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Biochemical Study on Anti Thyroid Peroxidase in Type 2 Diabetic patients with thyroid disorders

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Abstract:

Type 2 diabetes mellitus (T2DM) is the most frequent endocrinal disease commonly associated with thyroid disorders. The study is conducted at the Specialized Center for Endocrinology and Diabetes in Baghdad, during December 2014 up to October 2015. This study was done to investigate the prevalence of anti-thyroid peroxidase (Anti-TPO) antibody in patients suffered from type 2 diabetes with thyroid disorders. The study groups included a total number of 80 subjects consisting of 60 type 2 diabetic patients divided into 20 hyperthyroidism subjects (group 1), 20 hypothyroidism subjects (group 2), 20 euthyroidism subjects (group 3) and 20 healthy controls (group 4). The fasting blood samples were analyzed for (T3, T4, TSH) by using Vitek Immuno diagnostic Assay System (VIDAS). Enzyme Linked Immunosorbent Assay (ELISA) is used to detect anti-thyroid peroxidase (Anti-TPO) antibody. The results show that age, gender and BMI (body mass index) have significantly higher levels in the patients groups as compared to the healthy group at ($p < 0.01$). Among 60 type 2 diabetic patients, the hypothyroidism group showed a highest mean value (333.57 ± 104.77) of anti-TPO when compared to other groups. The levels of T3 and T4 were significantly higher in hyperthyroidism group, while the level of TSH was significantly higher in hypothyroidism group.

Key words: Type 2 diabetes, thyroid disorders, anti-thyroid peroxidase.

Introduction:

T3 and T4 are the most significant hormones produced from thyroid gland which play a central role in organizing the metabolic function, development and growth. Invariably, the imbalance of thyroid gland activity hypo and hyper leading to increase or decrease of thyroid hormones secretion which resulting a thyroid dysfunction [1,2].

In 1927, both Coller and Huggins made an investigation about the relation between diabetes mellitus and thyroid dysfunction. It had been shown in a surgery process, when they removed a part of thyroid gland had a perfection effect on the regeneration of glucose tolerance in patient suffering from hyperthyroidism and worsening diabetes

mellitus[3]. DM is a group of chronic disease characterized by an elevation of blood glucose that results from defect in insulin production and insulin action or both [4]. DM is classified into three principle types involving; type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM) and gestational diabetes and there are also other forms of DM separately from these three types [5]. Coller and Huggins (1927) and many other researchers proved that many individuals who have thyroid disorder were also had diabetes, this means that level of glucose in human blood affected with raised or reduced either or both thyroid hormones [6].

Due to deep underlying association between thyroid dysfunction and diabetes mellitus and malfunction of human in Iraq as well in other parts of world, this study was designed to fulfill the aims of : Determination of fasting blood sugar (F.B.S) and effect of age, sex and body mass index B.M.I in the patients groups .Also investigation of lipid profile and Triiodothyronine (T3),Thyroxine (T4) and Thyroid stimulating hormone (TSH) in the patients and control groups, as well as determination the prevalence of anti thyroid peroxidase (anti-TPO) with T2DM to find the relationships among the studied parameters in patients groups.

Methods:

Sample Collection

A total of 80 samples (serum) were collected from Iraqi individuals who attended to Specialized Center for Endocrinology and Diabetes in Baghdad during the period from December 2014 up to October 2015. Subjects include 20 healthy individuals (11 female and 9 male) and 60 patients with T2DM that divided into three subgroups :20 patients with hypothyroidism (13 female and 7 male), 20 patients with hyperthyroidism

(15 female and 5 male) and 20 patients with no thyroid disorders (Euthyroid group) (18 female and 2 male).Type 2 diabetics diagnosed at ages above 40 years ,while the healthy individuals were with ages (20-55) years .All individuals had no other disease . All of the subjects were interviewed personally to fill especially designed questionnaire form.

Laboratory Tests:

By using VIDAS and Sandwich ELISA method instruments, Serum analyzed for determination the levels of (FBS, T3,T4, TSH and Anti-Tpo). Fasting serum glucose was determined by using Accu-chek active monitor and lipid profile was determined by the sea separation method technique.

