SEROPREVALENCE OF AUTO-IMMUNE THYROIDITIS IN TYPE 1 DIABETIC CHILDREN IN DAMIETTA GOVERNORATE

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ABSTRACT

Background**: Type 1 diabetes mellitus (T1DM) is a common Pediatric autoimmune disorder. Children with type 1 diabetes mellitus are more prone to develop other organ-specific autoimmune diseases, among which autoimmune thyroiditis (AIT) is more frequently encountered.* ***Objective: *the present study designed to determine the seroprevalence and biochemical characteristics of autoimmune thyroiditis among children with insulin-dependent diabetes mellitus in Damietta Governorate in the period from October2011 to October2012.* ***Patients and Methods**: This cross sectional analytic study included 90 diabetic children receiving insulin therapy. They were selected from diabetic outpatients' clinic of Health Insurance Hospital in Damietta governorate; 54 of them were females and 36 of them were males. All patients were subjected to history taking, examination to detect major complications of diabetes & signs of other autoimmune disorders especially thyroiditis and laboratory investigations in the form of Hemoglobin A1C, Thyroid stimulating hormone (TSH), Anti-thyroid peroxidase antibody (TPO), Anti-thyroglobulin antibody (Tg) and Free tri-iodothyronine (T3)& Free thyroxin (T4) for patients with abnormal TSH. Thyroid ultrasound for all subjects with seropositive anti- thyroid autoantibody was also performed.* ***Results:*** *Mean age of studied cases was 11.2±3.3 years. Most of our cases were more than 6 years (87.8%), with duration of diabetes less than 3 years (62.2%), had positive family history of diabetes (70%), and negative family history of autoimmune diseases (66.7%). Prevalence of seropositive thyroiditis was 10 cases (11.1%) out of 90 patients, 8 (8.9%) of them shows picture of thyroiditis with neck ultrasound. Among patients with seropositive thyroiditis, there was no significant risk as regard sex of the patients (8 females and 2 males; P: 0.17), age (all patients more than 6 years; P: 0.2), and duration of diabetes (P: 0.59). There was significant difference as regard positive family history of autoimmune diseases (7 cases; P: 0.009). Regarding thyroid function status, 2.2% were suffering from hypothyroidism and were complaining only from goiter; TSH level was only increased in the two cases with low free T4 and low free T3. There was statistically significant difference as regard TPO Abs mean in negative cases and positive thyroiditis cases (2.74±8.03 and 263.69±231.77) respectively, and TG Abs mean in negative cases and positive thyroiditis (7.48±7.31 and 205.69±211.51).* ***Conclusion**: The presence of thyroid*

antibody positivity and the subsequent development of subclinical autoimmune thyroiditis were prevalent among the children with T1DM of our study. Positive family history of thyroid disease and abnormality in neck ultrasound were significant risk factors. ***Recommendations****: All patients with T1DM should be screened for autoimmune thyroiditis upon diagnosis, and in case of thyroid antibody positivity they should be regularly followed up in terms of their thyroid function and growth status.*

INTRODUCTION

Type 1 diabetes mellitus is an organ-specific auto-immune disease **(kamboj, 2013)** characterized by cell destruction resulting in glucose intolerance and finally insulin dependence. Genetic susceptibility and environmental triggering factor seem to be responsible for type 1 diabetes **(Atkinson and Eisenbarth, 2001).** The incidence of this condition is increasing by 2% to 5% per year worldwide, especially in the youngest age group (<5 years of age) **(Unwin et al., 2010).**

Children with type 1 diabetes mellitus are more prone to develop other organ-specific autoimmune diseases, among which autoimmune thyroiditis (AIT) is more frequently encountered **(Kordonouri et al., 2002).**

The thyroid is highly vulnerable to autoimmune diseases. The incidence of chronic autoimmune thyroiditis (CAT) has increased dramatically over the past few decades, affecting up to 5% of the general population. In children, CAT is the most common cause of acquired hypothyroidism in non endemic goiter areas **(Saranac et al., 2011).**

Autoimmune thyroiditis (AIT) is the most common thyroid disorder in the pediatric age range. The disease results from defects in immune regulation and a cascade of events progressing from lymphocyte infiltration of the thyroid, to T-cell- and cytokine-mediated thyroid follicular cell injury, and apoptotic cell death **(Brown, 2013).**

Auto antibodies are directed towards specific thyroid gland proteins, which are thyroglobulin (Tg), a fundamental component of thyroid colloid, and thyroid peroxidase (TPO), an enzyme participating in the production of thyroid hormones **(Sinclair, 2008).**

During the last few decades, some investigators have expressed concern about the autoimmune thyroid diseases along with type 1 diabetes are common autoimmune endocrine disorders that collectively affect approximately 3% of

the population **(Pearce and Merriman, 2009).**

So, the aim of our study was to determine the seroprevalence and biochemical characteristics of autoimmune thyroiditis among children with insulin-dependent diabetes mellitus in Damietta Governorate.

