Sapporo Medical Journal
Volume 54, Issue 08, August, 2020

Evaluation of pancreatic dysfunction in non-diabetic patients with thalassemia major

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Abstract— Introduction: Frequent transfusions in people with thalassemia put them at risk for the effects of increased iron load, leading to dysfunction of a number of organs such as the heart and endocrine glands including the liver and pancreas in these patients. Therefore, the aim of this study was to investigate the rate of pancreatic dysfunction in non-diabetic patients with thalassemia major. **Materials and Methods:** This study was a group historical study. Forty patients with non-diabetic thalassemia major (case group) and 40 healthy children based on age and BMI were included and for both groups the Oral Glucose Tolerance Test (OGTT) was concluded; glucose, fasting insulin, C-peptide levels, Serum ferritin and plasma sugar were measured two hours after receiving glucose. The insulin resistance index (HOMA IR) and beta cell function index (HOMA-B) were calculated based on the evaluation model (HOMA). SPSS software version 20 and SAS version 9.1 were used for statistical analysis of the data. **Results:** average fasting blood glucose ($P = 0.050$), insulin ($P <0.001$), C-peptide ($P <0.001$) as well as insulin resistance index ($P <0.001$) and beta cell function index ($P <0.001$) in the case group was significantly higher. According to the OGTT test, none of the individuals in the case-control groups had diabetes, the prevalence (IGT) in the case group and control was 22.5% and 2.5%, respectively, which were significantly higher in the case group ($P = 0.007$). According to the following analysis of ROC curve, four factors including transfusion number, HOMA-IR index, fasting glucose level and blood insulin level, had a high value in predicting impaired OGTT in patients with thalassemia major. **Conclusion:** It can be concluded that fasting glucose and insulin measurement and HOMA IR calculation are screening methods that reduce the need for OGTT in all thalassemia major patients and identify high

risk patients before irreversible damage to pancreatic cells, in order to take the necessary measures to prevent diabetes.

Keywords: Pancreas, thalassemia major, diabetes.

Introduction

Thalassemia is a disorder caused by a decrease or failure to produce globin chains. This reduction in production results from mutations in the genes of globin chains and disrupts oxygen delivery in infected patients (1,2). About five percent of the world's population suffers from hemoglobin-related diseases, of which thalassemia carriers account for about 1.7% (3). The disease affects both males and females equally, accounting for about 4.4 per 100,000 births (4,5). Under physiological conditions, alpha and beta genes play the most important role in the production of the hemoglobin chain. Two pairs of alpha genes and one pair of beta genes are involved in the synthesis and formation of normal hemoglobin chains (6,7). The classification of this disease is based on the reduction or non-production of each of the globin chains α, β, βδ or δβγ (8,9). Patients are specified with symptoms of chronic and severe anemia, lack of proper growth, spleen and liver enlargement, and bone disorders especially in the head and face, which are associated with changes in appearance (10).

Beta thalassemia is a type of hemoglobin disorder that, like alpha thalassemia, causes a decrease or non-production of hemoglobin. The gene encoding this chain is located on the short arm of chromosome 11 and is about 50 kilobits. This gene contains three exons that are separated by two introns (11). So far, more than 700 mutations have been reported in the beta gene, and it is estimated that one percent of the world's population, or about 200 million people, carry the beta thalassemia gene (12). Based on clinical manifestations, beta thalassemia is divided into three groups: minor, intermedia and major, and the latter is the most severe type of anemia in this group of patients which is caused by the inheritance of two defective alleles ($\beta^0\beta^0$ and $\beta^0\beta^+$) (13).

The importance of examining pancreatic disorders in thalassemia patients has led to a large number of findings emphasizing that the development of diabetes in beta thalassemia patients is provoked through both direct iron toxicity to pancreatic B-cells and insulin resistance (14). Chronic and severe iron poisoning in patients with thalassemia major causes endocrine dysfunction. One of these disorders is pancreatic damage by iron deposition, which leads to diabetes mellitus in 7 to 10% of patients over the age of 20 (15).

At present, oral glucose tolerance testing is recommended for prognosis and diagnosis of diabetes mellitus, which is performed periodically for all patients older than 14 years. Patients with a family history of diabetes should be tested at shorter intervals. It has been shown that early detection of glucose tolerance disorder and the use of iron and dietary repellents could normalize glucose tolerance testing or delay the onset of diabetes (16). However, pancreatic dysfunction in these patients is not limited to endocrine system, pancreatic exocrine secretion is also affected along with lack of lipase secretion, elastase field for malnutrition, weight loss, and secondary infections (17). Considering the above, in this study we decided to investigate the rate of pancreatic dysfunction in non-diabetic patients with thalassemia major.