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to study the effect of different factors in the parameters. Least significant difference –LSD test was used to express the significant between means. Correlation coefficient values were estimated to find the association between the different parameters in this study. All the statistic analysis and the finding results were made by the supervision of the bio-statistician; Dr. Naser Al-Anbary (College of Agriculture/ Baghdad University).

Results and Discussion:

Distribution of the samples of the study according to age in different groups

It can be shown from Table (1) that the incident opportunity of thyroid disorders was raised with the advance of age .The majority of thyroid disorders was recorded in the age group (30-49) years with 10 (50%), 8 (40%) in the hyperthyroidism and hypothyroidism groups, while 3 (15 %), 6 (30%)in the euthyroidism and control groups, respectively. Followed by age group (50-60) years with 8(40%), 9 (45%)in

the hyperthyroidism and hypothyroidism groups, while 8 (40%), 4(20%) in the euthyroidism and control groups, respectively. While the lowest percentage of thyroid disorders was recorded in age groups (Less than 30)

and (More than 60). These results were at high significant differences ($P < 0.01$) between each of the studied groups. Moreover the thyroid disorders were found more abundant in females 56(70%) than in males 24(30%).

Table 1. Distribution of samples according to the age in the different groups

Age groups	Frequencies and %	Group				χ^2 and P-value H.S: Highly significant P=0.0001
		Hyperthyroidism	Hypothyroidism	Euthyroidism	Healthy control	
Less than 30	Frequency %	0 0.00%	0 0.00%	0 0.00%	10 50.00%	$\chi^2 = 13.253$ ** 0.0001 H.S.
30-49	Frequency %	10 50.00%	8 40.00%	3 15.00%	6 30.00%	
50-60	Frequency %	8 40.00%	9 45.00%	8 40.00%	4 20.00%	
More than 60	Frequency %	2 10.00%	3 15.00%	9 45.00%	0 0.00%	
Mean \pm SD		50.40 \pm 8.26	53.55 \pm 7.43	59.55 \pm 7.91	33.60 \pm 13.63	

**:($P < 0.01$).

Results in the present study were in agreement with AL-Omairi (2010) [7] and vadiveloo *et. al.*(2013) who found that thyroid disorder incidence rates raised with individuals ages [8]. On the other side ,Ahmed *et. al.* (2009) found that the levels of thyroid hormones in the patient's life is increased in the first decade (30-49) and decreased in the second and third decades [9].

Distribution of samples according to BMI in different groups .

The thyroid disorders can be classified into four groups underweight [16.00-17.00 kg/m²], normal weight [18.50-25.00 kg/m²], over weight[25-30 kg/m²] and obese [>30 kg/m²]. It can be observed from table (2) that the highest percentage of obese patients was recorded in hypothyroidism 17 (85%). There was a highly significant difference between healthy and patients groups at ($p_{value} < 0.01$) according to their BMI.

Table 2. Distribution of samples according to BMI in different group

BMI groups	Frequencies and %	Group				χ^2 and P-value
		Hyperthyroidism	Hypothyroidism	Euthyroidism	Healthy control	
Under weight	Frequency %	4 20.00%	1 5.00%	3 15.00%	5 25.00%	$\chi^2 = 9.327$ ** P = 0.00218 H.S.
Normal weight	Frequency %	7 35.00%	1 5.00%	5 25.00%	3 15.00%	
Over weight	Frequency %	6 30.00%	1 5.00%	4 20.00%	5 25.00%	
Obese	Frequency %	3 15.00%	17 85.00%	8 40.00%	7 35.00%	
Mean \pm SD		27.77 \pm 3.82	34.28 \pm 4.24	29.98 \pm 5.95	29.61 \pm 5.95	

**:($P < 0.01$).

These results were matched with Mohammed Alfkhami *et. al* (2010) [10]. And with a study performed in Turkey

by Bastemira *et. al.* (2007), who noted that TSH level was positively associated with the degree of adiposity [11]. The

development of obesity can be related with many causes such as unhealthy life style and some diseases such as hypothyroidism. In vertebrates, T3 and T4 hormones considered the major regulators of energy metabolism. Therefore, any defect in thyroid hormones levels are frequently correlated with body weight change [12].

