Study of Insulin HormoneLevel and β-Cell Function in Children withβ-Thalassemia Major

Abstract

Background: Diabetes and Impaired Glucose Tolerance are amajor complication of iron overload in patients with beta thalassemia major.
Aim: To study the serum level of Insulin hormone and beta cell function in β- Thalassemia major patients.
Methods: This study was carried out on 50 children that were divided into:-**Group (1):** thirty child with beta thalassemia with age ranged between (5 to 10 years), **Group (2):** Twenty apparently healthy matched age children were also included as control group. Both groups were subjected to history taking, full clinical examination, anthropometric measures, laboratory investigation that included:-, complete blood picture, Serum iron, serum ferriten,fasting serum glucose, 2h postprandial serum glucose, fasting serum insulin by ELISA(Sandwish assay procedure.) IRI, BFI.
Results: There was highly statistical significant difference between the patient and control groups in the insulin resistance index (IRI), but there was decrease in beta cell function index (BFI) in patients group with no significantdifference.
Conclusion: Thalassemic child is at risk of IR which is responsible for abnormalities in glucose metabolism so proper management and regular follow up is necessary to early diagnosis and prevention of complications.

Key words:thalassemia,diabetes mellitus, impaired glucose tolerance andoral glucose tolerance test

Introduction

Thalassemia is inherited blood defect having lifelong implications in the life of the patients and their relatives. This hereditary condition results in defective production of the quaternary protein "haemoglobin-Hb". Hemoglobin, a component of red blood cells.Hb is made up of a pair of α and β globin chains. Thus, the levels of both the globin chains should be equal (1)**.**

Beta thalassemia is characterized by reduced or absent β globin chain synthesis. The gene encoding for β globin is located on chromosome 11. More than 200 point mutations and rarely some deletion can cause β thalassemia (2)**.**

However,frequent blood transfusions lead to chronic iron overload which cause endocrine problems, especially metabolism of glucoseas diabetes mellitus (DM) and impaired glucose tolerance (IGT)**(3).**

Levels of glucose in blood are regulated by two endocrine hormones secreted by the organ pancreas: insulin and glucagon. Insulin is required to lower the blood glucose levels. Hence, it helps in the breakdown of glucose. Glucagon on the other hand is increasing the blood glucose levels. Both the hormones work together and maintain the normal glucose concentration in the blood (3)**.**

Accumulation of iron in the pancreas causes the defective insulin secretion and leads to insulin deficiency. However, other studies also showed the presence of hyperinsulinemia and IR (rather than insulin deficiency) suggesting the involvement of insulin resistance in producing impairment to glucose homeostasis. Hyperinsulinemia may result from reduced hepatic clearance, which may be associated to iron overload (4)**.**

The causes for glucose metabolism abnormalities are not clear : either IGT with hyperinsulinemia and reduced insulin sensitivity are indicative of insulin resistance in these patients, while insulin deficiency secondary to iron induced pancreatic β-cell defects are considered as another possible mechanism of glucose abnormalities (5)**.**

Aim of the work :

The aim of this work was to evaluate β-cell function of pancreas and glucose metabolism abnormalities in β- Thalassemia major patients.

Patients and Methods:

A-Patients:

This study was conducted on 30 children with beta thalassemia major who were admitted to Hematology unit, Pediatric department, Tanta University Hospitals, and 20 healthy children of matched age and sex as a control group. This study was conducted after taking a consent from parents of studied patients and approval from ethical comittee of research center in Tanta university hospitals.

*** Inclusion Criteria:**

Children between (5-10) years old diagnosed with β-Thalassaemia clinically and hemoglobin electrophoresis who are receiving regular blood transfusion (over period of 12 month) to maintain a Hb level not more than 9.5g /dl and receiving Desferoxamine as achelator by intravenous infusion orExjade orally.

*** Exclusion Criteria:**

1. Children with hepatitis B or C.
2. Family history of DM in 1st degree relatives.
3. Children who receiving medication known to cause glucose intolerance such as steroids.

B- Methods:

All children included in this study were subjected to:

I-History taking with a special account on:

-Age of patients.
- Age of diagnosis of β-thalassemia .
- Age of the first blood transfusion.
-Duration of regular transfusion therapy.
-Age of start of chelation therapy.
-Type of chelation used.
-Dose and regularity of chelation therapy.

