AUTOIMMUNE THYROIDITIS IN TYPE 1 DIABETES MELLITUS

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We have compared the frequency of thyroid antibodies among diabetic (DM) patients with type 1 and type 2. Diagnosed type-1 DM patients, having no previous of history were taken as subjects and divided into early adulthood (18 to 35 yrs) and later adulthood (after 35 yrs) groups. Matched subjects with DM type II are taken as controls. In all subjects, serum concentration of free T3 and free T4, TSH, Thyroid peroxidase antibody (TPO-Ab) and Thyroglobulin antibodies (TG-Ab) were determined. It has been observed that the serum FT3 levels was lower in type-1 diabetics patients as compared to type II DM. In addition there was a slightly increase in the values of anti-TPO and anti-TG antibodies in later adult hood of type I DM when compared to the values of early adult hood of type I DM. And there was significant increase in the values of anti-TPO and anti-TG antibodies in later adulthood of type I DM when compare to the values of later adulthood of type II DM. It has been suggested that estimation of thyroid antibodies should be done periodically for every diabetic patients.

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Introduction

Diabetes mellitus is a group of metabolic diseases characterized by high blood sugar (glucose) levels that result from defects in insulin secretion, or action, or both. Elevated levels of blood glucose (hyperglycemia) lead to spillage of glucose into the urine, hence the term sweet urine. In 2014 the global prevalence of diabetes was estimated to be 9 % among adults aged (18+ years).[1]

Autoimmune thyroiditis is a group of inflammatory thyroid disorders with either hypothyroid, euthyroid or hyperthyroid state.[2] Diabetes mellitus type 1 is often accompanied by autoimmune diseases. Autoimmune thyroid diseases are amongst the most common.[3,4] Recent studies confirmed an increased incidence of autoimmune thyroid diseases even in type-2 diabetes mellitus. Various experimental, clinical studies as well as genetic and epidemiological studies showed the immunological and genetic basis of relationship between diabetes mellitus and thyroid diseases. Currently there is a lot of evidence about the importance of genetic factors in autoimmune diseases.[5]

At least two major clinical forms of chronic autoimmune thyroiditis can be distinguished, Hashimoto and atrophic. The Hashimoto type is characterized with small goiter, elevated anti-thyroperoxidase antibodies (anti-TPO), less commonly anti-thyroglobulin antibodies (anti-TG), and a typical ultrasound picture of thyroiditis. The function of the thyroid gland can be normal, however hypothyroidism can develop later. The atrophic form is a less common type of chronic autoimmune thyroiditis characterized by early development of hypothyroidism and ultrasound signs of thyroid gland atrophy and production of fibrotic tissue. Serum levels of anti-TPO and anti-TG are also typically elevated.[4]

The coexistence of hypothyroidism might cause disturbances in the metabolic control of patients with diabetes.[6] Even subclinical hypothyroidism (slightly elevated TSH without impairment of T4 and T3 levels) is associated with higher frequency of symptomatic hypoglycemia.[7] This finding can be explained by basis of the well known physiological effects of thyroid metabolism on carbohydrates metabolism. Thyroid hormones stimulate intestinal absorption of glucose, further glycogenolysis and hepatic insulin catabolism are also enhanced. These mechanisms have a hyperglycemic effect and subtle changes in thyroid hormone levels might interfere with these actions, thereby increasing the risk of hypoglycemia.[8,9]

The relationship between type I diabetes mellitus and autoimmune thyroid disease was first described in the early 1960s by Pettit and Landing.[9,10] The association of type 1 diabetes mellitus with autoimmune thyroid disease has been well documented in many populations.[11-15] The occurrence of thyroid autoantibodies against microsomes (AMA) and thyroglobulin (ATA), frequently seen in Hashimoto's thyroiditis and Graves' disease, has also been reported in type 1 diabetes mellitus with varying frequency.[16-18] Several subsequent cross sectional studies from various parts of the world have been reported.[19,20] Type 1 diabetes mellitus may be associated with additional autoimmune disorders including autoimmune thyroid disease,[21] coeliac disease[22] and Addison's disease.[23]

In this study we aimed to compare the frequency of thyroid antibodies among diabetic patients with type 1 and type 2 diabetes.

