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ORIGINAL ARTICLE

Auto Immune Markers in Type 1 Diabetes and Thyroid Dysfunction in Egyptian Patients

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ABSTRACT

Background: *Type 1 diabetes mellitus (T1DM) is a common endocrine disorder associated with aberrant immune responses to specific β-cell auto-antigens. Markers of the process are various including auto-antibodies to glutamic acid decarboxylase (GADA) and to islet cell (IA-2). Other autoimmune diseases such as thyroid dysfunction are more common in T1DM. Autoimmune thyroid diseases are the most prevalent immunological diseases in patients with type 1 diabetes.* ***Objectives:*** *This study was planned to clarify the occurrence of autoimmune thyroid dysfunction and auto-antibodies in Egyptian patients with T1DM in patients without any history of thyroid disease and to assess its relation with diabetes-specific auto-antibodies.* ***Methodology:*** *This is a prospective case-controlled clinically-based study. Sixty patients diagnosed with diabetes type 1 according to WHO criteria for diagnosis of diabetes mellitus (DM) and 30 healthy controls matched for age, sex and body mass index (BMI) were recruited from the outpatient clinic of Endocrinology Department, Al-Zahraa Hospital, Al-Azhar University. All patients and controls were subjected to; Random blood glucose level, Glycated Hemoglobin (Hb A1c) assay level, Anti glutamic acid decarboxylase (anti-GAD), C-peptide level and Anti islet cell antigen (IA-2) auto antibodies, Serum free T3, free T4, TSH and Anti-Thyroid Autoantibodies (antithyoid peroxidase- TPO and anti-thyroglobulin-TG) were also done.* ***Results:*** *We reported that 45 patients (75.0%) were positive to autoantibody for GAD, 17 patients (28.3%) were positive for IA-2, 15 patients (25.0 %) for TPO, and 10 patients (16.66 %) were positive for TG-AB. All subjects with overt hypothyroidism (5 patients / 8.33%) had positive GAD and thyroid auto-antibodies, subclinical hypothyroidism (SCH) were reported in 15 patients (25%) and One subject only (1.67%) had clinical hyperthyroidism.* ***Conclusions:*** *We found a high prevalence of diabetes-specific auto-antibodies in T1DM patients. Subclinical thyroid dysfunction was evident in a considerable number of T1DM patients. These results indicate the importance of regular thyroid screening in T1DM patients.*

INTRODUCTION

Thyroid diseases and diabetes mellitus are the two most common endocrine disorders encountered in clinical practice [1,2]. It was suggested that 15% to 30% of patients with type 1 diabetes mellitus (T1DM) have autoimmune thyroid dysfunction (AITD) [3,4].

It was well known that, type 1 diabetes mellitus (T1DM) has an immunological background. This autoimmune background includes several auto-antibodies which directed mainly against beta cells of pancreas and its product of insulin which leads to damage of the beta cells and causes absolute insulin deficiency [5].

However, it is not assumptive that the destruction of the beta cells is caused by auto-antibodies. Like all auto immune diseases, the etio-pathogenesis of this disease is multi-factorial, more complexed and exceeding being a single disease [6].

Recently, it becomes clear that genetic susceptibility loci are shared between several autoimmune diseases [7]. Gravies disease (GD), Hashimoto thyroiditis and idiopathic thyroid failure are the syndromes of autoimmune thyroid disease (AITD) sharing immunological abnormalities, histological changes in the thyroid gland, and also the genetic predisposition [8].

Usually no isolated autoimmune disease was observed as a single disease but other immune diseases happened together[9]. Majority of patients with type 1 diabetes are clinically euthyroid.

It is recommended to screen those patients for auto-antibodies against thyroid tissues and hormones. Screening for anti-thyroid peroxidase (anti-TPO), anti-thyroglobulin antibodies (anti-TG) and thyroid hormones levels has been recommended[10].