PATIENTS AND METHOD

The present study was a cross sectional analytic study which was conducted in diabetic outpatients clinic of Health Insurance Hospital in Damietta governorate in the period from October 2011 to October 2012 every other day. It consisted of 90 diabetic children with type1 DM receiving insulin therapy ranging from 4 to 15 years of age.

Diagnosis of type 1 diabetes was based on Criteria for the diagnosis of diabetes, obtained from American Diabetes Association **(ADA, 2012)**, 1) HbA1C ≥ 6.5 percentage, 2) Fasting plasma glucose ≥ 126 mg/dl (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 hr. 3) 2-h Plasma glucose ≥ 200 mg/dl (11.1 mmol/l) during an oral glucose tolerance test. The test was performed as described by the WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water. 4) In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥ 200 mg/dl (11.1 mmol/L). In the absence of unequivocal hyperglycemia, the results were confirmed by repeat testing. Patients receiving medications affecting thyroid function (as steroids, iodide and carbamazol) ,Patients with chronic liver & kidney disease and immunocompromised patients were excluded.

All children were subjected to the following:

1. **Detailed history taking:** personal history, diabetic history with special emphasis on age at onset of DM (defined as first injection of insulin), duration of DM, daily insulin requirements and symptoms suggesting complications of DM. Symptoms suggesting other autoimmune disorders and family history of IDDM or other autoimmune disorders specially thyroid diseases were also reported.
2. **Thorough clinical examination:** including anthropometric assessment [Height, weight and body mass index (BMI)]. Special attention has been paid for signs of diabetic complications and signs of other autoimmune disorders specially

thyroiditis (goiter, short stature and decline in school performance).

3. **Laboratory investigations:** three milliliters of venous blood were collected from each enrolled child. One ml was taken into a clean glass tube containing 1.5 mg Ethylene diamine tetra acetic acid (EDTA) for determination of Hemoglobin A1C. The remaining blood was put in plain tube then centrifuged at 3000 rpm for 10 minutes and the clear serum obtained was taken for the other investigations: Thyroid stimulating hormone (TSH), Anti-thyroid peroxidase antibody (TPO Ab) and Anti-thyroglobulin antibody (Tg Ab). Finally, free triiodothyronine (T3) and free thyroxin (T4) for patients with abnormal TSH were performed.

   Hemoglobin A1C was determined by using chromatographic-spectrophotometric ion exchange ***(Hanas et al., 2010).*** Anti-thyroglobulin antibody was determined by using Enzyme Linked Immunosorbent assay (ELISA) for the quantitative determination of antibodies against thyroglobulin ***(Ruf et al., 1989).*** Anti-thyroid peroxidase antibody was determined by using Enzyme Linked Immunosorbent assay (ELISA) for determination of antibodies against thyroid peroxidase ***(Ruf et al., 1989).*** Regarding thyroid stimulating hormone, The DRG TSH ELISA is an enzyme immunoassay for the quantitative in vitro diagnostic measurement of TSH in serum was used ***(Barker, 1948).***

4. **Radiological investigations**: thyroid ultrasound (US) was done for all subjects with seropositive anti- thyroid auto-antibodies.

Ethical consent: the study was approved by the Hospital Ethics Committee in accordance with local research governance requirements, and it was explained to the prospective participants. All participating mothers signed an informed consent form.

Statistical analysis: Data collected were reviewed, coded, and entered PC where statistical analysis were done using SPSS

(statistical package for social science) version 14. For descriptive statistics, frequency number and percentage are used for qualitative data. Mean and standard deviation (SD) for quantitative data were used. Chi square test, Student t test for 2 independent groups, ANOVA (analysis of variance) test for more than 2 groups, Pearson's correlation test, and regression analysis for prediction of risk factors, the level $p < 0.05$ was considered the cut-off value for significance ***(Dawson and Trapp, 2001).***

