



Insulin-Dependent Diabetes Mellitus and Thalassaemia

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ABSTRACT

Background: Thalassaemias are disorders of hemoglobin structure or synthesis. The risk of endocrine dysfunction as insulin dependent diabetes mellitus (IDDM) in patient affected with thalassaemia may be induced and other different related disorder may occur.

Objective: The aim of this study was to evaluate the prevalence of IDDM among Saudi thalassaemic children, also to detect insulin release among previous subjects.

Material and Methods: This study was performed on 40 thalassaemic children; also 40 apparently healthy subjects were included as a control group. History taking and clinical examination were performed among all studied groups. A morning venous blood sample was withdrawn for measuring of IL-1 beta and Tumor necrosis alpha (TNF- alpha) using ELISA technique. Oral glucose tolerance level was performed (OGTT) also, serum glucose, insulin release and serum ferritin was measured.

Results: The levels of IL- 1beta and TNF- alpha were 13.1 ± 14.5 and 328 ± 341.4 respectively. Serum ferritin was highly raised among thalassaemic children, with high statistical significant differences between patients and controls. No statistical significant differences were recorded between the investigated groups according to age, weight, height, sex and consanguinity, while significant difference was recorded according to liver Spain. The mean blood glucose of thalassaemic children and control were 3.8 ± 0.7 & 5.3 ± 0.9 in 0 minutes, 5.8 ± 0.5 & 8.3 ± 1.5 in 1 hour, and 4.7 ± 0.7 & 7.8 ± 1 in 2 hour respectively. Statistical significant differences were recorded at 0, 1 and 2 hours. Insulin release after 5 minutes of intravenous glucose injection was less than the control (492.31 ± 385.34 & 713.9 ± 594.0) in thalassaemic and control respectively, non statistical significant differences was recorded between them.

Conclusion: Thalassaemic children may be susceptible to diabetes or pre –diabetic stage, this may predispose by multiple blood transfusion and deposition of iron in islet in beta –cell which decrease insulin level and causes hyperglycemia.

KEY WORDS: Insulin-Dependent Diabetes; Mellitus and Thalassaemia.

INTRODUCTION

Insulin dependent diabetes mellitus is the effect of T cell dependent autoimmune destruction of insulin production beta cells in the pancrease. Insulin is one of the islet autoantigens responsible for activation of T-lymphocyte functions, inflammatory cytokine production and development of IDDM (Tchorzewski et al., 2001). Information about the inflammatory state of an individual can become of clinical relevance since factors that determine, inflammation can be modified (Chatz et al, 2010). The proinflammatory cytokines TNF- and IL-6 play an important role in the pathogenesis of insulin- development diabetes mellitus (Alexandrak et al.,2008), while TNF- is also involved in promoting insulin resistance, development or progression of IDDM (Glowinska and Urban ,2003).

Insulin-dependent diabetes mellitus (IDDM) is a frequent complication in patients with β -thalassaemia major. It is believed to be a consequence of the damage inflicted by iron overload to the pancreatic β -cell (Rewers et al., 2002). Liver disorders and genetic influences seem to be additional predisposing factors to diabetes mellitus in patients with β -thalassaemia. Ethnic variations are frequently reported on prevalence and complications of diabetes mellitus in the β -thalassaemia patients (King, 2008).

Dowlati et al., (2010) reported that, cytokines are central mediators of inflammation by controlling innate and adaptive immune responses as well as tissue damage, defense, repair, and remodeling. Type 1 diabetes is an inflammatory disease of the pancreatic islet, in which insulin-producing β -cells are preferentially destroyed to varying degrees by the concerted action of autoreactive T-cells and monocytic cells. Many cytokines play an

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important role in the etiopathogenesis of type 1-DM, among these is IL-2 which induces its action through its binding to specific interleukin-2 receptors (IL-2Rs) that are present on the surface of T-cells (Chatz et al., 2010). Lars Groth and Thomas ,(201 1) found that , a number of cytokines have been shown to be important for the development of type 1 diabetes both at the level of the immune system and at the level of the target β -cells (The actual mechanism of β -cell destruction is still unclear, and classical T-cell effector pathways as well as many proinflammatory cytokines have been proven dispensable in transgenic animal models