This study was planned to clarify the occurrence of autoimmune thyroid dysfunction and auto-antibodies in Egyptian patients with T1DM in patients without any history of thyroid disease and to assess its relation with diabetes-specific auto-antibodies.

METHODOLOGY

This prospective case-controlled clinically-based study was conducted between July 2015 and June 2017. The study population included 60 patients (33 males and 27 females) diagnosed with diabetes type 1 according to WHO criteria for the diagnosis of diabetes mellitus (DM) and 30 healthy controls (18 males and 12 females) matched for age, sex and body mass index (BMI). All patients and controls were recruited from the Outpatient Clinic of Endocrinology Department, Al-Zahraa Hospital, Al-Azhar University.

The study protocol was approved by the Ethics Committee of the faculty of Medicine, Al-Azhar University. Informed detailed consent was obtained from all patients and controls to be included in the study.

Inclusion Criteria:

Diabetes was diagnosed according to the World Health Organization (WHO) 1999 G criteria and American Diabetes Association guidelines [11].

Terms and Classifications: those for whom insulin treatment was started within three months from diagnosis were classified as having type 1 diabetes [12]. The majority of patients were newly diagnosed with short duration of diabetes and did not have any known endocrine autoimmune disorders diagnosed before enrolment.

Exclusion Criteria:

Patients were excluded if they had any acute or chronic systemic illnesses as judged by the investigator or if they were receiving drugs (such as lithium or steroids) that could interfere with thyroid function tests or if they were pregnant [13].

Study Procedures:

History taking, physical examination, weight, height and Body mass index (BMI) were calculated for all patients and control subjects whose were subjected to: Random blood glucose level, glycated Hemoglobin (HbA1c) assay, C-peptide level, serum free T3, free T4 and TSH. Laboratory tests for anti glutamic acid decarboxylase (anti-GAD), anti islet antigen (IA-2). Anti thyroid peroxidase (anti-TPO) and anti thyroglobulin antibodies (anti-TG) were also done [14].

Sample collection and storage:

Serum separator tube (SST) were used and samples allowed to clot for 90 minutes at room temperature, centrifuged for 15 minutes at 1000 ×g., serum were separated and assayed immediately or divided and stored at -20°C. Repeated freezing-thawing was avoided. EDTA samples were also collected for HbA1c estimation.

Thyroid hormones: Serum FT_3, FT_4 and TSH were measured by electro-chemiluminescence immunoassay (ECLIA) using Cobas e411 (Roche diagnostics, GmbH, Mannheim, Germany). [40].

HbA1c level: was measured by a quantitative turbidimetric inhibition immunoassay (TINIA) method using (Cobas Integra 400, Roche, Swizerland).

Anti Glutamic Acid Decarboxylase (anti-GAD):

It was measured by ELISA[41] using commercially available kit (RSP Ltd, Cardiff, UK) with reference value < 5.0 U/ml.

Thyroid Peroxidase antibodies (ani-TPO), Thyroglobulin antibodies (anti-TG):

Anti TPO and anti TG were measured by ECLIA using Cobas e411 (Roche diagnostics, GmbH, Mannheim, Germany) with reference values for TPO < 16 IU/ml and for TG < 28 IU/ml.

Anti Islet cell antibody (IA-2):

It was measured by ELISA using commercially available kit(IA2 ELISA Kit Cusabio / USA)

Calculation of Results:

For calculation the valence of human islet cell antibody (ICA, IA-2), compare the sample well with control. Negative Control OD values must less than 0.1 and Positive Control OD Values must more than 0.8. Or else repeat the test. If OD negative ＜0.1, calculate it as 0.1. λ While OD sample/ OD negative ≥ 2.1: Positive λ While OD sample / OD negative ＜ 2.1: Negative [41]

RESULTS

The study population included 60 patients (33 males and 27 females) diagnosed with diabetes type 1 according to WHO criteria for the diagnosis of diabetes mellitus (DM) and 30 healthy controls (18 males and 12 females) matched for age, sex and BMI. The Age at diagnosis of DM (years) was 15±8.5, (Table 1 and Figures 1, 2).

