DETECTION OF AUTO-ANTIBODIES SPECIFIC TO ISLETS CELLS IN PATIENTS WITH THYROID DYSFUNCTION BY USING BIOCHIPS TECHNIQUE

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Abstract

The aim of this study was to detect the auto immune Type 1 diabetes among the disorders with thyroid dysfunction (hyperthyroidism and hypothyroidism).The study included 50 patients suffering from thyroid dysfunction, their aged was from (1-30) years. They were selected randomly from the Al-kindy center for infertility and indocrinology. The control group consisted of (20) healthy subjects comparable for age and sex of study groups. Thyroid function tests (thyroid-stimulating hormone [TSH], thyroxin, and triiodothyronine) measured in by using miniVIDAS system and auto-antibodies for Beta cells in pancreas estimated in the same patients by using immunofluorescent method (IFAT), the method named BIOCHIPS Mosaic. Results of thyroid function test revealed that 25 patients were evaluated to have hyperthyroidism and 25 patients with hypothyroidism and the results of BIOCHIPS showed hyperthyroidism group was having auto-antibodies specific to islets cells with 8 percent of all the subjects (two from 25 patients) .While there was no signs of auto antibodies for islets cells in hypothyroidism group. The study revealed that the prevalence of thyroid dysfunction was higher in women than men.

Key words: hyper and hypothyroidism, autoimmune Diabetes Type 1, auto- antibodies islets cells.

Introduction

Type 1 diabetes mellitus (DM) one of the most common chronic diseases in childhood, is caused by insulin deficiency resulting from the destruction of insulin–producing pancreatic Beta cells in the islets of langerhans. As the disease progresses, the pancreatic islets are destroyed by antibodies and lymphocytes infiltrating them, causing the production of insulin to stop. (1, 2).Type1 is characterized into two subgroups i.e. type 1A and type 1B. Type 1A results from autoimmune B-cells destruction, usually leads to insulin deficiency, when as type1B DM occurs due to lack of immunologic marker inductive of an autoimmune destruction process of the B-cells (3).Type1 DM is often connected with other autoimmune diseases, the most concomitants disease is autoimmune thyroiditis, several studies confirmed the higher prevalence of autoimmune thyroiditis in patients with type1 diabetes compared with non-diabetic population (4,5). The disease occurs in 4-6% of children and 20-40 % of adults with type1 DM, while the highest prevalence has been reported among middle aged women (6,7). The differences in the prevalence rates reported in various studies are probably due to a different genetic background associated with differences in the selection of patients and for also antibody determination. The prevalence of auto immune thyroiditis in the general population ranges from 1-7 % (8, 9). Two major clinical form of chronic autoimmune thyroiditis can be distinguished–Hashimoto and Graves diseases (10). Up to 20 percent of patients with type 1 DM have positive anti - thyroid antiboidies (anti thyroid peroxidase anti-TPO and /or anti thyroglobulin anti -TG), and 2 to 5 percent of patients with type 1 diabetes developed auto immune hypothyroidism (11-14). The prevalence of autoimmune thyroiditis is higher in girls with diabetes compared to boys, the girls were more likely than boys to have anti-TOP and anti -TG antibodies (14). Cross-sectional studies have reported that the risk of thyroid dysfunction in patients with type1 DM is two to three folds higher than in the general population (15).Type1 DM may be seen in association with hypothyroidism and hyperthyroidism, in Downs's syndrome and also in patients with congenital rubella. Autoimmune thyroid diseases and immune mediated diabetes which is more common in women and occurs in early to middle adulthood is characterized by autosomal inheritance and presence of autoimmune Addison's disease and seen in cases of

haemochromatosis (16, 17, 18). Thyroid dysfunction is common among diabetic patients and can produce metabolic disturbance (17).