Materials and methods

This study is a group historical study. The study population included healthy children without thalassemia major referred to the subspecialty pediatric clinic of "Hazrat Ali Asghar Hospital ,Tehran, Iran " as a control group and children with beta thalassemia major referred to the above clinic who based on the oral glucose tolerance test (OGTT) did not diagnose with diabetes, and at least 7 years had passed since their blood transfusions, and they had entry and exit criteria.

Inclusion criteria

1. Having informed consent
2. hepatitis B and C negative in all people

Exclusion criteria

1. Having other metabolic diseases, including hypo and hyperthyroidism
2. Taking medications that interfere with glucose metabolism

Procedure

All patients were monitored as part of a regular blood transfusion program every 20 to 25 days to keep the average hemoglobin level above 9 g / dL before transfusion. All patients with thalassemia were treated with iron chelator or defroxamine 5 nights a week. The response to iron chelator treatment was assessed by the mean serum ferritin level taken by blood tests every four months. It should be noted that to measure the parameters, the time of fasting for 8 hours has been observed as much as possible. Fast glucose levels, two-hour plasma glucose after glucose uptake (1.75 g / kg), c-peptide, and insulin and ferritin levels were measured in all participants. Based on the measured values, HOMA (Homeostasis Model Assessment) was used to assess insulin sensitivity.

Insulin resistance Index (IRI)

$$\text{HOMA IR} = \frac{G_0\ (\text{mg/dl}) \times I_0(\text{mu/ml}) \times 0/0555}{22.5}$$

I_0= fasting insulin, G_0 =fasting glucose

Insulin Release index was used to evaluate the secretion of B cells in the fasting state based on the HOMA model, which is as follows;

$$\text{HOMA} - \text{B} = \frac{[20 \times I_0(\text{mu/ml})]}{[(G_0(\text{mg/dl}) \times 0/0555) - 3/5]}$$

Patients were then divided into three groups with Impaired fasting glucose (IFG) Impaired glucose tolerance (IGT) and Normal fasting glucose (NFG) and HOMA-B division between the two groups were compared. Estimation of HOMA-IR insulin sensitivity was assessed in 3 groups and also the amount of plasma C-peptide levels correlation with ferritin, HOMA-B and HOMA- IR were measured.

Data analysis method

The results were expressed as a mean and standard deviation for quantitative variables and as a percentage for class qualitative variables. Comparison between quantitative variables was performed by T-Test or if there was abnormal distribution by Mann-Whitey test. Comparisons between qualitative variables were also performed using Chi-square test or Fisher's exact test. The correlation between quantitative variables was investigated using Pearson Correlation Coefficient and Spearman Rank Correlation. SPSS software version 20 and SAS version 9.1 were used for statistical analysis of the data. Level of less than 0.05 was considered significant.

Ethical considerations

Patients were explained about the goals and how the plan was being implemented, and they were assured that the information about their illness would only be provided to the plan implementer. They were assured that if they did not accept the invitation to participate in the study, their treatment would be routine. Also, there were no charges for this study. All stages of the project were approved by the ethics committee of the Center for Growth and Development of the Institute of Metabolic Endocrinology of Iran University of Medical Sciences.

Results

In the present study, a total of 80 people entered the study and were divided into two groups. Group one included 40 patients with non-diabetic major thalassemia (case group) and group two evaluated 40 healthy individuals without thalassemia major (control group). In terms of age, the average age of patients in the two case and control groups was 6.9 ± 22.2 years and 6.99 ± 22.8 years, respectively, and there was no difference between the two groups (P-value = 0.665). In terms of pancreatic parameters, the mean fasting blood glucose in the two case and control groups was 9.41 ± 94.93 and 8.04 ± 91.03 mg / dL, respectively, and considering that the P-value was 0.05, it is statistically significant. Average blood glucose two hours after receiving the glucose was shown in Figure 1 and was 18.11 ± 124.93 and 11.49 ± 121.7, respectively, with no difference between the two control groups (P-value = 0.345).