About 50% of beta thalassaemic patients have increased serum TNF, and the changes after BMT are related to the occurrence of immune mediate complications. The persistence of low TNF concentrations after successful engraftment may be due to the preparative regimen and the lack of adverse immune reactions (Meliconi et al., 1992). Böber et al, (2001) declared that, thalassaemia major is a common and serious medical problem, worldwide however, there are few data concerning the various endocrine disorders which occur in this condition. Böber et al surveyed Italian centres in order to establish the prevalences and times of onset of endocrine disorders in patients with beta-thalassaemia major, insulin dependent diabetes mellitus they detected the disease among 4.9% of examined subject , the prevalence of , insulin dependent diabetes mellitus was recorded in different centers The study reported that several endocrine evaluation in thalassaemic patients must be carried out regularly , especially in those patients over the age of 10 years with iron overload and poor compliance with chelation therapy. The prevalences of some complications, such as insulin dependent diabetes and hypothyroidism, were lower than previously recorded. Hence, it is to be hoped endocrine complications will be less common in the future, for patients who have started chelation therapy during the first years of life. Because of the improved survival of thalassaemic patients with insulin dependent diabetes, and the high incidence of multiple endocrine complications, it is important to carry out careful follow-up studies for the early detection of any other associated complications to facilitate correct treatment. Some people with thalassaemia develop diabetes, which adds another burden to managing their health. It is very important for people with thalassaemia to do what they can to prevent diabetes and to follow their treatment plan should they develop diabetes.

The main cause of diabetes in people with thalassaemia is iron overload in addition to these other factors and the presence of liver disease and viral infections, which are common with thalassemia (Suvarna et al., 2006).

MATERIALS AND METHODS

Subjects

This study includes 40 Saudi thalassaemic children (group I) and 40 control healthy children nearly same age and sex (group II), completely free clinically from Pediatric department King Abdulaziz University Hospital. All the previous groups were subjected to the following:

- 1- Complete history taking, family history of thalassaemia, and other infection.
- 2- Clinical examination includes sex maturity, high, weight, liver and spleen.
- 3 -Venous blood samples from thalssaemic children for measuring IL- 1beta and TNF-alpha using ELISA. Also, serum ferritin was measured among investigated groups.
- 4- After 3 days on carbohydrate diet and overnight fast, an oral glucose tolerance test was performed (1.75g / kg body weight) and serum glucose measured 1-2 hours after an overnight fast both groups were sampled. Then both groups were injected with glucose (0.3 g/kg) using 25% glucose solution and another venous sample was taken 5 minutes to evaluate rapid insulin release using radioimmunoassay technique.

Methods

Detection of IL-1 beta and TNF alpha

Using ELISA technique (Medgenix Diagnostic, Belgium) (Joachim et al., 2003).

-Blood samples were collected in sterile tubes and serum samples were separated, and serum kept at -20C till analyzed.

-The samples added to wells containing antibody, after incubation

Allowing the formation of a sandwich (coated Mabs IL-1 beta Mabs2-HRP)(horse radish peroxidase)

- The microtiter plates washed to remove excess unbound enzyme labeled antibodies.
- The elevation solution Tetramethyl-benzydine (TMB) is added and incubated.
- The reaction stop by adding H₂SO₄, the microtiter plates were read at appropriate wavelength.
- Standard curve is plotted and IL-1 beta concentration in samples were determined

Insulin release estimation

Insulin was measured by radioimmunoassay (RIA) using kits (Diagnostic product corporation, Los Angles, code no. M097) (German, 1993): In this test:

- 125I-labelled insulin competes with insulin in the patient sample for sites on insulin-specific antibody to the wall of a polypropylene tube.
- After incubation, isolation of antibody-bound fraction is achieved by decanting the supernatant.
- The tube is then counted in gamma counter, the count being inversely proportionally to the amount of insulin present in the patient sample.
- The quantity of insulin in the sample is then determined by comparing the count to standard curve.

RESULTS

Table (1): Comparison of patients and controls according to mean standard deviation of different data.

Different data	Controls (40)	Thalassaemic children (40)	Significance
Age(year)	12.64 ± 3.4	11.6 ± 4.3	Non sig.
Weight (Kg)	40.4 ± 13.4	33.5 ± 8.6	Non sig.
Height(cm)	147 ± 14.4	140 ± 15.6	Non sig.
Liver Spain	6.4 ± 0.7	11.7 ± 2.7	sig.