Materials and methods

Subjects

The samples were collected from the Central hospital, Al-dawadmi, KSA, and measurements were conducted in the Department of Clinical Biochemistry, College of Applied Medical Science, Al-dawadmi, Shaqra university, KSA. Fifty diagnosed type-1 diabetes mellitus patients are chosen for our study. Patients classified into two groups each consists of 25 subjects (M=12, F=13) of early adulthood (18 to 35 yrs) and 25 subjects (M=13, F=12) of later adulthood (after 35 yrs). Twenty-five age matched subjects (M=11, F=14) with diabetes mellitus type II were taken as controls. All the subjects had no history of previous thyroid diseases. Informed consent was obtained from all the subjects. Fasting blood samples were collected by venipuncture technique and for separation of serum, the blood is centrifuged at 3000 rpm for 5 min.

The separated serum is used to estimate serum TSH, FT3, FT4, anti TPO antibodies and anti TG antibodies.

Measurements of anti-TPO (IgG)

Anti-TPO (IgG) was measured by using enzyme linked immunosorbent Sandwich assay (ELISA). The ELISA procedure was done according to the manufacturer's instruction (DRG, Germany). Highly purified human thyroid peroxidase (TPO) was bound to microwells. 100 µl of calibrators, controls and patients sera were added in duplicate to each well and incubated for 30 minutes at room temperature. After washing three times, 100 µl of conjugate (anti-human IgG labeled with horseradish peroxidase) was added to each well and incubated for 15 minutes at room temperature. After washing three times, 100 µl of substrate solution, (Tetramethylbenzidine 'TMB') was added to each well. The reaction mixture was then incubated for 15 minutes at room temperature in the dark. 100 µl of stop solution (1 M hydrochloric acid) was then added to each well. Finally, the optical density was measured using microplate reader instrument (Expert Plus, EC) at 450 nm. The mean absorbance (O. D) for each set of duplicate calibrators, controls and patients sera was calculated. The IgG concentration of the unknown was determined from the standard curve. Any concentration >30 IU mL^{-1} was considered as positive.

Measurements of anti-thyroglobulin (anti-TG-Ab) measurement

Anti-thyroglobulin was measured by using ELISA assay. The ELISA procedure was done according to the manufacturer's instruction (DRG, Germany).

The microwells were coated with purified native human thyroglobulin (hTg). Anti-Tg autoantibodies (TG-Ab), when present in the sample, will bind to the solid phase. After removing non specific antibodies by a washing process, the immune complexes are detected by alkaline phosphatase conjugated polyclonal antibodies to human IgG. After removing the unbound conjugate by another washing step, the chromogen/substrate is added, which turns from clear to yellow color if the antibody being tested is present. The intensity of the yellow color, directly proportional to the amount of antibody present in the patient sample, is measured using a spectrophotometer with a 405 nm filter. Patient sample concentrations are read from a calibration curve.

The mean absorbance (O.D) for each set of duplicate calibrators, controls and patients sera was calculated. The IgG concentration of the unknown was determined from the standard curve. In healthy normal subjects 99 % of all values were below 20 IU mL^{-1}.

Measurement of thyroid stimulating hormone (TSH), free T3 and free T4

Thyroid stimulating hormone (TSH), free T3 (FT3) and free T4 (FT4) were measured by an automated analyzer (Elecsys 2010 platform, Roche Diagnostics GmbH), as per the procedure indicated by the manufacturer.

Statistical Analysis

Comparison between means was performed by Student's *t*-test and comparison between frequencies was carried out by chi-squared test. A '*p*' value of 0.05 or less was interpreted as significant for the analysis.

Results

Data in Table 1 showed that, there was a significant decrease in the values of FT3 in both groups of type I DM when compared to the FT3 values of later adult hood of type II DM (Figure 1). There is no statistical difference in the values of FT4 and TSH between both the groups of type I diabetes mellitus and type II diabetes (Figures 2 and 3).

There was a slight increase in the values of anti-TPO and anti-TG antibodies in later adult hood of type I DM when compared to the values of early adult hood of type I DM. And there was significant increase in the values of anti-TPO and anti-TG antibodies in later adulthood of type I DM. when compare to the values of later adulthood of type II DM (Figures 4 and 5).

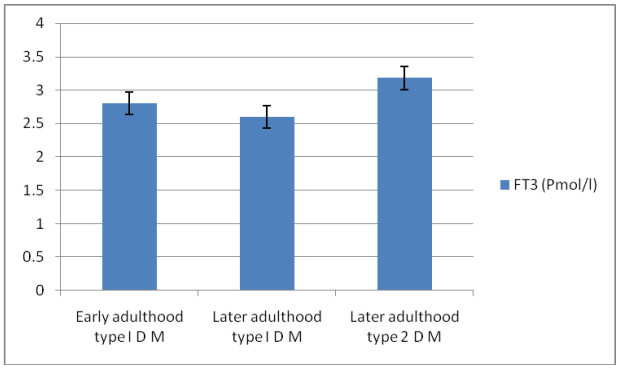


Figure 1. The mean level of free T3 (p mol/l) in different groups.