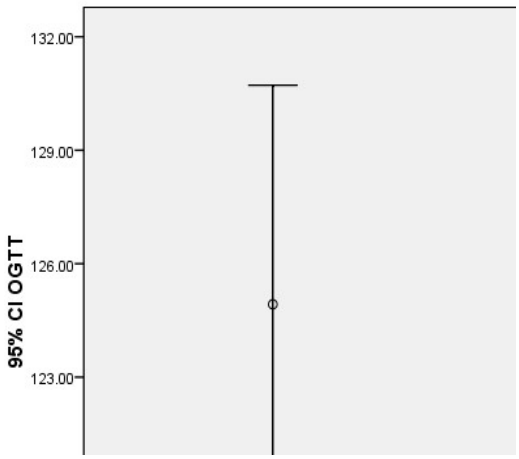

Figure 1: Average OGTT in both cases and controls

The mean insulin resistance index or IR HOMA in the case group was 2.1 ±0.81 and the control group was 1.13 ± 0.58, which was significantly higher according to the results obtained in the case group (P00 value = 0.001). The mean performance index of beta cells or HOMA-B in the case group and control was 107.83 ± 41.08 and 15.99 ± 8.2, respectively, and considering that the P-value was 0.001, it was statistically significant. The mean blood insulin levels in both of the case and control groups were 8.9 ± 3.17 and 4.95 ± 2.27 mg / dL, respectively, and were statistically significant given that P-value = 0.001. Also, the mean level of peptide C in the two case and control groups was 4.51 ± 1.69 and 2.69 ± 1.02 g / dL, respectively, which is statistically significant (P-value = 0.001).

In Figure 2 in the case group, there is a significant but inverse correlation between OGTT and blood insulin levels (r Correlation of 0.668, P-value = 0.001).

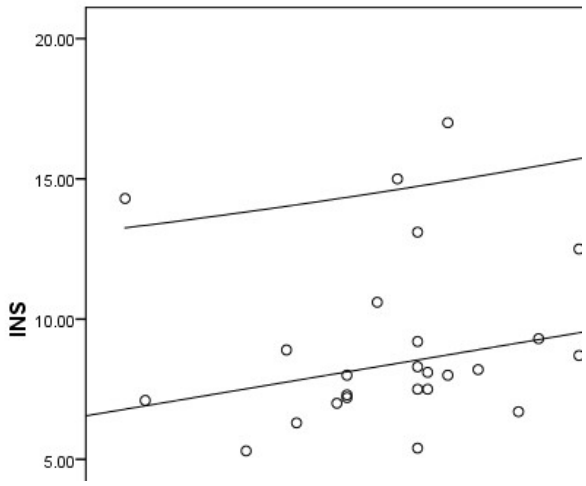

Figure 2: Linear correlation between blood sugar two hours after glucose uptake and blood insulin levels in the case group

Also, in Figure 3, there is a significant and direct relationship between OGTT and HOMA IR index (r correlation equal to 0.39, P-value = 0.014).

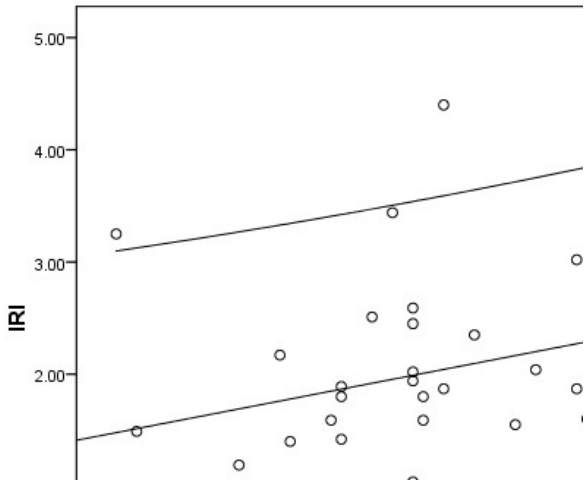

Figure 3: Linear correlation between OGTT and IRI index in the case group

And in Figures 4 and 5 in the case group and control of fasting blood glucose level with HOMA IR index with direct correlation (r correlation equal to 0.429, P-value = 0.006) and with HOMA-B index with inverse correlation (r correlation equal to -0.62, P-value = 0.001).\