2-Thorough clinical examination with a special account on:

- Weight, height, span, upper and lower body segment.
- Head and neck for: Pallor, Jaundice, mongoloid facies.
- Abdominal examination for: hepatomegaly and splenomegaly.

3- **Laboratory investigation including**:

-Complete blood count.
-Serum iron.
-Serum ferritin.
-ALT,AST,BUN,Creatinine.
-Fasting serum glucose and 2-h post prandial serum glucose.
- Fasting serum insulin.

Statistical analysis: Results were analyzed using SPSS (ver. 25.0; IBM, Chicago, IL, USA). Quantitative data was displayed in the form of mean ± standard deviation (SD). Qualitative data was demonstrated through figures of frequency and percentage. Charts were used to illustrate data and relations where appropriate and $p < 0.05$ was accepted as indicating statistical significance.

Results:

This case control study was conducted in Hematology Unit, Pediatric Department, Tanta University Hospital after being approved by the department ethical Committee. The study population included 50 children who were divided into two groups:

Control group: This included twenty (20) healthy children of matched age and sex.

Patient group : This included thirty (30) children diagnosed with β-Thalassaemia since more than two years by clinical features and hemoglobin electrophoresis and receiving regular blood transfusion (over period of 12 month).

Table (1): Demographic data of the Patient groups.

	Patient group (n=30)	Control group (n=20)	Test value	P value
Age(years)	7.5± 1.83	7.5± 1.73	0.00	1.00[1]
Gender Male Female	19(63.3%) 11(36.7%)	11(55%) 9(45%)	0.347	0.556[2]
Family historyofconsanguity Positive Negative	9(30%) 21(70%)	2(10%) 18(90%)	**3.182**	**0.003*[3]**
Height(cm)	124.95±11.5	127.5±16.8	0.598	0.553[1]
Weight(kg)	31.7±7.3	32.8±7.4	0.481	0.632[1]
BMI(kg/m^2)	18.8±2.5	20.4±1.97	**2.147**	**0.037*[1]**

1. Abbreviations: **BMI:** body mass index**.**
2. Independent t-test used; 3. Chi-square test used; 4. Fisher exact test.

*Statistical significant when p-value <0.05.

There was no statistically significant difference between studied groups as regard age and sexwhile there was significant lower BMI in patients group P>0.05 in comparison to control group table(1).

Table (2): Incidence of clinical presentation in Patient group.

	Patient group

	n=30	%
Pallor	20	66.7
Jaundice	9	30%
Mongoloid Face	12	40%
Splenomegaly	15	50%
Hepatomegaly	11	36.7%
Splenectomy	15	50%

Pallor and splenomegaly were the most common presenting symptoms (66.7% and 50%, respectively). While splenectomy was presented in 50% of patients.

Table (3): Comparison between studied groups as regard complete blood picture:

	Patient group (n=30)	Control group (n=20)	Test value	p-value
Hemoglobin (g/dl) Range	7.3 ± 0.8 (5.6-10.2)	13.9± 1.4 (11.5-16)	**5.73**	**<0.001*** [1]
MCV (fl) Range	53.2±2.5 (46-60)	72.4±3.2 (62-83)	**4.84**	**<0.001*** [1]
MCH(pg) Range	18.8±1.8 (12-25)	22.3±1.2 (18-28)	**3.53**	**<0.001*** [1]
MCHC(g%) Range	35.2±3.0 (26-44)	48.3±2.7 (45-57)	**2.98**	**<0.001*** [1]
WBCs($10^3/\mu L$) Range	9.817±1.765 (4-11)	10.514±0.816 (4-11)	0.986	0.412[1]
Platelets ($10^3/\mu L$) Range	179.342±23.645 (150-230)	218.752±19.824 (160-280)	1.06	0.308[1]

Independent t test
* Statistical significant when p-value <0.05.

As regard Hb, MCV, MCH and MCHC were significantly lower among Patient group (p<0.001)while, WBCs and platelets count were lower among Patient group but with statistically insignificant differences as p >0.05.