RESULTS

In the present work, descriptive characteristics of the studied patients revealed that their ages ranged from 4 to 15 years and duration of diabetes from 0 (newly discovered cases) to 12 year with mean duration 3.1 years and mean BMI: 18.1±4.7; TSH value ranged from 0.05 mlU/l to 10mlU/l, TPO antibody value from 0.2 IU/ml to 694 IU/ml, TG antibody value from 0.6IU/ml and the maximum was 734IU/ml, HbA1C mean was 8.4% **(Table 1).** Regarding general characteristics, it was found that most of them were more than 6 years (87.8%), females were more frequent than males (60% and 40%) respectively, most of them (62.2%) had duration of diabetes less than 3 years, with positive family history of diabetes (70%), and negative family history of autoimmune diseases (66.7%), 10 patients (11.1%) were seropositive to thyroiditis, 8 (8.9%) of them shows picture of thyroiditis with neck ultrasound **(Table 2).** AS regard risk factors among studied cases, there was no significant risk regarding sex of the patients; (8 females and 2 males; P: 0.17), age (all patients more than 6 years; P: 0.2), and duration of diabetes (P: 0.59). There was significant difference as regard positive family history of autoimmune diseases (7 cases; P: 0.009) **(Table 3).** Finally, we found no statistically significant difference between seronegative and seropositive cases as regard TSH and HbA1C while there was statistically significant difference as regard TPO mean in negative cases and positive thyroiditis (2.74±8.03 and 263.69 ±231.77) respectively and TG mean in negative cases and positive thyroiditis (7.48±7.31 and 205.7±211.5) respectively **(Table 4).**

Table (1): Descriptive statistics of the studied cases (n=90).

Variable	Minimum	Maximum	Mean±SD
Age (years)	4.00	15.00	11.2±3.3
Duration of DM (years**)**	0.00	12.00	3.1±2.3
BMI	11.00	35.90	18.1±4.7
TSH (mlU/L**)**	0.05	10.00	2.3±1.6
TPO Ab (IU/ml)	0.20	694.00	31.7±110.9
TG Ab (IU/ml)	0.60	734.00	29.5±92.2
HbA1c	3.00	14.00	8.4±2.1

Table (2): General characteristics of the studied cases

Variables	No.	%
Age :		
Less than 6ys.	11	12.2
More than 6ys.	79	87.8
Sex :		
Male	36	40
Female	54	60
DM duration:		
Less than 3ys.	56	62.2
More than 3ys.	34	37.8
Family history DM:		
Negative	27	30.0
Positive	63	70.0
Family AID		
Negative	60	66.7
Positive	30	33.3
Seropositive for thyroiditis:		
negative	80	88.9
positive	10	11.1
US neck:		
Normal	82	91.1
Thyroiditis	8	8,9

Table (3): Risk factors (Kakleas et al., 2009) of thyroiditis among studied cases

	Normal	Thyroiditis	X^2	P
Sex:				
Males (No., %)	34 (42.5)	2 (20)	1.875	0.171
Females (No., %)	46 (57.5)	8 (80)		
Family history of AID				
Negative (No., %)	57 (71.3)	3 (30)	6.8	0.009*
Positive (No., %)	23 (28.7)	7 (70)		
Age (years)				
≤ 6 years	11 (13.8)	0 (0)	1.56	0.21
> 6 years	69 (86.2)	10 (100)		
Duration of DM (years)				
≤ 3 years	49 (61.3)	3 (30)	0.29	0.59
> 3 years	31 (38.2)	7 (70)		

*significant

Table shows significant risk for thyroiditis with positive family history of autoimmune disease, while there was non-significant risk regarding sex, age, and duration of diabetes

Table (4): Comparison between diabetic seropositive and diabetic seronegative cases as regard some biochemical parameters

Variables	negative (Mean ±SD)	Positive (Mean ±SD)	T test	P value
TSH	2.12±1.26	3.54±2.91	1.525	0.160
TPO Ab	2.74±8.03	263.69±231.77	3.560	0.006*
TG Ab	7.48±7.31	205.69±211.51	2.963	0.016*
HbA1c	7.48±7.31	8.24±2.23	0.176	0.864

*significant

Table shows significant increased TPO and TG antibodies in seropositive cases,while there was no significant difference as regard TSH.