Table 1: Demographic characteristics of the study population

Demographic characteristics	T1DM n=60)	Controls (n=30)	P value
Age (years)	22.6±6.1	23.6±7.3	>0.05*
Sex: - **Males** - **Females**	 33 (55%) 27 (45%)	 18(60%) 12 40%)	>0.05**
BMI	28.7±11.7	25.5±8.2	>0.05*
Age at diagnosis of DM (years)	15±8.5		
Duration of DM (years)	16±9.1		

Data are represented as mean±SD or numbers (%). BMI: body mass index.

**** Student's t-test. ** Chi-square test***

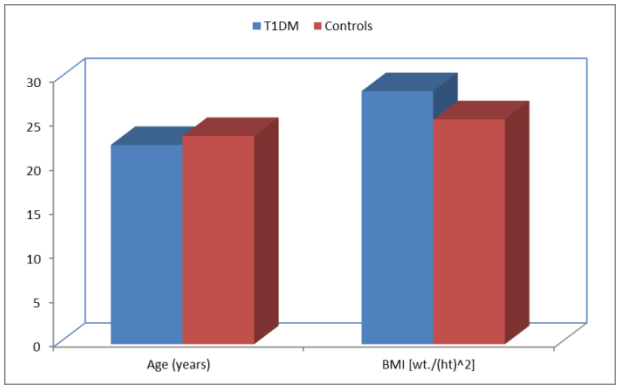

Fig. 1: Bar chart between T1DM and controls according to age and BMI.

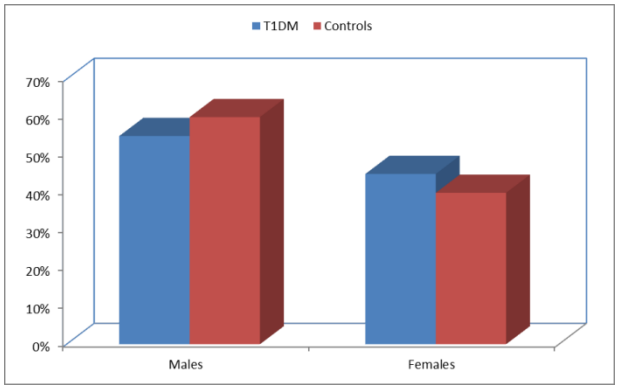

Fig. 2: Bar chart between T1DM and controls according to sex.

It was found that 45 patients (75.0%) were positive autoantibody for GAD, 15 patients (25.0 %) for TPO, 10 patients (16.66 %) were positive for TG-AB and 17 patients (28.3%) were positive for IA-2. All subjects with overt hypothyroidism had positive anti-GAD and thyroid auto-antibodies. One subject (1.67%) had clinical hyperthyroidism with strongly positive GAD, TG-AB, and TPO. Cases showed positivity of both GAD-Ab and IA-2 Ab were 15 (25%), Cases showing positivity of both IA-2 and TG-Ab was 9 (15%) Cases showing positivity of both TPO and TG-Ab were 8 (13.33%). And cases showed positivity of both TPO + TG + GAD + IA-2 were 3 (5.0%), (Table 2 and Figure 3).

