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Original Article

Effect of Iron overload on Glucose Regulation in Transfusion dependent Beta Thalassemia major Patient

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ABSTRACT

Objective: To evaluate the effect of iron overload on regulation glucose metabolism (diabetes and prediabetes) by using fasting blood glucose level and 2- hour postprandial plasma glucose level.

Methods: This cross-sectional study was carried out at Jamila Sultana Thalassemia center for a period of 6 months. 54 (25 males and 29 females) patients of 12-26 years age, of Thalassemia major on regular blood transfusions and iron chelating therapy were included in the study. Demographic data was taken and standard Body Mass Index was measured for each patient. Fasting plasma glucose levels were checked following an overnight fast, after that each patient was given an oral glucose challenge of 1.75gm/kg body weight with a maximum dose of 75gm. Post-prandial plasma glucose levels were checked two hours after this oral glucose challenge. The data were entered on Statistical Package for the Social Scientists vr. 21 for analysis. Mean and standard deviation were calculated for all quantitative data, frequencies and percentages were calculated for qualitative data.

Results: 13% of the patients had impaired fasting blood glucose, 5.6% patients had impaired 2-hour postprandial levels and 2 patients had their levels were in diabetic range. Patients' age and Body Mass Index showed a positive correlation with 2- hour postprandial glucose level with highly significant p- value (<0.001).

Conclusion: The findings highlight the importance of regular follow-up of patients with β-thalassemia major patients for early detection and management of associated complications.

Key words: Iron overload, Beta Thalassemia, Impaired glucose level, Glucose regulation, Diabetes, Pre-diabetes.

Introduction

Beta Thalassemia (BT) is the most common autosomal co-dominant disorder with 5-8% carrier rate in Pakistan. Every year 5000-9000 children are born with beta thalassemia major (TM).[1] It is caused by mutation in beta globin gene resulting in deficiency or absent beta chain. The condition results in severe anemia due to hemolysis and ineffective erythropoiesis, requiring lifelong transfusion. Multiple transfusions result in iron overload and deposition of iron in various tissues. With advancing age, iron deposition is markedly elevated and causes toxic effects in various organs like heart, liver and endocrine glands.

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This results in significant morbidity and mortality especially in patients without monitoring of meticulous iron chelation therapy. Iron chelation therapy and hydroxyurea are the main stay of treatment. Proper iron chelation therapy is mandatory to reduce serum ferritin level and to prevent iron deposition in these vital organs.[2] Patients with poor compliance to chelation therapy develop complications of iron overload earlier. Regular assessment of iron status is thus essential for effective management of these patients and serum ferritin is most reliable and widely used indicator of iron overload in various organs.[3,4]

Endocrine complications are among the most important complications associated with suboptimal chelation therapy. The frequent endocrine complications include hypogonadism, delayed puberty, growth retardation, Diabetes Mellitus (DM),

impaired thyroid, parathyroid and adrenal functions. Early detection and treatment of endocrine complications is of utmost importance to prevent irreversible damage to these organs. DM and glucose intolerance are one of the commonest complications in Beta Thalassemia patients due to iron overload. The prevalence of diabetes and glucose intolerance is 9.4% and 7.1% in Beta Thalassemia major patients and 2% and 24% in Beta Thalassemia Intermedia (TI) patients.[5,6] Various mechanisms are involved in pathogenesis of Diabetes in Beta Thalassemia Major patients. These include, hemochromatosis resulting in insulin deficiency, insulin resistance and impaired glucose metabolism in liver due to iron overload, and hepatitis C infection.[7] Immune mechanisms and inflammatory markers, and iron overload are associated with impaired insulin sensitivity. Risk factors for development of Diabetes in these patients include age, serum ferritin level, frequency of transfusions, compliance with iron chelation therapy, family history of Diabetes and infection with hepatitis viruses.[8]A ferritin level of > 3000 has been found to be associated with high risk of development of Diabetes.[9]Iron accumulation in the tissues causes free radical injury leading to lipid peroxidation of cell membrane, mitochondrial and lysosomal membrane.[10] It is recommended that iron mediated Diabetes can be partially reversed if treated earlier. Early screening and detection of glucose impairment and insulin resistance is recommended in all thalassemic patients from 8-10 years, so that the disease can be halted.[11,12] According to American Diabetes Association guidelines, in conditions associated with increased red blood cell turnover, such as Thalassemia, sickle cell disease, pregnancy (second and third trimesters), hemodialysis, recent blood loss or transfusion, or erythropoietin therapy, only plasma blood glucose criteria should be used to diagnose diabetes.