DISCUSSION

Regarding the general characteristics of the studied patients, most patients were females (60%) probably as a result of estradiol effect which is seemed to accelerate the progression of autoimmune diseases via enhancing the pathway of T helper type 2 (Th2) cells, while androgens had a protective effect **(Fox, 1992).** The age of the studied children varies from 4-15 years and most of their ages more than 6 years (87.8%), most of them (62.2%) with duration of diabetes less than 3 years. The incidence of T1DM is increasing by 2% to 5% per year worldwide, especially in the youngest age group **(Unwin et al., 2010).** Environmental influences are important factor in the development of type 1 diabetes in young age, Exposure to one or more environmental triggers alters the immune system in such a way that susceptibility is converted to path physiology and destruction of β cells begins **(Virtanen et al., 2000).**

Regarding the family history of diabetes, most of studied children (70%) were with positive family history of diabetes, but only (33.3%) with family history of autoimmune diseases. IDDM is considered a complex genetic trait; that is, not only do multiple genetic loci contribute to susceptibility, but environmental factors also play a major role in determining risk. A large body of evidence indicates that inherited genetic factors influence both susceptibility and resistance to the disease. There is significant familial clustering of diabetes mellitus, with an average prevalence risk in siblings of 6% compared to 0.4% in the general population **(Hanukoglu et al., 2003).**

Regarding BMI, it ranges from 11 to 35.9 with mean 18.1±4.7 this results was found also by **Kakleas et al. (2009)** who reported in their study that there was no significant effect of anti-thyroid antibody positivity on the growth and BMI status of the children with diabetes was observed (BMI 21.7 versus 20.5 kg/m2, P = NS).

Regarding the seropositive cases to TPO antibodies and or TG antibodies, 10 (11.1%) among our patients were seropositive for auto- thyroid antibodies. The same finding was found by other many studies as the prevalence of thyroiditis in **Hansen et al. (2003)** study was 13% compared with prevalence of 10–22% in previous studies of children and adolescents with T1DM **(Kordonouri et al., 2002).** However, in our young patients with a relatively short

duration of diabetes, we expect an increasing prevalence of TPO antibody with time as demonstrated by others **(Holl et al., 1999).**

The prevalence rate of anti-thyroid antibodies in the T1DM patients of **Kaloumenou et al. (2007)** study was 18.75%, while a significant percentage (55.5%) of them presented subclinical auto-immune thyroiditis relatively early in the course of the disease. Their findings are in agreement with previous studies in this age-group, reporting a prevalence of thyroid antibody positivity of 10%–23.4% **(Kordonouri et al., 2005)** and **Kordonouri et al. (2002),** and of SAIT of 45% **(Prina Cerai et al., 1994).**

The most prevalent autoimmune disease associated with IDDM is AIT. Its prevalence varies from 8% to 50%, depending on the age, sex and ethnic origin of the subjects. It has been reported that follow-up of AIT is important not only for the detection of hypothyroidism, but also for the early diagnosis of papillary carcinoma and thyroid lymphoma. It has been suggested that an immunologic mechanism stimulates lymphocytic infiltration into the thyroid through an autoimmune mechanism, thereby promoting the pathogenesis of papillary carcinoma **(Soyucen et al., 2010).**

Regarding the general characteristics of the seropositive thyroiditis patients, all ages more than 6 years, **Kakleas et al. (2009)** reported that an increase of the age at diabetes diagnosis by 1 year increased the probability of thyroid antibody positivity by 15%. Moreover, a significant positive association between the prevalence of anti-Tg antibodies and current age was observed additionally, the presence of one type of anti-thyroid antibody increased the probability for the development of the other type of anti-thyroid antibody. This observation suggests that autoimmune disease is the final phase of a process starting with auto-recognition, passing through immunity with the appearance of auto-antibodies, and finally leading to cell destruction and autoimmune disease. Moreover it is known that the maximum autoimmune activity is observed during puberty **(Talal, 1978).**

Regarding the duration of diabetes, we found that most of them (70%) had duration of diabetes more than 3 years, and most of them with positive family history of autoimmune diseases (70%). Many authors found that thyroid autoimmunity is increased

with longer duration of diabetes **(Kordonouri et al., 2002).** We found (70%) of our studied patients with duration of diabetes more than 3 years. This could be explained by the fact that in adolescent girls, apart from diabetes duration, the presence of female hormones may significantly contribute to the development of thyroid autoimmunity **(Kaloumenou et al., 2007).**

Regarding the general characteristics of the seropositive thyroiditis patients, we found that females are more than males (80% and 20% respectively). Similarly, **De Block et al. (2003)** reported a 3-fold risk of anti-TPO antibody positivity in female adolescents and young adults with diabetes in comparison with males. Also in the general population, girls are more prone to develop thyroid disease than boys **(Kaloumenou et al., 2007) ..**