Table (1) shows that, the mean age of thalassaemic and controls .No Statistical significant differences were found between age, weight and height, while significant difference was recorded according to liver span among the two investigated groups.

Table (2): Comparison of examined patient and control according to different data

Different data	Controls (40)		Thalassaemic children (40)		Significance
	No.	%	No.	%	
Sex					Non sig.
Male	12	30	28	70	
Female	28	70	12	30	
Consanguinity					Non sig.
Negative	28	70	22	55	
Positive	12	30	18	45	

Table (2) reveals, non significant differences were recorded according to Sex and consanguinity.

Table (3) Comparison of patient and control according to OGTT.

Glucose level (mmol/L)	Control	Thalassaemics children	Significance
Glucose (0min)	3.8 ± 0.7	5.3 ± 0.9	Sig.
Glucose (1 h)	5.8 ± 0.5	8.3 ± 1.5	Sig.
Glucose (2h)	4.7 ± 0.7	7.8 ± 1.0	Sig.

Table (3) shows, that the mean blood glucose of thalassaemic children and control were 3.8±0.7 & 5.3 ±0.9 in 0 min., 5.8 ±0.5 & 8.3±1.5 in 1 h, and 4.7±0.7 & 7.8±1 in 2 h respectively . Statistical significant differences were recorded at 0, 1 and 2 hours.

Table (4): Patients and control in association with insulin released.

Insulin released(mIU/dl)	Controls	Thalassaemic Children	Significance
Before glucose	7.6 ± 3.5	9.0 ± 8.5	Non sig.
5 min after glucose injection	45.1 ± 28.1	36.7 ± 27.2	Non sig.

Table(4): shows that, the mean insulin levels in venous blood, at 0 time for thalassaemic children and controls were 9.0± 8.5 & 7.6± 3.5 mIU/dL respectively. After 5 min the mean between the two previous groups were 36.7±27.2 & 45.1±28.1 mIU/dL respectively .Non statistical significant differences were detected between both groups.

Table (5): Comparison of Thalassaemic children and control according to insulin release percent increment.

Insulin release percent increment	Controls	Thalassaemic children	Significance
Mean±standard deviation	713.9 ± 594.0	490.30 ± 383.32	Non sig.

Table (5) shows that, the mean percent increment was 492.31 ± 385.34 & 713.9 ± 594.0 in thalassaemic and control respectively, with no statistical significant differences between them.

Table (6): Comparison of patient and control according to serum level of IL-1 beta, TNF alpha and serum ferritin.

Interleukin pg/ml	Control	Thalassaemic children	Significance
IL-beta	22.2 ± 19.3	13.1 ± 14.5	Non sig.
TNF alpha	382 ± 376.7	328 ± 341.4	Non sig.
Ferritin ($\mu\text{g/l}$)	63.0 ± 72.9	1872 ± 1032	sig.

Table (6) shows that, the mean serum level of interleukin -1 beta in thalassaemic children and control were 13.1 ± 14.5 & 22.2 ± 19.3 and 328 ± 341.4 & 382 ± 376.7 for TNF alpha. No statistical significant differences between them. Serum ferritin was highly raised among thalassaemic children, with high statistical significant differences between patients and controls.

DISCUSSION

Cytokines through modulating T-cell can induce cell infiltration (insulinitis) and inhibit insulin release by Beta cell (Albert et al., 2006). In thalassaemic children a frequent blood transfusion initiate immune reaction against the beta-cells or by excessive deposition of iron by the foreign cellular elements in the transfused blood. In this study, the mean percent of insulin increment was 492.31% & 713.9% among thalassaemic and control respectively, with non statistical significant differences between them. In thalassaemic patients frequent blood transfusion was important initiation of immune response against the beta-cells, by the excessive iron deposition or cellular elements in transfused blood (De Sanctis et al., 2004).