Table 1. Comparison between mean levels of FT3, FT4, TSH, TP-Ab and TG-Ab.

Parame-ter	Early adulthood DM 1	Later adulthood DM 1	Later adulthood DM 2	'*p*' value
FT3 (pmol L^{-1})	2.8 ± 0.71	2.6 ± 0.67	3.18 ± 1.02	<0.05 S
FT4 (pmol L^{-1})	9.75 ± 0.15	9.81 ± 0.25	9.50 ± 0.05	N.S
TSH (µl U mL^{-1}	3.91 ± 0.25	3.85 ± 0.45	4.1 ± 0.33	N.S
TPO (units)	26.98 ± 2.61	29.28 ± 2.40	19.49 ± 1.92	<0.0001 H.S
TG Ab (units)	25.55 ± 2.20	27.52 ± 1.8	22.75 ± 1.91	<0.0001 H.S

S= Significant, H.S = highly significant, N.S = not significant

From Table 2 we can note that in early adulthood type 1 DM a total 5/25 (20 %) patients had positive TPO antibodies (2 men and 3 women) while only 3/25 (12 %) patients had positive TG antibodies (1 men and 2 women), if we look to late adulthood type 1 DM there are total 7/25 (28 %) patients had positive TPO antibodies (3 men and 4 women) and total 4/25 (16 %) patients (1 men and 3 women) had positive TG antibodies, similarly late adulthood type 2 DM had total of 6/25 (24 %) patients with positive TPO antibodies (3 men and 3 women) and 5/25 (20 %) (2 men and 3 women) patients had positive TG antibodies (Figure 6**)**.

The results (Table 2) also indicate that in the early adulthood type 1 DM the percentage of positive subjects to TPo-Ab is more higher in females 3/13 (23 %) than in males 2/12 (16.6 %). In the late adulthood type 1 DM, the percentage of females positive TPo-Ab is higher 4/12 (33.3 %) than in males 3/13 (23 %) and it is also still higher in late adulthood type 2 DM in female 3/14 (21.4 %) than in males 3/11 (23 %) (Figure 7**)**. We also found (Table 2) that in the early adulthood type 1 DM the percentage of positive subjects to TG-Ab is more higher in females 2/13 (15.3 %) than in males 1/12 (8.3 %).

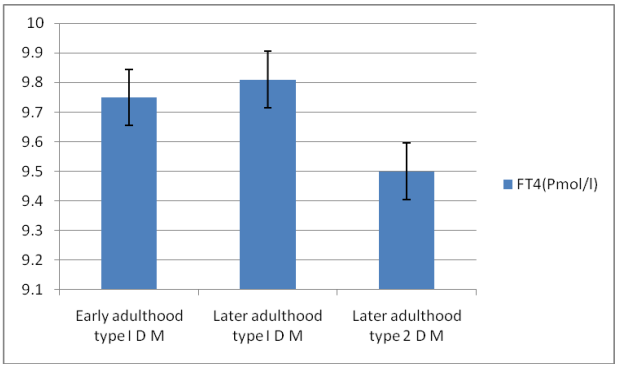


Figure 2. The mean level of free T4 (*p* mol L^{-1}) in different groups.

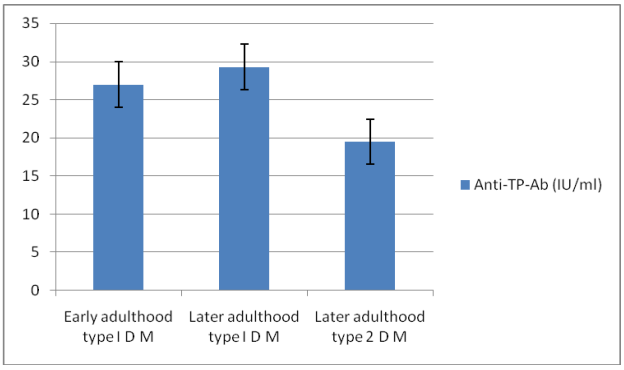


Figure 4. The mean level of TP-Ab (IU mL^{-1}) in different groups.