Table (4):comparison between studied groups as regard Liver and kidney function tests :

	Patient group (n=30)	Control group (n=20)	Test value	p-value
Creatinine (mg/dL) Range	0.79±0.15 (0.42-1.1)	0.70±0.14 (0.40-1.0)	2.158	0.36[1]
BUN (mg/dL) Range	14.53±2.7 (7-22)	14.18±3.05 (6.8-21.5)	0.427	0.671[1]
AST (U/L) Range	48.4±19.2 (39-110)	24.7±3.12 (14-35)	**5.473**	**<0.001*1**
ALT (U/L) Range	68.7±27.7 (42-115)	24.8±3 (15-36)	**7.032**	**<0.001*1**

BUN: blood urea nitrogen; **AST:** aspartate aminotransferase; **ALT**: alanine aminotransferase;
Independent t test

* Statistical significant when p-value <0.05.

β -Thalassemia major patients had significantly higher levels of ALT (68.7 ± 27.7 vs. 24.8 ± 3; p<0.001), and AST (48.4 ± 19.2 vs. 24.7 ± 3.12; p<0.001) in comparison to the control group.

Table (5): Serum insulin levels among study groups

	Patient group (n=30)	Control group (n=20)	Test value	p-value
Plasma insulin (mg/dL) Range	10.23±1.76 (7-14)	8.01±0.68 (6-10)	**5.572**	**<0.001*** [1]
FBS (mg/dL) Range	97.6±8.75 (81-119)	90.5±5.37 (70-105)	**3.216**	**0.002*** [1]
2hpp (mg/dL) Range	97.2±8.27 (80-116)	96.1±16.4 (78-110)	**0.326**	**0.031*** [1]
IRI Range	2.73±0.26 (2-3.5)	1.76±0.14 (1.3-2)	**17.399**	**<0.001*** [1]
BFI Range	117.8±11.8 (80-150)	123.5±10.9 (90-155)	1.742	0.08[1]
OGTT Normal IFG IGT DM	20(66.7%) 7(23.3%) 0(0%) 3(10%)	19(95%) 1(5%) 0(0%) 0(0%)	**6.156**	**<0.001*** [2]

Abbreviations: **OGTT:** oral glucose tolerance test**; FBS:** fasting blood sugar; **2hpp**: 2-hour postprandial plasma glucose; **IRI:** insulin resistance test; **BFI**: b cell function index
Independent t test; 2.Fisher exact test.
*Statistical significant when p-value <0.05.

As regardPatient group had significantly higher levels of FBS (97.6 ± 8.75 vs. 90.5 ± 5.37; p = 0.002) in comparison to the control group. They also have a significantly higher insulin resistant index than the control group (2.73 ± 0.26 vs. 1.76 ± 0.14; p< 0.001).

As regard BFI,was reduced in β -Thalassemia patients but the difference was not significant. The OGTT of the 30 β -Thalassemia patients, showed that 10 (33.3%) had abnormal results, three (10%) were diabetic and 7 (23.3%) had IFG. Fig(2,3,4,5)

Figure (1): Serum insulin level among study groups

Figure (2): FBS level among study group s

Figure (3): 2hpp level among study groups

Figure (4): IRI level among study groups

Figure (5): OGTT results among study groups

Table (6): Iron levels among study groups

	Patient group (n=30)	Control group (n=20)	Test value	p-value
Serum Iron	160.8±22.12	79.6±11.24	**17.06**	**<0.001*[1]**
Serum Ferritin	3008.03±279.2	69.8±34.3	**57.01**	**<0.001*[1]**

Independent t test

* Statistical significant when p-value <0.05.

As regard, Patient group had significantly higher levels of serum iron and ferritin (p<0.001) with mean and standard deviation of(160.8±22.12 vs. 79.6±11.24, 3008.03±279.2 vs. 69.8±34.3, respectively).