In our study, there was impaired glucose tolerance, the mean of oral glucose load (OGTT) was 7.8 mmol/L (140 mg/dl), the cases were not over diabetes but classified as impaired glucose tolerance which is prediabetic stage. The thalassaemic response to IV glucose load was decreased, where the insulin release increment 5 min after the load was 490.30% of its value before IV load compared to 713.9% for controls. Also, insulin released after 5 minutes of glucose administration was $36. \pm 27.2$ and 45.1 ± 28.1 among thalassaemic patients and controls respectively. These results in agreement with Dmochowski et al., (1993), who declared that, the difference in insulin levels was apparently due to reduced hepatic insulin extraction in thalassaemia. Thalassaemia patients were studied prospectively at 6-month intervals for 6-12 months, and repeated measures analysis of variance indicated that across a 6-month interval, there was a decrease in the total integrated insulin response; they concluded that, patients with thalassaemia major have significant insulin resistance, which may be compensated for by an elevated circulating insulin level. Dmochowski et al., reported that, the elevated insulin level in response to tolbutamide appears to be due to reduced hepatic extraction of insulin and not to an enhanced insulin secretory response. Over time, patients with thalassaemia experience a reduction in their circulating insulin levels. Persistent insulin resistance along with a progressive reduction in circulating insulin levels may lead to glucose intolerance and diabetes mellitus, which have a high prevalence in patients with thalassaemia major. In thalassaemia, treated with high transfusion which is consequence of hepatic liver cirrhosis secondary to hemosiderosis and iron deposition in the Beta-cells.

This study indicate that, the mean liver span was (11.7 ± 2.7 and 6.4 ± 0.7), statistically significant differences between cases and controls, this revealed that, hepatomegaly associated with cirrhotic liver which lead to impaired glucose tolerance after OGTT. Many studies indicate the association between iron overload and impaired glucose tolerance, they suggested that, in Beta thalassaemic cases beta-cells dysfunction and alpha-cells over activity (glucagon released) may lead to development of diabetes mellitus (Gamberini et al., 1998). Our study compared patients and controls according to OGTT, the study declared that the mean blood glucose of thalassaemic children and control were 3.8 ± 0.7 & 5.3 ± 0.9 in 0 min., 5.8 ± 0.5 & 8.3 ± 1.5 in 1 h, and 4.7 ± 0.7 & 7.8 ± 1 in 2 h respectively, statistical significant differences were recorded at 0, 1 and 2 hours. The study found an impaired glucose tolerance after OGTT which may be explained by hepatomegaly and cirrhotic liver, although the level of serum ferritin was $1872 \mu\text{g/l}$ which indicate of iron overload. Our results confirmed with that detected by El-Hazmi et al., (2011) and Walter et al., (2006).

El-Hazmi et al., reported that either hyperinsulinaemia or hypoinsulinaemia was encountered in the majority of thalassaemic patients. The prevalence of diabetes mellitus was 6% compared to 2% in the β -thalassaemia minor and normal children. Impaired glucose tolerance (IGT) occurred at a significantly higher (24%) frequency in the β -thalassaemia major compared to 2% in the β -thalassaemia minor patients and normal controls, respectively. The prevalence of diabetes mellitus was significantly lower in the Saudi thalassaemic patients compared to the results obtained from patients of other ethnic groups reported. Liver function abnormalities were

more frequent in this group than in the β -thalassaemia minor and the controls. It appears from the inference of our results that combinations of factors including iron overload and liver damage predispose the β -thalassaemia major patients to β -cell damage and, hence, to impaired glucose tolerance. Walter et al., found that, blood transfusion therapy is life-saving for patients with β -thalassaemia and sickle cell disease (SCD), but often results in severe iron overload. Blood transfusions and iron chelation therapy, patients continue to be at high risk for iron overload and iron-induced toxicities.

Iron-related cardiac disease remains the most common cause of death in thalassaemia (Borgna-Pignatti et al, 1998). In addition, over 70% of patients with thalassaemia suffer from primary or secondary amenorrhoea, hypogonadism, osteoporosis and other endocrine disorders (Bronspiegel-Weintrob et al, 1990).

In pilot data from a recent study, Vichinsky et al (2005) confirmed this finding and demonstrated that the strongest predictors of organ injury in thalassaemia were duration of iron exposure and the specific diagnosis. In a recent study involving 30 thalassaemia and 43 SCD patients with severe haemosiderosis, they confirmed that greater cardiac and endocrine disease occurred in the thalassaemia patients. Although the specific role of iron overload in mediating injury was primary in thalassaemia but it was also, important to enhance gastrointestinal iron absorption seen thalassaemia patients that may initiate different iron trafficking. In order to investigate the relationship of iron overload and specific disease to injury, they examined biomarkers of oxidative stress, inflammation and tissue injury. However, while each class of biomarker is traditionally thought to represent one of these processes, it is important to note that they are not distinct entities and have considerable interaction, i.e. oxidative stress can initiate tissue injury and/or inflammation. This study showed that mean standard deviation according to age was, (12.64 \pm 3.4), weight (40.4 \pm 13.4) and length (147 \pm 14.4), non statistical significant differences were found between age, weight and height among the two investigated groups, our results nearly corresponding to that detected by Walter et al., (2006), they found non statistical difference between cases and controls according to age (23.6 \pm 8.6), length (154.2 \pm 9.1) and weight(52.3 \pm 8.9),