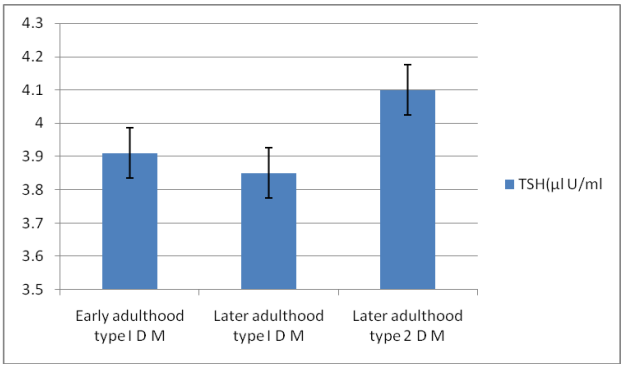


Figure 3. The mean level of TSH (µl U mL^{-1}) in different groups.

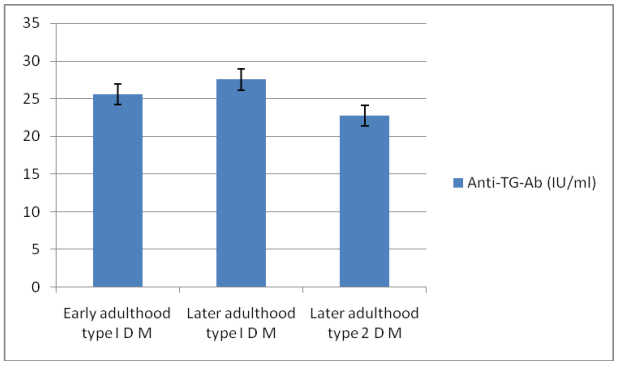
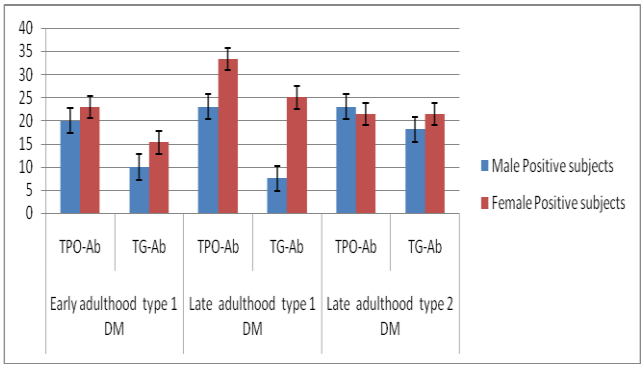


Figure 5. The mean level of TG-Ab (IU mL^{-1}) in different groups

Table 2. Percentage of positive subjects to TPo-Ab and TG-Ab in different diabetic groups.

Group	Item	Male Positive subjects	female Positive subjects	Total
Early adulthood type 1 DM	TPO-Ab	2/12 (20 %)	3/13 (23 %)	5/25 (20 %)
	TG-Ab	1/12 (8.3 %)	2/13 (15.3 %)	3/25 (12 %)
Late adulthood type 1 DM	TPO-Ab	3/13 (23 %)	4/12 (33.3 %)	7/25 (28 %)
	TG-Ab	1/13 (7.6 %)	3/12 (25 %)	4/25 (16 %)
Late adulthood type 2 DM	TPO-Ab	3/11 (23 %)	3/14 (21.4 %)	6/25 (24 %)
	TG-Ab	2/11 (18 %)	3/14 (21.4 %)	5/25 20 %)



No. of subjects = 25 in each group, Positive TPo-Ab subjects ≥30 IU mL^{-1}

Figure 6. Percentage of male and female subjects positive to TPo-Ab and TG-Ab in early and late adulthood type (1) DM with respect to late adulthood type (2) DM.

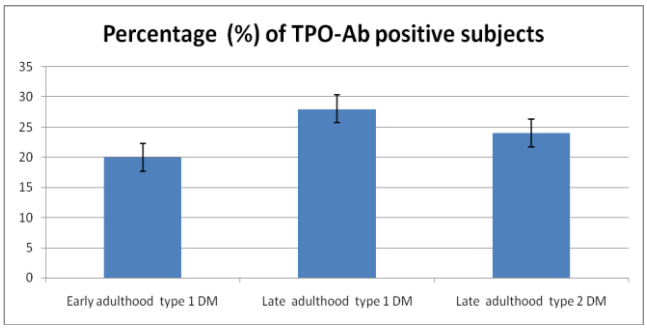


Figure 7. Percentage of TPo-Ab positive subjects in different types of diabetic (No. of subjects = 25 in each group).