Table 2: Positivity for the all Antibodies

	Number of Cases	Percentage of Cases
Anti GAD Ab	45	75.0%
IA2	17	28.3%
TPO	15	25.0%
TG	10	16.66%
TPO + TG	8	13.33%
TG + IA-2	9	15.0%
GAD + IA-2	15	25.0%
GAD + IA2 + TPO + TG	3	5.0%

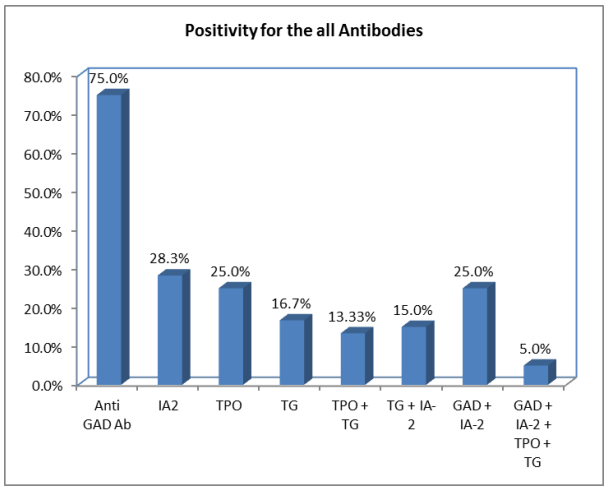

Fig. (3): Bar positivity for the all antibodies distribution of the study group.

Table 3: Laboratory data of results from the studied subjects (Units & %).

Laboratory data	T1DM (n=60)	Controls (n=30)	P value
Blood glucose, Random (mg/dL)	401.9±191.0	96.3±10.1	＜0.001
TSH (µIU/mL)	9.3±4.1	2.1±1.4	0.223
FT3 (pg/mL)	1.4 ± 0.55	2.6 ± 0.70	＜0.001
FT4 (ng/dL)	1.2±0.8	1.3±0.1	0.749
C-peptide (nmol/L)	0.17±0.03	0.6±0.27	＜0.001
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HbA1c (%)	10.2±2.9	5.3±0.5	＜0.001
GAD positive n(%)	45 (75%)	1(3.33%)	<0.001
IA-2 positive n(%)	17(28.3)	0(0%)	<0.001
TPO Ab positive n(%)	15(25.0%)	1(3.33%)	<0.001
TG Ab positive n(%)	10 (16.6%)	0(0%)	<0.001
TPO Ab+TG Ab	8 (13.33%)	0(0%)	<0.001
GAD + IA-2	15(25.0%)	0(0%)	<0.001
GAD + IA-2 + TPO + TG	3(5%)	0(0%)	>0.05

HbA1c: glycosylated hemoglobin, TSH: thyroid stimulating hormone, FT4: free thyroxine T4, GADA: glutamic acid decarboxylase antibody, Anti Glutamic Acid Decarboxylase (anti-GAD), Anti Islet Antbody (IA-2), Thyroid Peroxidase (TPO) ,Thyroglobulin auto-antibodies (TG-Ab)

Table 4: Thyroid status of patients with T1DM (n=60)

Thyroid status	Number	Percent
Hyperthyroidism	1	1.67%
Hypothyroidism (symptomatic)	5	8.33%
Subclinical hypothyroidism	15	25%
Euthyroid	39	65%

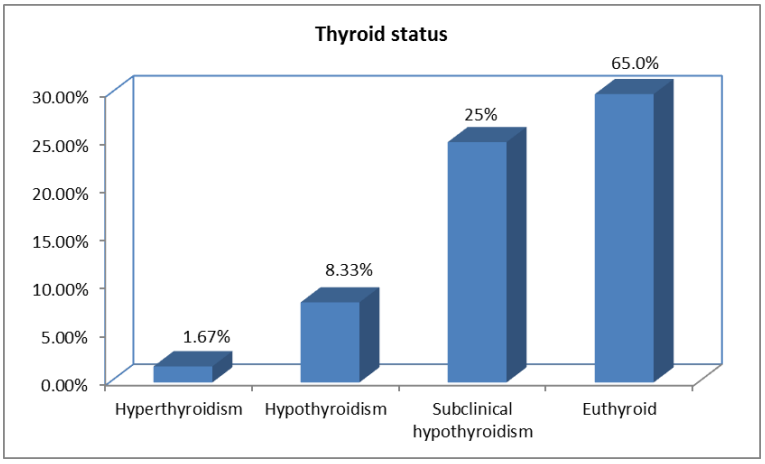

Fig. (4): Bar chart thyroid status distribution of the diabetic patient group.