Levels of T3, T4 and TSH hormones in different groups

Regarding the thyroid hormones (T3 and T4) and thyroid stimulating

hormone (TSH), their levels are shown in Table (3). The highest mean value of T3 is (2.78 ± 0.46 nmole/L) and T4 is (165.95 ± 15.16 nmole/L) and lowest mean value of TSH is (0.0163 ± 0.006 umole/L) were recorded in hyperthyroidism group, while the lowest mean value of T3 is (1.26 ± 0.10 nmole/L) and T4 is (99.80 ± 8.01 nmole/L) and highest value of TSH is (25.31 ± 6.17 umole/L) were recorded in hypothyroidism group when compared to other groups.

Table 3. Levels of the hormones in patients and control groups.

Group	No.	Sex		Age group	Mean \pm SE		
		male	female		T3 nmole/L	T4 nmole/L	TSH umole/L
Hyperthyroidism	20	5	15	40-65	2.78 ± 0.46 a	165.95 ± 15.16 a	0.0163 ± 0.006 b
Hypothyroidism	20	7	13	40-65	1.26 ± 0.10 b	99.80 ± 8.01 b	25.31 ± 6.17 a
Euthyroidism	20	2	18	40-70	1.54 ± 0.10 b	123.35 ± 4.76 b	1.84 ± 0.21 b
Healthy control	20	9	11	20-55	1.47 ± 0.08 b	108.90 ± 4.33 b	2.16 ± 0.21 b
LSD value					0.697 **	25.803 **	8.698 **
					** (P<0.01).		
					a : Highly mean value same symbol (b ,b ,b) : Non significant differences different symbol (a, b) : Significant differences		

Data illustrated in Table (3) showed that the distribution of T3 and T4 concentration among diabetic patients, with highly significant differences at ($P_{\text{value}} < 0.01$) when compared with control group. Furthermore, we found highly significant differences between studied groups according to TSH concentration at ($P_{\text{value}} < 0.01$). Our results agreed with Koch *et. al.* (2009) who found that thyroid hormones (T3 and T4) levels were decreased and TSH levels were increased in hypothyroidism patients [13]. While cappa *et.al.* (2011) mentioned that many factors such as

age, gender, pregnancy, bacterial infection play an affected role in the thyroid diseases [14].

Fasting blood glucose (FBS) levels in different groups

Results obtained from Table (4) showed that the highest mean values of FBG concentration were recorded in patients groups, hypothyroidism (161.67 ± 14.93) and euthyroidism (157.15 ± 10.89) and hyperthyroidism (112.83 ± 1.82) groups when compared to control (94.15 ± 1.82) group. A highly significant difference was found in FBG concentration at ($P_{\text{value}} < 0.01$).

Table 4. Effect of difference group in FBS, BMI and TPO

Group	Mean \pm SE		
	F.B.S (mg/dl)	BMI (kg/m ²)	Anti-TPO (IU/ml)
Hyperthyroidism	112.83 ± 6.65 b	27.77 ± 0.85 b	134.56 ± 34.72 b
Hypothyroidism	161.67 ± 14.93 a	34.28 ± 0.95 a	333.57 ± 104.77 a
Euthyroidism	157.15 ± 10.89 a	29.98 ± 1.33 b	109.41 ± 45.65 b
Healthy control	94.15 ± 1.82 b	29.60 ± 1.33 b	28.32 ± 9.39 b
LSD value	27.795 **	3.206 **	168.73 **
** (P<0.01).			

Obesity is a predictor of impaired fasting glucose (IFG). In most of overweight people or obese, the metabolism has many complications because the excess levels of visceral fat (abdominal fat). Abdominal obesity is positively correlated with insulin resistance and metabolic syndrome. The

hyperinsulinemia is the most factor influenced the development of impaired fasting glucose. Some studies have reported that abdominal obesity is one of the risk causes for increasing the IFG and T2DM [15]. These results were in agreement with other studies carried out in Iraq by Hussien (2012) who found that FBG of diabetic patients have a higher significance when compared with healthy group [16]. Also these findings were compatible with other studies by Hanfi in Egypt (2011) [17] and also in agreement with Patil *et al.* (2010) who observed that the levels of FBG were elevated despite elevated C-peptide levels in obese individuals, proving the insulin resistance role [18]. The loss of triiodothyronine (T3) within the cells leads to an elevation of TSH levels, which causes a reduction of Glucose transporter type 4 (GLUT-4) activity in the skeletal muscles and adipose tissues; thus insulin resistance is stimulated which was noted in high percentage of obese patients [19].