Material and method

The study include 50 patients which was suspected with thyroid dysfunction of age (1-30) years and 20 healthy blood donors taken as a healthy control group. The groups suspected with thyroiditis were subjected to the following: Determination of free T3, T4 and TSH in serum by using miniVIDAS (bioMetrieux). And estimation of auto-antibodies IgMAG (profile) for islet cells in pancreas by using Indirect Immun-ofluorescence Test (IFAT), method named BIOCHIP Mosaic from UROIMMUN Company applied as the leaflet kit.

a- The principle:

The test kit is designed exclusively for the invitro determination of humane anti-bodies in serum or in plasma. The determination can be performed qualitatively or quantitatively. Frozen sections of Monkey pancreas are incubated with diluted patient samples. If the reaction is positive, specific antibodies of classes IgA, IgG and IgM attached to the tissue antigens. In a second step, the attached antibodies are stained with fluorescein-labelled anti-human antibodies and made visible with the fluorescence microscope.

b- Procedure:

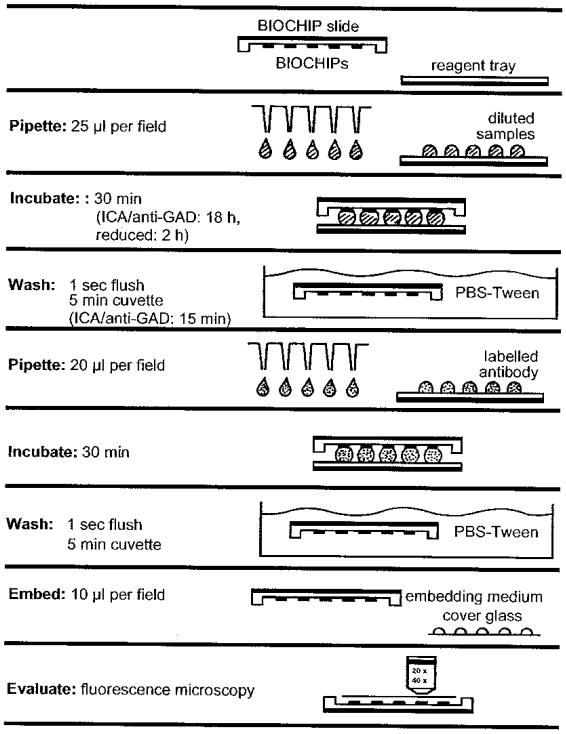
- Serum or plasma was diluted at a ratio 1:10 in PBS-Tween.
- 25 ML of diluted samples were added to reaction field of the reagent tray.
- BIOCHIP slides fitted into the corresponding recesses of the reagent tray and incubated for 18hr at room temperature.
- The BIOCHIP slides rinsed with a flush of PBS-Tween and immersed immediately in a cuvate containing PBS-Tween for at least 15min with shaking.
- 20 ML of fluorescein-labelled anti-human globulin was added to each reaction field of a clean reagent tray by using stepper pipette.
- BIOCHIP slide removed from cuvate and dried with a paper towel and immediately put into the recesses of reagent tray. Incubation for 30min at room temperature.
- BIOCHIP slide rinsed with a flush of PBS-Tween by using a cuvate with PBS-Tween for at least 5min with shaking then BIOCHIP slide was counterstained with diluted drops of Evan blue.

- -10 ML of embedding medium was added per reaction field.

c- Calculation of results:

BIOCHIP slide were examined under HOX-Magnification of a fluorescent micro-scope. Their dark green staining identified positively labeled cells.

TITERPLANE Technique: AS follow:



Steps explained BIOCHPs Technique.

Statistical analysis

Comparison of paired data from the groups of the subjects was done using T-test (t), while correlation between groups were analyzed using person correlation coefficient formula .Statistical tables include observed frequencies with their percentage. SPSS and Microsoft excel programs were used for T-test and correlation coefficient calculation respectively.

Results

The results of this study showed there was none significant differences ($p>o.o5$) in hypo and hyperthyroidism patients which was

accumulated in age group (21-30) years (15, 60%) and (20, 80%) respectively in Fig.(1). Non significant differences appeared (p>0.05) when comparison between Gender of hypo and hyperthyroidism patients and it was found that the female was the predominant are (22, 88%) and (19, 76%) respectively as noted in Fig.(2).