Table 5: Diabetes specific autoantibodies and thyroid autoantibodies in patients with evident and subclinical hypothyroidism

Auto-antibodies	Evident hypothyroidism	Subclinical hypothyroidism	P value
GADA positive (n=45)	4/45 (8.89%)	12/45 (26.67%)	<0.01
IA-2 positive (n=17)	1/17 (5.89%)	4/17 (23.5%)	<0.001
TPO Ab positive (n=15)	3/15 (20%)	7/15 (46.67%)	<0.001
TG Ab positive (n=10)	2/10 (20%)	3/10 (30%)	>0.05
TPO Ab+TG Ab (n=8)	3/8 (37.5%)	3/8 (37.5%)	>0.05
GAD + IA-2 (n=16)	1/15 (6.66%)	4/15 (26.66%)	<0.01
GAD + IA2 + TPO + TG + (n=3)	1/3 (33.33%)	0/3 (0%)	<0.001

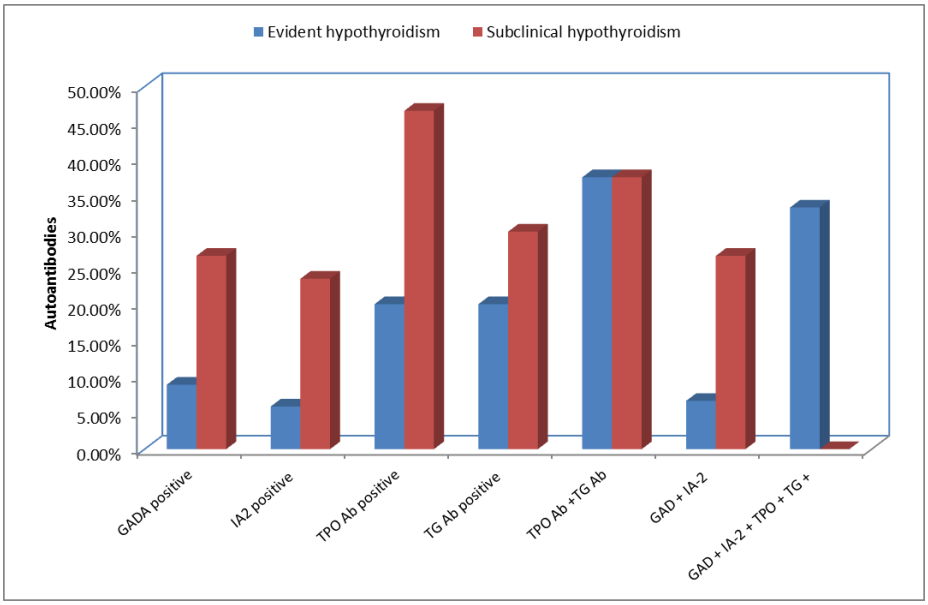

Fig. 5: Bar chart between evident hypothyroidism and subclinical hypothyroidism according to auto-antibodies.

DISCUSSION

The incidence of thyroid dysfunction (high TSH) either clinical or subclinical in our study among T1DM group were present in 20 patients out of 60 (33.3%) which is in agreement with many previous studies like Hwang GB, et al [15] who reported 30.4%, Lee et al [16] who reported 38.8%, Kang et al [17]who reported 28.8%, Jung et al [18] who also reported 26% prevalence of AITD among T1DM patients and also with Perros et al [19] who reported 31.4% as an incidence of thyroid dysfunction among T1DM female patients, but our results were lower than the study done by Heba et al [20], who found it 65%, while it is more higher than the study of Hansen et al [21] who found 5% of patients with T1DM had thyroid dysfunction.