Anti thyroid peroxidase (Anti-TPO) in different groups.

Table (5) represented the distribution of Anti-TPO in the four study groups, the table shows that the highest occurrence of Anti-TPO were recorded (333.57 ± 104.77), (134.56 ± 34.72) in hypothyroidism and hyperthyroidism groups respectively when compared to euthyroidism (109.41 ± 45.65) and healthy (28.32 ± 9.39) groups. A highly significant difference was found in Anti-TPO level in thyroid patients when compared with euthyroidism and healthy control groups at ($P_{\text{value}} < 0.01$).

Table 5. Effect of difference group in FBS, BMI and TPO

Group	Mean \pm SE	
	BMI (kg/m ²)	Anti-TPO (IU/ml)
Hyperthyroidism	27.77 \pm 0.85 b	134.56 \pm 34.72 b
Hypothyroidism	34.28 \pm 0.95 a	333.57 \pm 104.77 a
Euthyroidism	29.98 \pm 1.33 b	109.41 \pm 45.65 b
Healthy control	29.60 \pm 1.33 b	28.32 \pm 9.39 b
LSD value	3.206 **	168.73 **

** (P<0.01).

Anti-thyroid peroxidase antibodies (anti-Tpo Ab) are considered as one of the specific autoantibody of generalized autoimmune thyroid dysfunction in the human body which is directed to thyroid follicular [20]. Tpo is a membrane bound enzyme, glycosylated hemoprotein which is responsible for synthesis of thyroid hormones by stimulating both of the iodination process of thyroglobulin and coupling the residues of iodotyrosyl to generate the thyroid hormones tri-iodothyronine (T3) and thyroxin (T4) [21]. These results were compatible with the study made in United Kingdom in (2008), that found a highly significant difference between patients and healthy groups [22]. This study also agreed with Abdelgadir A. Elmugadam *et al.* (2010) [23]. Hasan HG (2011) mentioned that the Tpo activity has qualitative abnormalities in patients with congenital thyroid disorders [24].

Person's correlation coefficient between study parameters at the studied samples with comparison significant

Table (5) represented in term of simple correlation coefficients (person's correlation coefficients), that there were variously significant levels for the extracted responding coefficients between a study parameters responding which indicated meaningful interactions. The results invited searching for that significant interaction in order to construct the veritable measurement scale for studying the thyroid disorders in type 2 diabetic patients.

Table (5) showed significant positive correlation in FBS with lipid profile;

cholesterol, triglyceride, HDL, VLDL at ($P_{\text{value}} < 0.01$) and significant difference with BMI at ($p_{\text{value}} < 0.05$) and significant negative correlation was found with Tpo, LDL, T3, T4, TSH. Results obtained from Table (5) showed that B.M.I has a significant positive correlation with TSH at ($P_{\text{value}} < 0.01$), while a significant difference was found with the following: Anti-Tpo, triglyceride and VLDL at ($P_{\text{value}} < 0.05$)

and significant negative correlation with HDL and T3. Finally, the table showed a significant negative correlation between Anti-Tpo and the following: cholesterol, triglyceride, HDL, VLDL, T3, T4. The analysis of this study was showed a significant difference of TSH level and lipid profile (triglyceride, LDL, VLDL) at ($P_{\text{value}} < 0.05$), except HDL which has a significant negative correlation.

Table 5. person's correlation coefficient between the parameters under study.

	FBS	BMI	TPO	CHOLS	TRIG	HDL	LDL	VLDL	T3	T4	TSH
FBS	-	0.22 *: S	0.18 NS	0.33 ** : HS	0.44 **: HS	0.31 ** : HS	-0.17 NS	0.46 ** : HS	-0.12 NS	-0.18 NS	0.05 NS
BMI		-	0.23 *: S	0.07 NS	0.21 *: S	-0.11 NS	0.14 NS	0.21 *: S	-0.18 NS	-0.28 ** : HS	0.21 ** : HS
TPO			-	-0.05 NS	-0.04 NS	-0.04 NS	0.02 NS	-0.06 NS	-0.07 NS	-0.07 NS	0.18 NS
CHOLS				-	0.14 NS	0.21 *: S	0.31 ** : HS	0.13 NS	-0.15 NS	-0.25 *: S	0.11 NS
TRIG					-	-0.27 ** : HS	0.21 *: S	0.91 ** : HS	-0.03 NS	-0.07 NS	0.25 *: S
HDL						-	-0.60 ** : HS	-0.10 NS	-0.12 NS	-0.01 NS	-0.16 NS
LDL							-	0.04 NS	-0.11 NS	-0.21 *: S	0.24 *: S
VLDL								-	-0.05 NS	-0.04 NS	0.22 *: S
T3									-	0.70 ** : HS	-0.22 *: S
T4										-	-0.35 ** : HS
TSH											-