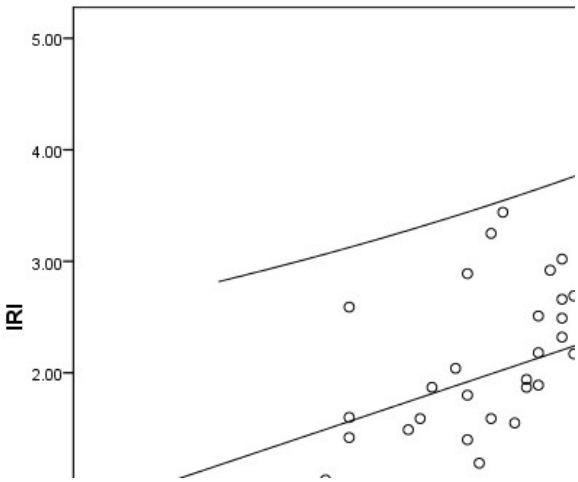

Figure 4: Linear correlation between FBS and IRI index in the case group

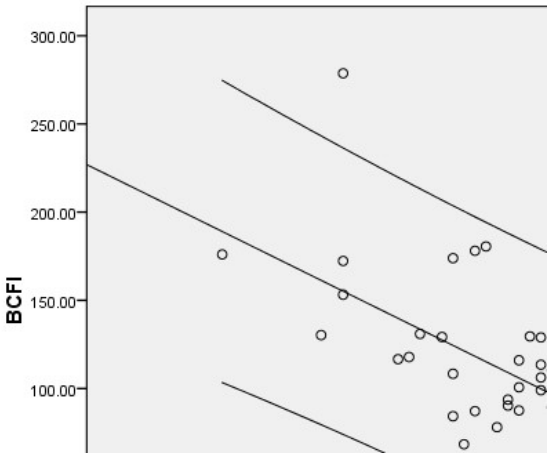

Figure 5: Linear correlation between FBS and HOMA-B index in the case group

Serum ferritin and peptide C concentrations were not correlated with any of the glucose, insulin, ALT, HOMA IR, or HOMA-B indices. In the healthy group, there was no correlation between OGTT and none of the glucose, insulin, ALT, HOMA IR, or HOMA-B indices. The case group was then divided into two groups, IFG and NFG, and compared with the control group in terms of fasting insulin levels, HOMA IR, and HOMA-B. In the results obtained in Figure 6, the levels of insulin and HOMA IR in the control group with each of the IFG and NFG groups were significantly different (P-value = 0.001), but statistically there was no significant difference between the IFG and NFG groups.

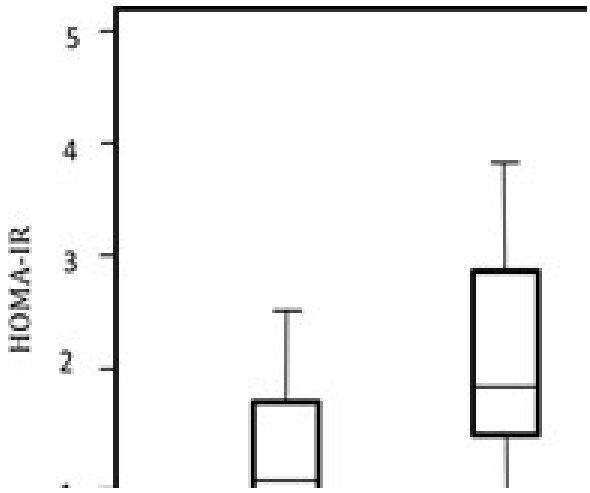

Figure 6: HOMA-IR relationship with the case and control groups

In Figure 7, the HOMA-B index in the control group had a significant difference with each of the IFG and NFG groups (P-value = 0.001) and also a significant difference was obtained between IFG and NFG groups (P-value = 0.001).

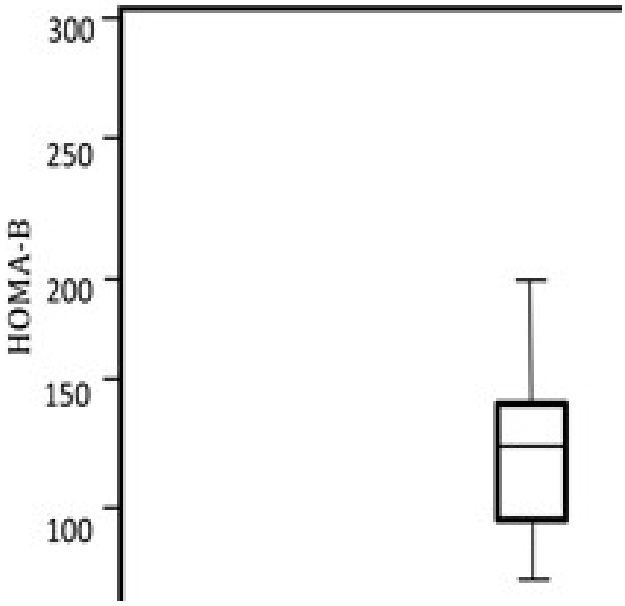

Figure 7: HOMA-B relationship with the case and control groups

Based on OGTT above 140 mg / dL, the prevalence of OGTT was disturbed in the case group and the control group was 22.5% and 2.5%, respectively, which was significantly higher in the case group (P-value = 0.007). Accordingly, the prevalence of impaired OGTT is 11.3 times higher in patients with thalassemia (Confidence Interval 1.36 to 94.34) than in healthy individuals. Based on ROC analysis, the four factors of patients age (submaximal level 0.775) fasting blood glucose levels (0.748 below the curve), blood insulin level (0.767 below the curve) and HOMA IR (0.79 below the curve) had a significantly higher OGTT prediction disorder in the case group.