Also, in our study, the prevalence of sub-clinical hypothyroidism (SCH) was 25% among T1DM group and came zero% among control subjects. These results were discrepant from another study In Egypt done by Metwalley & El-Saied[22] who found that 6.3% prevalence of subclinical hypothyroidism in T1DM patients if we compared to 1.6% in control healthy group. Also, in this study, the prevalence of overt hypothyroidism was 8.33% which and subclinical hypothyroidism was 25%. These results was found that were near to the results obtained by Sanyal et al [23] who reported overt hypothyroidism (6%) and subclinical hypothyroidism (32%) among T1DM group.

On the other hand, we reported that 1 case only showing hyperthyroidism (1.67%) which is in agreement with previous results obtained by Perros et al.[19] and Mouradian & Abourizk [24] which was between (1-2%).

Regarding TPO and TG antibodies in our study, we reported 25% positivity for TPO and 16.6% for TG antibodies in T1DM group. This result is in agreement with Sanyal et al.[23] who reported 24% prevalence for TPO and 16% prevalence of TG among T1DM patients and also with Ghawil et al.[25] who documented 23.4% TPO positivity and 7% anti-TG positivity among Libyan T1DM patients.

Our result of TPO positivity in T1DM group is lower than reported by Kordonouri et al.[26] who reported 38%, Umpierrez et al.[27] who reported 33% and by Sharifi et al[28] who reported 39.6% positivity of TPO among Indian T1DM patients. While our results of TPO positivity were higher than reported by Hansen et al.[21] who reported 13% in T1DM patients, by Hwang et al.[15] who reported 14.7% and by Mantovani et al.[29] who reported 16.7% TPO positivity in Brazilian T1DM patients. The difference in prevalence in these studies may be related to the number of cases and duration of diabetes at the time of sampling.

Regarding the relation between prevalence of hypothyroidism among TPO positive cases, in our study, 10 cases out of 15 cases with positive TPO showed hypothyroidism either symptomatic or subclinical (66.7%) (Table 5 and Figure 5). It was stated by Umpierrez et al.[27] that 83% of female subjects and 51% of male subjects with positive TPO show hypothyroidism.

In our study, there is association between positive TPO and/or TG antibodies and high TSH levels; this result is in agreement with other reported studies [30-32]. This may be due to the effect of anti-TPO on the thyroid gland either directly through tissue destruction or indirectly through T-cells infiltrating the thyroid gland which is associated with TPO antibodies [30].

It was stated that the prevalence of GADA positivity is much higher in patients with AITD than in general population[33,34]. The combination of T1DM and AITD is called autoimmune poly-endocrine syndrome type 3[35,36]. Hwang et al.[15] reported that GADA positivity at the onset of T1DM was predicted of AITD development, also, Jin et al.[37] reported that a high GADA titer was a significant predictor of AITD in T1DM patients.

Regarding GADA & IA-2 positivity, in our study, it was found that the prevalence of positive GADA in T1DM group was 75% which is in agreement with Catassi et al.[38] who reported 66% prevalence and with Sanyal et al.[23] who reported positive GADA in 78% of T1DM patients. While, the prevalence of GADA positive cases was much higher in our study (75%) than in the study done by Kochupillai and Goswami [39] who reported 38%. On the other hand we reported that the prevalence of positive IA-2 among T1DM group was 28.3% which is in agreement with that reported by Sanyal et al.[23].

Also, regarding combination of positivity of TPO + TG + GADA + IA-2, we reported that combination prevalence was 5% among T1DM group and this result was in agreement with that obtained by Sanyal et al.[23] who reported 4% for that combination.

CONCLUSION

We found a high prevalence of diabetes-specific auto-antibodies in the T1DM patients. Subclinical thyroid dysfunction was evident in a considerable number of T1DM patients. These results indicate the importance of regular thyroid screening in T1DM patients.

Conflicts of interest: There is no fund received and no conflict of interest.

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