Table 1: Diagnostic Criteria for Diabetics and Pre-Diabetics[13]

Parameters	Impaired	Diabetes
Fasting Glucose*	100–125 mg/dL (5.6–6.9 mmol/	>126 mg/dL (7.0 mmol/L)
2hr post prandial **	140–199 mg/dL (7.8–11.0 mmol/L)	>*200 mg/dL (11.1 mmol/L)*
HbA1c level	5.7–6.4% (39–47 mmol/mol)	> A1C $6.5% (48 mmol/mol

*Fasting is defined as no caloric intake for at least 8 hours.**Post prandial should be performed using a glucose load containing the equivalent of 75-g anhydrous glucose dissolved in water. The present study was designed to evaluate the effect of iron overload on regulation glucose metabolism (diabetes and prediabetes) by using fasting blood glucose level and 2 hour postprandial plasma glucose level.

Methods

This cross-sectional study was carried out at Jamila Sultana Thalassemia center for a period of 6 months. 54 patients of Beta Thalassemia major coming for regular follow up were included in the study. Sample size calculated from WHO sample size calculator where confidence level = 95%, Anticipated population proportion = 29.4% [2], Absolute precision required = 12%. Patients of Thalassemia major patients of both genders, presenting in routine follow up at Jamila Sultana Thalassemia center, more than 12 years of age and on regular blood transfusions and iron chelating therapy were included in the study and selected by purposive sampling. Acutely ill patients with ongoing severe infections like pneumonia and UTI, patients on steroids or those treated with bone marrow transplantation were excluded from the study. Informed consent was taken from each patient or his/her guardian. Demographic data was taken and weight, height and BMI were measured for each patient. Fasting plasma glucose levels were checked following an overnight fast after which each patient was given an oral glucose challenge of 1.75gm/kg body weight with a maximum dose of 75gm. Post-prandial plasma glucose levels were checked two hours after this oral glucose challenge. Each patient's Performa was filled by the same doctor. All the available serum ferritin levels for each patient were also noted from the available records. The data were entered on Statistical Package for the Social Scientists (SPSS) version 21 for analysis. Mean and standard deviation were calculated for all quantitative data (age, blood sugar levels). Frequencies and percentages were calculated for qualitative data.

Operational Definitions

Abnormal Glucose Regulation: It includes a fasting blood glucose level of 100 mg/dl or more and a post-prandial blood glucose level of 140 mg/dl or more. These blood glucose levels were determined through a standard oral glucose tolerance test (OGTT). Pre diabetics show impaired levels

Diabetes Mellitus: It includes fasting blood glucose level of 126mg/dl or more and a post prandial blood glucose level of 200mg/dl or more (table 1).

Thalassemia Major: Patients who were diagnosed to have thalassemia before the age of 2 years, based on

hemoglobin electrophoresis (which showed markedly raised levels of hemoglobin F and reduced levels of hemoglobin A1 and required regular blood transfusions since then.

Results

Out of 54 patients, 25 (46.3%) were males and 29 (53.7%) were females. Mean age of the patients was 16.13+ 3.670, ranging from 12-26 years. Table- 2 shows BMI, Serum ferritin levels, fasting glucose and 2-hourpostprandial plasma glucose levels. As shown in the table, mean ferritin in these patients was 4912.9+1661.469, mean fasting glucose was 87.055+10.458 and mean 2-hourpostprandial glucose was 105.01+36.792. The results of impaired fasting and 2-hour postprandial levels in study population showed that13% of patients had impaired fasting blood glucose and 5.6% patients had impaired 2-hour postprandial levels and 2 patients had their levels in diabetic range. Table 3 shows correlation of age, BMI and Serum ferritin levels with plasma glucose level. As shown in the table, age and BMI show a positive correlation with 2- hour postprandial glucose level, whereas correlation with serum ferritin was not found to be statistically significant.

Table 2: Evaluation of Different Parameters of study population (n=54)

Parameter	Range	Mean + SD
Age (in years)	12-26	16.1+3.670
Weight (in Kg)	21-72	32.7 + 8.639
Height (in feet)	3.80-5.70	4.70 + 0.406
BMI (kg/m2)	12.52-24.91	16.04 + 2.329
Serum Ferritin (ng/l)	2174-11057	4913 + 1661.4692
Fasting Blood Glucose (mg/dl)	72-124	87.1 + 10.458
2-hour Blood Glucose (mg/dl)	58-284	105 + 36.792

Table 3: Correlation of serum ferritin level and other parameters with glucose level (n=54)

	Fasting Glucose level (Pearson correlation)	P value	2 hour post prandial glucose level (Pearson correlation)	P value
Serum Ferritin Level	0.92	0.509	0.045	0.745
BMI	0.398	0.003	0.493	**0.000**
Age	0.199	0.148	0.616	**0.000**

