same time a progressive reduction in circulatory insulin level occur which ultimately lead to diabetes. So, it appears

that insulin resistance develops before any derangements in glucose level appear. This raised insulin level helps to counteract insulin resistance and maintain glucose homeostasis. With continuation of disease process, pancreatic beta cell damage starts leading to lowering of insulin level and increase in glucose level. Dmochowski K et al²¹ postulated that decreased hepatic uptake of insulin is the major factor for developing insulin resistance. Presence of insulin resistance has been shown also by Hafez M et al¹⁰ and Pappas S et al¹⁸. However, insulin resistance has not been found in some studies.

In our study, FSI and IR correlated with parameters related to iron overload like total amount of packed cells received, serum ferritin and spleen size revealing role of iron overload as risk factors for development of insulin resistance.

In our study, insulin Sensitivity in cases was found to be significantly lower than the controls (p value 0.001) by independent t test. Some workers have found similar results^{19,21}. %S correlates negatively with total amount of packed cells received, spleen size and serum ferritin. As we know insulin sensitivity is the reverse of insulin resistance, this finding is expected.

On the other hand, we found beta cell function to be significantly higher in cases than controls. There is dearth of literature regarding beta cell function. Jaruratanasirikul S et al¹² showed a higher but not significant beta cell function index in patients with a normal glucose tolerance test than those with an abnormal glucose metabolism having liver and pancreatic cell damage. Suverna J et al⁴ found Beta cell function Index to be higher in cases as compared to controls but was not statistically significant ($p = 0.077$). However, to the contrary of our findings, Hafez M et al²⁰ found significantly lower mean HOMA beta cell function

($p=0.007$) among cases than controls. The present study not only rules out hyposecretion of insulin in our patients, but may also indicate beta cell hyperfunction to explain rise of insulin level in the face of insulin resistance. Over time, further iron overload may increase insulin resistance and may also initiate damage to pancreatic beta cells with subsequent hyposecretion of insulin to give the full picture of impaired glucose metabolism. Unlike pancreas, liver is rich in reticuloendothelial cells where iron is stored. This might explain earlier liver damage and appearance of insulin resistance than hyposecretion of insulin.

FSI, IR, %S, %B was found to be significantly lower among EBT patients compared to BTM. It is known that EBT is a very heterogeneous disease with variable clinical manifestations ranging from mild thalassemia intermedia with little or no symptoms to a very severe transfusion dependent thalassemia major syndrome. Our pool of E beta thalassemia patients have milder phenotype reflected by low transfusion requirement and not very high serum ferritin. So they are not overtly

loaded with iron yet. This may be the reason that they do not show any statistically significant difference in terms of insulin related parameters when compared with control group.

Our beta thalassemia major patients showed evidence of insulin resistance but did not have overt impaired glucose tolerance or diabetes mellitus in spite of being heavily iron overloaded. From our study, it is evident that insulin resistance develops as the earliest abnormality in glucose metabolism in beta thalassemia major patients. Iron overload plays a significant role in its development. These patients should be monitored periodically to assess status of glucose metabolism. E beta thalassemia patients, who form a significant part of thalasseemics in eastern part of India, have milder clinical manifestation reflected by low transfusion requirement and low serum ferritin. This group of patients does not show any evidence of insulin resistance or abnormality in serum glucose level. Chelation therapy should be started in all thalassemia patients so that iron overload related problems can be retarded.

Table 3: Findings of some of the related studies

Study	Study group(yrs)	Sample size	Ferritin	Patients with IGM	IGM onset(yrs)	IR	Reference
Present study	5-12	4	4360±1705	0	-	present	-
Suvarna J et al	8-15	30	7623±2381	0	-	present	4
Gulati R et al	3-22	37	4920±2758	3	5.5-18	-	5
Jaruratanasirikul S et al	13.6±3.9	48	5206±3291	6	11 to?	-	12
Chern JPS et al	2-36	89	3107.3±2530.7	23	7-27	-	11
Khalifa AS et al	10-31	56	3648±2219	12	18.33 [¥]		9
Arrigo T et al	17-42	29	-	4	18 [€]	absent	10
Soliman TA et al*	14-22	16	-	5	-	absent	18

* 12 patients were identified having IGM using continuous glucose monitoring system in the same study group. Insulin resistance found in 3(out of 16) patients. [¥] indicates mean age. [€] indicates lowest age of onset.

CONTRIBUTIONS

Dr Atanu Pan and Dr Badal Chandra Mandal conceptualized the study and reviewed the manuscript critically. AP and SSN collected the data, searched literature and drafted the manuscript. AD and PM helped in performing the investigations, searching the literature and also reviewed the manuscript.

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