Our results showed that, the mean serum level of interleukin -1 beta in the thalassaemics and controls were nearly the same (13.1 \pm 14.5 & 22.2 \pm 19.3 for IL-beta and 328 \pm 341.4 & 382 \pm 376.7 for TNF alpha) also, non statistical significant differences were recorded between them. However, the normal serum concentration of IL-beta and TNF alpha in our study together with the absence of circulating Islet-cell antibody, indicate the absence of the role of the immune system in the etiology and pathogenesis of IDDM in our studied cases. These results were not confirmed with Wanachiwanawin et al (1999) and Meliconi et al., (1992) they reported that, the increased levels of both cytokines may be explained by an ongoing inflammatory reaction at the onset of IDDM. IL-1 beta potentiated by other cytokines as TNF-alpha and IFN-gamma, is an important effector molecule, both in early and late events in the immune mediated processes that lead to beta-cell destruction and IDDM, this cytokines combination leads to formation of nitric oxide by human islets which mediate the inhibitory effects of cytokines on glucose stimulated insulin secretion by human islets.

Eizirik et al., (1994) explained that, the cytokines have been proposed as inducers of beta-cell damage in human insulin-dependent diabetes mellitus via the generation of nitric oxide (NO), this concept is mostly based on data obtained in rodent pancreatic islets using heterologous cytokine preparations. Many studies examined whether exposure of human pancreatic islets to different cytokines induces NO and impairs beta-cell function. Islets from human pancreata were exposed to the following human recombinant cytokines, alone or in combination: IFN-gamma, TNF-alpha, IL-1, and IL-1 beta. After 48 h, none of the cytokines alone increased islet nitrite production, but IFN-gamma induced a 20% decrease in glucose-induced insulin release. Eizirik et al., (1994) reported that, combinations of cytokines, notably IL-1 beta plus IFN-gamma plus TNF-alpha, induced increased expression of inducible NO synthase mRNA after 6 h and resulted in a fivefold increase in medium nitrite accumulation after 48 h. These cytokines did not impair glucose metabolism or insulin release, but there was an 80% decrease in islet insulin content. An exposure to IL-1 beta plus IFN-gamma plus TNF-alpha increased NO production and decreased both glucose-induced insulin release and insulin content. Inhibitors of NO generation, aminoguanidine or NG-nitro-L-arginine, blocked this cytokine-induced NO generation, but did not prevent the suppressive effect of IL-1 beta plus IFN-gamma plus TNF-alpha on insulin release and content.

Many authors' (Borgna-Pignatti et al., 2004) explained the reduction in circulating insulin level which leads to glucose intolerance and diabetes mellitus. This beta-cell destruction is mediated by neither islet cell antibody (ICA) nor by cytokines production especially IL-1 beta and TNF-alpha. Although, iron deposition is confirmed by many studies but others (Julie et al., 2011), reported that iron chelating program does not prevent the development of abnormal glucose tolerance in chronically transfused patients. On the other hand it was explained that early chronic hypoxia (low Hb level) and iron overload (high Hb level) after hypertransfusion program, both may cause with different processes an impairment of glucose metabolism (Yanan and Bor Luen 2011). Giovanni et al., (2002) and Ong et al., (2008) concluded that, inflammatory reaction occur among thalassaemic children which

lead to increase both IL-beta and TNF alpha which may lead to insulinitis and disturbances in oral glucose tolerance which may lead to IDDM. It was found that the percentage of IL-2 receptor positive circulating T-cells was significantly increased in diabetic children than non diabetic group (Dinarelo et al., 2010 and Oloomi., 2006).

Conclusion

Thalassaemic children may be susceptible to diabetes or pre-diabetic stage, this may predispose by multiple blood transfusion and deposition of iron in islet in beta-cell which decrease insulin level and causes hyperglycemia.

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