Discussion

Iron overload is the main problem in patients of Beta Thalassemia major, resulting in various metabolic complications which are usually seen after 10 years. Diabetes mellitus is the most common among these; and its etiology is multifactorial (genetic factors, insulin deficiency, insulin resistance, and liver dysfunction secondary to viral hepatitis).[14]In this study, among thalassemic adolescents with mean ferritin level of 4912.9+1661.469, 13% of patients had impaired fasting blood glucose, 5.6% patients had impaired 2-hour postprandial levels and 2 patients had their levels in diabetic range. In a study done by Najafipour et al on 56 patients of Thalassemia major with mean age of 15.62+/-4.44 years, Diabetes mellitus was present in 5 patients (8.9%), impaired fasting glucose was found in 16 patients (28.6%) and an impaired glucose tolerance test was found in 4 patients (7.1%).[15]Variousrisk factors for diabetes has been reported in these patients and include age, increased amount of blood transfusion, serum ferritin level, compliance with iron-chelating therapy, family history of diabetes and pubertal status. Other studies have shown similar findings, reporting 10%, 10.4% and 5% cases with DM, and 7.1%, 14.6% and 8% cases with IGT respectively.[16-18] In our study its association has been found with age and BMI. Najafipour et al, also reported that the risk factors for impaired glucose metabolism were age, amounts of blood transfusion and duration of blood transfusion. The high prevalence of diabetes mellitus in patients with thalassemia is not only due to direct impairment of insulin excretory function by chronic iron overload. It has been reported that immune system activation against pancreatic beta cells due to pancreatic iron deposition through oxidative damage, act as an environmental factor that triggers the autoimmune response which, in turn, contributes to selective beta-cell damage. This shows that good iron chelation is an important factor for determination development of diabetes in these patients.

Chronic Hepatitis C has also a strong association with abnormal GTT; Khalifa et al reported 100% positivity for Hepatitis C in patients with abnormal GT. [17]Another cross-sectional study was carried out on prevalence of diabetes in HCV positive and HCV negative cases of Beta Thalassemia major patients and it was reported that the prevalence of Diabetes among adult beta Thalassemia patients was higher in HCV

positive cases as compared to HCV negative patients (15.2% vs. 1.9%, p: 0.02). However, no difference was observed in the younger (8-15 years) HCV positive and negative groups. It is probable that the existence of hemosiderosis makes the effect of HCV infection on glucose metabolism clinically more evident.[18]

A study done on Glucose homeostasis and relationship of oxidative stress in children and adolescents with β-Thalassemia major in Egypt reported that patients who are not on irregular chelation therapy had significantly higher fasting, 2-h post-load plasma glucose, serum ferritin, ALT, fasting insulin and HOMA-IR. They also observed that oxidative stress markers OSI and plasma MDA (plasma malondialdehyde, an oxidant marker) levels were significantly elevated while serum TAC (Total antioxidant capacity) level was significantly decreased in thalassemic patients compared with healthy controls. (P<0.001 for each). Moreover, HOMA-IR was positively correlated with age, serum ferritin, ALT, MDA, and negatively correlated with TAC.[19]Further such studies can be done in our population too.

Intensive combined chelation therapy (oral plus sub cutaneous) over a period of 24 to 36 months brings about improvement in the Glucose Metabolism Disorders.[20]A study conducted by Platis reported an overall prevalence of IFG =16.7% and that of DM 12.5%. They also reported that dry liver iron concentration (LIC) had significant correlation with serum ferritin levels (r = 0.512; p = 0.011) and impaired fasting glucose was significantly higher in BTM patients with very high LIC (>30 mg Fe/g dry liver) versus those with lower LIC (p = 0.044).[21]A cross-sectional study of impaired glucose metabolism was carried out in 48 β-thalassemic patients receiving hyper-transfusions. The prevalence of impaired glucose tolerance in thalassemic patients receiving hyper-transfusions with suboptimal iron chelating therapy was 12.5%. The study also reported that the clinical characteristics of thalassemic patients who developed impaired glucose tolerance were wasting, stunting, higher ferritin levels, splenectomy, and lower insulin level due to pancreatic β-cell damage.[22]

Conclusion and Recommendations

The study emphasizes the importance of regular follow-up of patients of β-thalassemia major for early detection and management of associated complications. Because not all of the patients with thalassemia major could be correctly diagnosed by fasting glucose alone, we preferred to use the oral glucose tolerance test rather than fasting blood glucose for the diagnosis of abnormal glucose tolerance in thalassemic patients. It is thus recommended that A 2-hour oral glucose tolerance testing, preferably combined with insulin secretion determination, should be performed at 10-12 years of age and annually thereafter. Moreover, if fasting serum glucose is >110 mg/dl at any stage, OGTT is indicated.

Conflict of interest: None to declare

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- **Mohammad Usman** was involved in Conception, Study Designing, Planning, Study Conduction, Analysis and discussion
- **Aftab Ahmad Khan** participated in Study Conduction, analysis and discussion
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