* (S): ($P < 0.05$)., ** (H.S): ($P < 0.01$)., NS: Non-significant.

These results agreed with Bakker *et al.* (2001) who showed that thyroid autoimmunity may also have a central role in the elevation of lipid profile levels [25]. It has been reported that thyroid dysfunction in males and postmenopausal females increase the level of TPO level, these results matched with Bairaktari (1999) [26]. This study showed that there was an association between FBS and lipid profile, that matched with Nayak *et al.* (2011) who found that the 2 diabetic patients had significantly abnormal levels of lipid profile [27, 28].

Conclusion:

* Prevalence of thyroid disorders increase with advanced age of patients

and females represent the vast majority of thyroid disorders than males.

* There was a positive correlation between Anti- TPO and body weight.

* F.B.S was found to be related with high body weight in hypothyroidism group.

* Anti-TPO incidence rate increases in hypothyroidism group more than other study groups.

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دراسة كيموحيوية لإنزيم (Anti-thyroid peroxidase) في مرضى السكري النوع الثاني المصابين باعتلال الدرقية

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**المركز الوطني لبحوث السكري، الجامعة المستنصرية

الخلاصة:

مرض السكري النوع الثاني يعتبر من أمراض الغدد الصماء الأكثر شيوعاً و الذي يرتبط عموماً مع اضطرابات الغدة الدرقية. أجريت الدراسة في المركز التخصصي لأمراض الغدد الصم والسكري في بغداد/الجامعة المستنصرية خلال الفترة كانون الأول 2014 ولغاية تشرين الأول 2015. هذه الدراسة أجريت للتحري عن نسبة (Anti-TPO Ab) في المرضى الذين يعانون من مرض السكري النوع الثاني وأمراض الغدة الدرقية. المجاميع المدروسة تضمنت 80 مريضاً، كان من ضمنها 60 مريض يعاني من مرض السكري والذي قسم إلى (20) مريض كانوا يعانون من فرط الغدة الدرقية (المجموعة الأولى)، (20) مريض كانوا يعانون من قصور الغدة الدرقية (المجموعة الثانية)، (20) مريض لديهم سوي الغدة الدرقية (المجموعة الثالثة) و (20) آخرين كانوا من الأصحاء (المجموعة الرابعة). أستعمل نموذج الدم أليصامي للكشف عن اختبارات (T3,T4,TSH) بواسطة تقنية الفايديس (VIDAS). تقنية الأمتزاز المناعي المرتبط بالإنزيم (ELISA) استعملت للتحري عن وجود مستضدات الغدة الدرقية (Anti-TPO) Ab. بينت النتائج بأن هناك اختلافاً معنوياً عالياً في معدل العمر، الجنس، ودليل كتلة الجسم (BMI) في مصول مجاميع المرضى مقارنة بمصول مجموعة الأصحاء. إن من بين 60 مريضاً، المجموعة التي كانت تعاني من فرط الغدة الدرقية كانت تمتلك أعلى نسبة (333.57 ± 104.77) من مستضد ال (TPO) بالمقارنة مع المجاميع البقية. كان هناك اختلافاً عالياً المعنوية في مستوى ال [T3,T4] عند المجموعة التي تعاني من فرط الغدة الدرقية، بينما في مستوى ال (TSH) كان هناك اختلاف عالياً المعنوية في المجموعة التي كانت تعاني من قصور في الغدة الدرقية.

الكلمات المفتاحية: سكر النوع الثاني، الأخطاء الدرقية، إنزيم الدرقية Anti-thyroid peroxidase.