Table (7): Correlation betweenIRI, BFI and laboratory data

	IRI		BFI	
	r	p-value	r	p-value
BMI	**-0.282**	**0.047***	-0.130	0.369
AST	**0.523**	**<0.001***	0.339	0.139
ALT	**0.637**	**<0.001***	0.330	0.818
Serum iron	**0.806**	**<0.001***	0.254	0.075
Serum ferritin	**0.906**	**<0.001***	0.230	0.108
IRI	---	---	**0.807**	**0.035***
BFI	**0.807**	**0.035***	---	---
Age at thalassemia diagnosis	0.092	0.322	0.113	0.298
Mean duration of transfusion	0.107	0.210	0.098	0.427
Mean age at start of chelation	0.084	0.427	0.121	0.335

*Abbreviations: **BMI:**body mass index**; AST:** aspartate aminotransferase; **ALT**: alanine aminotransferase; **IRI:** insulin resistance test; **BFI**: b cell function index*

As regard BFIhad significant direct correlation with IRI. IRI had significant direct correlations with AST, ALT, serum iron and serum ferritin, while had significant negativecorrelation with BMI.Fig(6,7)

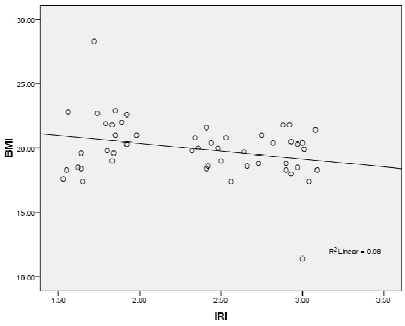

Figure (6): Correlation between IRI and BMI.

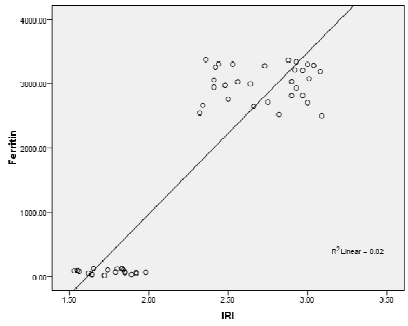

Figure (7): Correlation IRI and serum ferritin.

Discussion

β- Thalassemia major is a serious genetic disease which often treated by frequent blood transfusions (6). SO regular blood transfusions in b-TM has improved the quality of life and also increased survival of these patients; however,frequent blood transfusions lead to chronic iron overload that may cause endocrine problems, especially metabolism of glucose including diabetes mellitus (DM) and impaired glucose tolerance (IGT) (7).

The aim of this work was to evaluate β-cell function, Insulin resistance and abnormalities in glucose metabolism in β-Thalassemia major patients.

The current study was carried out onThirty (30) child with packed RBcs transfusion dependentβ-Thalassaemia Major who were admitted in Hematology Unit, Pediatric Department, Tanta University Hospital and

Twenty (20) healthy child of matched age and sexserved as a control.

In the current study the mean age of thalassemic children in our study was 7.5.1 ± 1.83 years Our study population had a relatively younger age group when compared to other studies, As in, **Chen, *et al***(8)where the mean the cases were 14.8 ± 6.9.

In the current study male predominance was found between Patient groups males represent (63.3%) while female represent (36.7).

This is in agreement with **Islam *et al*** (9)***with which reported a male to female ratio of 1.26.***

Our result is not in agreement with a similar study carried out in Hong Kong that found that the male to female ratio was equal among their studied thalassemic cases.(10)

In the current study it was found that BMI was significantly lower in Patient group than control group

This is in agreement with **Dey, Konwar, Sarkar**(11)who found that underweight (Low BMI) was a common finding in thlalassemia patients specially when they were older than 10 years of age.

The cause of under nutrition in thalassemia major patients is multifactorial. Endocrinopathies including hypothyroidism, hypo-gonadotropic gonadism, growth hormone deficiency, hypo-parathyroidism are the major causes of under nutrition in these patients.(11)**.**

In the current study we founded that mean age of start of chelation theapy was 4.32±2.52.

This is in agreement with **Al-Kherbash , Al-Awdi , Hasan**(12)**who** found that earlier initiationof chelating therapy in 19.7% of the cases at age less than 3 years led to the best outcome of the cases, with no mortality. Furthermore, out of the 19 (28.9%) cases in whom the first chelating therapy was started between 3 and 6 years of age, 16 (90%) patients remained alive and only 10% of these patients died. The mortality rate was high (50%) among those in whom the first chelating therapy was started at the age of 11 years and older. Thus, earlier initiation of chelating therapy led to better outcomes and reduced the mortality rate, with a highly statistically significant difference (*P*=0.0190).