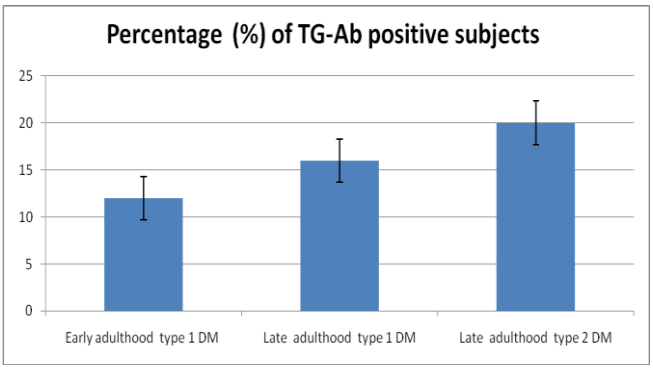


Figure 8. Percentage of TG-Ab positive subjects in different types of diabetic (No. of subjects = 25 in each group).

In the late adulthood type 1 DM, the number of females positive TG-Ab is higher 3/12 (25 %) than in males 1/13 (7.6 %) and it is also still higher in late adulthood type 2 DM in female 3/14 (21.4 %) than in males 2/11 (18.1 %) (Figure 8).

Discussion

Autoimmune diseases combined by development of specific immune response against one or more organs. Many factors are involved including genetic and environmental factors leading to a clinically evident disease.

It is well known that Type 1 diabetes mellitus is an autoimmune disease and can be associated with other autoimmune diseases.[24] Previous studies showed that patients with type 1 diabetes mellitus, is frequently reported to have autoimmune thyroid disease.[25]

In the present study, the serum levels of FT4 and TSH in early and late adulthood type 1 Diabetes Mellitus were not statistically different than that of later adulthood Type II diabetic patients, but the serum FT3 levels were found to be lower in type-1 diabetics as compared to type II diabetes mellitus. The decreased serum level of FT3 may be due to impairment of 5-monodeiodinase enzyme activity, which controls the peripheral conversion of T4 into T3.[26] Guillermo et al found subclinical hypothyroidism in 6.5 % of type-1 diabetic male patients. The likely explanation for this association with thyroid abnormalities is a common underlying predisposition leading to co-existing autoimmune destruction of pancreatic islet cells and autoimmune attack on thyrocytes.[27]

In addition the results showed that, there was a slightly increase in the values of anti-TPO and anti-TG antibodies in later adult hood of type I DM when compared to the values of early adult hood of type I DM. There was significant increase in the values of anti-TPO and anti-TG antibodies in later adulthood of type I DM when compare to the values of later adulthood of type II DM. In agreement with our results, a previous study conducted to determine the level of thyroid autoimmunity among clinically thyroid patients of type 1 and type 2 diabetics and to correlate the levels with pattern of diabetes showed that thyroid autoimmune process seems to be correlated more with type 1 diabetic.[28]

The prevalence of thyroid diseases in diabetic patients was found to be 2-3 times higher than in non-diabetic subjects.[29]

We also observed that in the early adulthood type 1 DM the percentage of positive subjects to TP-Ab and TG-Ab are more higher in females than in males and this with agreement with the previous studies that indicated that organ-specific endocrine autoimmunity develops more frequently in females, including type 1 DM and type 2 DM with thyroid auto-immunity and this may be due to inheritance of the production of serum anti-TPO in an autosomal fashion in females but not in males.[30,31]

The involvement of organ specific antibodies in the pathogenesis of the disease is secondary to tissue destruction by thyroid infiltrating T-cells is still unknown. It is also unclear that whether anti-TPO antibodies are able to induce hypothyroidism by blocking the enzyme thyroid peroxidise.[32]

The benefits of identifying thyroid dysfunction at an early stages as a clinical disorder even in asymptomatic patients are considerable particularly in view of high likely hood of progression to overt thyroid dysfunction. It could be concluded that estimation of thyroid antibodies should be done periodically for every type-1 diabetic patients.[33]

Conclusion

Patients with positive antibodies should be monitored for TSH elevation at yearly intervals with the goal of early detection to prevent the possible adverse effects on the human body metabolism. Without these regular and specific laboratory tests, early diagnosis of autoimmune thyroid diseases in routine diabetologic practice is a very difficult task.

Conflict of interests

All authors have declared that there is no conflict persists in this article.

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Competing interests

The authors declare that they have no competing interests.

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