Kaloumenou et al. (2007) suggested that all patients with T1DM should be screened for autoimmune thyroiditis upon diagnosis and then yearly, and in case of thyroid antibody positivity they should be regularly followed up in terms of their thyroid function and growth status. Several authors recommend annual screening for thyroid disease in all type 1 diabetes subjects with TSH measurement; this procedure is considered the most sensitive way to identify patients with thyroid dysfunction, as auto-antibodies may persist for many years without thyroid dysfunction **(Hansen et al., 2003 and Hoffman, 2003).**

However, **Hansen et al. (2003**) did not find any initial TPO-Ab–negative patients who developed thyroid disease after 3 years of follow-up. Other authors recommend screening using TSH and TPO-Abs. In a cohort of 58 type 1 diabetic patients enrolled in the Diabetes Control and Complications Trial and followed for 18years, **Umpierrez et al. (2003)** observed that TPO-Ab–positive subjects were 17.9 times more likely to develop thyroid dysfunction. These authors recommended annual screening using TSH determination, particularly in patients with positive TPO-Abs. **Barker (2006)** screened type 1 diabetic patients with TPO-Abs and thyroid function at onset and every 1–2 years thereafter and patients with positive TPO Abs every 6–12 months. Finally, a third group of authors' recommends TSH determination only in TPO-Ab positive patients **(Maugendre et al., 2000).**

CONCLUSIONS

The presence of thyroid antibody positivity and the subsequent development of subclinical autoimmune thyroiditis were prevalent among the children with T1DM of our study. The possible risk factors for its development were older age, female gender, and long diabetes duration. Subclinical hypothyroidism was not found to affect the children's growth and BMI status.

RECOMMENDATION

Screening for thyroid disease should be performed at diabetes onset in all pediatric patients with type 1 diabetes and yearly laboratory and ultrasound examinations are necessary to detect early thyroid dysfunction. Further studies are required to investigate thyroid autoimmunity in type1 DM in younger patients.

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دراسة معدل انتشار التهاب الغدة الدرقية المناعى لدى الأطفال المصابين بالنوع الأول من مرض السكرى بمحافظة دمياط

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مقدمة : يعد النوع الأول من مرض السكري من الأمراض المنتشرة لدى الأطفال كما ان هؤلاء الأطفال المصابين بهذا المرض عرضة للعديد من الأمراض المناعية الأخرى, ويعتبر التهاب الغدة الدرقية المناعى احد اكثر هذه الأمراض انتشارا فى الأطفال المصابين بالسكري.

الغرض من البحث : تقييم نسبة حدوث التهاب الغدة الدرقية المناعى فى الأطفال المصابين بالنوع الأول من السكرى.

طريقة البحث : تعد هذه الدراسة من نوعية الدراسات المقطعية التحليلية وقد اشتملت على 90 طفل مصاب بالسكرى منهم 54 انثى و36 ذكراً. وقد أجريت هذه الدراسة فى مستشفى التأمين الصحى للطلبة بمحافظة دمياط. تعرض جميع المرضى لأخذ التاريخ المرضى والفحص لاستبعاد وجود أمراض مزمنة أو مضاعفات لمرض السكرى, والفحوص المختبرية الاساسية فى شكل الهيموجلوبين السكرى ,ووظائف الغدة الدرقية, والأجسام المضادة للغدة الدرقية , وكذلك تم عمل اشعة تليفزيونية على الرقبة لكل المرضى الايجابيين للأجسام المضادة.

النتائج: وقد أظهرت الدراسة وجود 11% من الأطفال مصابين بارتفاع فى الأجسام المضادة للغدة الدرقية, منهم 2.2% يعانون من نقص فى افراز هرمون الثيروكسن مما يلزم معه بدء العلاج. وقد لوحظ ان نسبة الاناث أكبر من الذكور فى ذلك مع وجود تاريخ مرضى بالعائلة للسكرى وكذلك لأمراض مناعية أخرى.

الاستنتاجات : فان النتائج تثبت وجود أجسام مضادة للغدة الدرقية لدى الأطفال المصابين بالنوع الأول من السكرى بنسبة 11% مما ينبأ بحدوث التهاب مناعى بالغدة الدرقية ونقص فى افراز الثيروكسين مع مرور الوقت.

التوصيات: ينصح بضرورة قياس وظائف الغدة الدرقية والأجسام المضادة لها مع اكتشاف السكرى وبطريقة دورية .ولكن تفسير هذا التواجد للأجسام المضادة للغدة الدرقية مع مرض السكرى يحتاج لمزيد من الأبحاث والدراسات.