In this regard, the best cutoff for fasting blood glucose is 96.5 mg / dl (100% sensitivity and 71% specificity), for blood insulin levels equal to 9 micU / ml (87.5% sensitivity and 67.7% specificity) and for HOMA IR index 2.47 (Sensitivity was 87.5% and characteristics were 71%) and for patients aged 24.5 years, OGTT prediction was impaired in patients with beta-thalassemia. Other indicators, such as ferritin levels, peptide C, serum ALT levels, or HOMA-B index, did not have predictive capability for impaired OGTT.

Discussion

Glucose metabolism disorders and impaired glucose tolerance testing have been shown to be confirmed in patients with major thalassemia. In patients with beta-thalassemia, treatment with repeated injections is significantly expanded, but this is accompanied by an acute and chronic accumulation of iron, which is itself associated with metabolic and endocrine disorders. In fact, the accumulation of iron affects the tissues that regulate the hemostatic mechanisms of carbohydrates, such as the pancreas and liver. Some risk factors for endocrine disorders, including pancreatic beta cells, have also been identified, such as older age, Increased frequency of blood perfusion, high ferritin levels, familial diabetes mellitus, underlying liver dysfunction, and genetic changes (18).

What we studied in the present study was an assessment of pancreatic dysfunction in non-diabetic patients with major thalassemia beta. In the present study, we found that patients with beta-thalassemia experienced an increase in fasting blood glucose, an increase in insulin resistance index, an increase in beta cell dysfunction and a decrease in blood hemoglobin levels, which confirms significant disturbances in glucose regulation and metabolism mechanisms such as insulin resistance. On the other hand, the present study found that a significant proportion of patients with beta thalassemia major (22.5%) were diagnosed with OGGT disorder (2.5% in the control group) which will put them at risk for developing diabetes mellitus in the future. In line with the present study, other studies have confirmed or hindered the development of pancreatic dysfunction in thalassemia major. In a 2016 study by Mokhtar et al., 40% of patients with thalassemia major had impaired glucose tolerance testing that significantly exceeded the prevalence obtained in our study (19). In a 2015 study by Ghergherehchi et al., Patients with thalassemia

had a 33% higher prevalence of glucose tolerance than controls, which was in line with our study, also glucose levels were significantly higher in patients. But contrary to our study, an increase in liver enzymes was observed in their study, which did not change in our study (20).

In a 2015 study by Bas et al., There was a significant positive correlation between insulin resistance index or HOMA-IR and hepatic iron load, as well as an inverse correlation between T2 pancreas and fasting glucose concentration, which basically justified pancreatic dysfunction following iron overload (21). In Li et al .'s study, beta-thalassemia patients had lower pancreatic volume and fasting normal glucose levels were significantly higher than normal (22), which was consistent with our study. In a 2012 study of 59 patients, Noetzli et al. Had 12 cases of glucose tolerance disorder, which accounted for 20% of the prevalence, which was quite similar to the frequency of our study. Insulin resistance was also strongly associated with inflammatory markers as well as high iron overload (23).

Another result of our study was the significant HOMA-IR difference between the IFG and NFG and control groups, but the IFG and NFG groups did not differ significantly. The control group's HOMA-B also had significant differences with the IFG and NFG groups, on the other hand Significant differences were found between IFG and NFG groups, and the results were consistent with Angelopoulos' study (24). In contrast with our study was the study of Suvarna in 2006, which was performed in the age group of 8 to 15 years and no cases of IGT were observed (25).

Based on IGT, the best cutoff for fasting blood glucose was 96.5 mg / dl, and 9 micU / ml for fasting insulin levels and moreover a Noetzli study for IGT patients, indicated 97 mg / dl Cutoff for fasting blood sugar and 9 micU / ml fasting insulin levels, which was in line with our study (23).

Conclusion

Based on the results, it can be concluded that fasting glucose and insulin measurement and HOMA IR calculation are screening methods that reduce the need for OGTT in all thalassemia major patients and identify high-risk patients before irreversible damage to pancreatic cells to take steps to prevent diabetes.

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