In the current study, pallor, jaundice, mongloidfacies, hepatomegaly and splenomegaly were the main presenting clinical manifestations in almost patient group as there is 66.7% had pallor while 40% had mongoloid facies.

Splenomegaly appeared in 50% of patientgroup,while hepatomegaly appear in 36.7 % of patientgroup. Also, history of splenctomy appear in 50% of Patient group .

This is in agreement with**Galanello**(13)who found that patients with thalassemia major have a severe

microcytic hypochromic anemia with low mean corpuscular volume and mean corpuscular hemoglobin.

In the current study, it was found that mean serum iron and ferritin levels were significantly higher in Patient group than control group.

This in agreement with **Kurtoglu et al** (14) **and Taher et al** (15) who found significantly higher serum iron and serum ferritin and significantly lower TIBC in patients with thalassemia major compared with normal control individuals.

In the current study it was found that liver enzymes were significantly higher in Patient group with mean of (48.4±19.2 & 68.7 ±27.7) of AST and ALT respectively than the control group (24.7±3.12 & 24.8±3) .

This is in agreement with**Sedigheh et al** (16)who noted the similar findings from study in Iran with significantly raised liver enzymes (ALT, AST) in homozygous thalassemia major patients than in controls.

Also in agreement with **Suman et al**(17)who noted positive correlation was between number of transfusions and serum ferritin level with correlation coefficient (r=+.33). As Iron deposition in liver takes place, its functions are affected which are predicted by raised ALT and AST. There was a positive correlation between serum ferritin and liver enzymes (r=0.87±84).

Asharaf S et al (18)who observed during a study that some disturbances occur in liver functions in hepatitis negative thalassemia patients with iron overload.

Barton et al(19)noted similar results as serum ferritin increases liver enzymes also increases.

In the current study the Patient group had a significantly higher insulin resistance index with mean of (2.73±.26)while was (1.76±.14) in control groupwhile BFI was reduced in Patient group than control but the difference was not significant.

This is in agreement with **Suvarna, et al** (20)who noted 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.The increase in insulin levels has been postulated due to reduced hepatic insulin extraction rather than an increase in the secretory response.

This is in agreement with**Ghergherehchi and Habibzadeh,**(21) **who** documented that, β-TM patients had significantly higher IRI and elevated transaminases than the control group; but the decrease in BFI was not significant.

In the current study, it was found that Patient group showed abnormal. OGTT results in 26.3% of which is significantly higher than control group(5%).Three was diabetic (10%) and 7 were IFG (23.6)

This is in agreement with**Ghergherehchi and Habibzadeh,** (21)who documented that abnormal OGTT results were observed in 33.3% of b-TM patients, which was significantly higher than in the control group (7.5%). Diabetes mellitus and IFG prevalence in b-TM patients were 7.7 and 25.6%, respectively. The prevalence of abnormal OGTT, DM and IFG in this study is similar to the literature (**Noetzli et al., 2012**) (22).

In another study from Iran, the prevalence of DM and IFG was 5.1 and 7.7%, respectively (**Shams et al., 2010**) (23).It is possible that these differences were mostly related to the geographical location of the studied population, duration of the diagnosis, number of patients and chelation therapy in each study

Conclusion

From this work we can conclude that:

Children with beta thalassemia on long term blood transfusion is at risk of abnormalities in glucose metabolism as IGT,DMso proper management by proper chelation and regular follow up of glycemic indices is necessary to early diagnosis and prevention of complications

Recommendations

1. Regular follow up for patients with thalassemia for early detection of glucose abnormalities in order to improve their quality of life.
2. Medical education for thalassemic patients and their family members about importance of chelation therapy and the dangers of iron overload.
3. Wide scale study in multicenter is needed.

CONSENT AND ETHICAL APPROVAL

This study was conducted after taking a consent from parents of studied patients and approval from ethical comittee of research center in Tanta university hospitals.
History taking, carful clinical examination, specific investigational studies

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