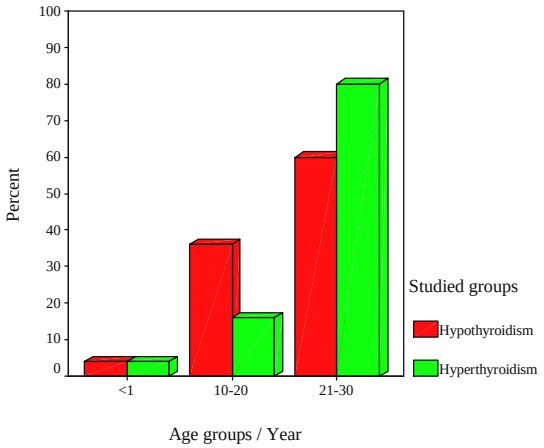


Fig.(1): Age distribution of Hypo and hyperthyroidism patients enrolled in the study.

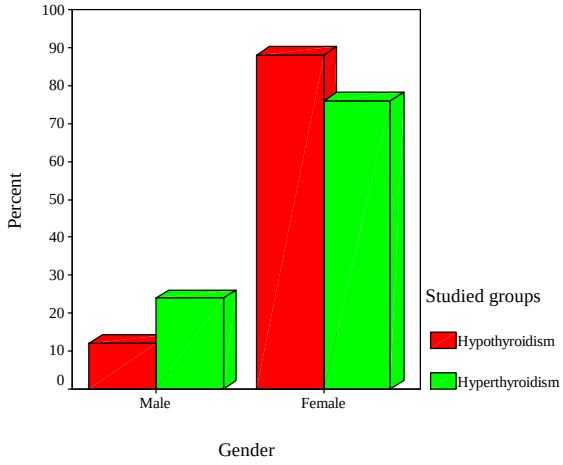


Fig. (2): Sex distribution of hyper and hypothyroidism Patients enrolled in the study.

The results of anti-islets cells Ab in sera of hypo and hyperthyroidism patients represented in negative results which was (25,100%) and (23, 92.0%) respectively, but the positive cases were only in hyperthyroidism patients (2, 8.0%) with non–significant differences (P.0.05) in Table (1). All hypothyroidism patients which have negative anti islets cells Ab with age group (21-30),(10-20) years were indicated with high frequently (15,60%) & (9,36%) respetively, showed in Table (2). While only two positive Anti-islets cells Ab (1, 4.0%) was found within age groups (<1) year and (21-30) year with highly significant differences in table and Fig.(3) by immunofluoirescent microscope.

Table (1)
The percentage of Anti-Islets cells antibodies in sera of hypo -hyperthyroidism patients and control group.

		Studied groups			
		Healthy control	Hypothyroidism	Hyperthyroidism	Total
Anti-Islet cell Ab.	Positive N			2	2
	%			8.0%	2.9%
	Negative N	20	25	23	68
	%	100.0%	100.0%	92.0%	97.1%
Total	N	20	25	25	70
	%	100.0%	100.0%	100.0%	100.0%

	Value	df	P-value
Chi-Square	3.706	2	0.157 NS

Table (2)
Presence of Anti-Islets cells antibodies in sera of patients with Hypothyroidism according to age.

Studied groups			Anti-Islet cell Ab. Negative	Total
Hypothyroidism	Age groups / Year <1	N	1	1
		%	4.0%	4.0%
	10-20	N	9	9
		%	36.0%	36.0%
	21-30	N	15	15
		%	60.0%	60.0%
	Total	N	25	25
		%	100.0%	100.0%

Studied group		Value	df	P-value
Hyporthyroidi	Chi-Square	11.840	2	0.003 HS

Table (3)
Presence of Anti-Islets cells antibodies in sera of patients with hyperthyroidism according to age.

Studied grou			Anti-Islet cell Ab. Positive	Negative	Total
Hyperthyroid	Age group / Year <1	N	1		1
		%	4.0%		4.0%
	10-20	N		4	4
		%		16.0%	16.0%
	21-30	N	1	19	20
		%	4.0%	76.0%	80.0%
	Total	N	2	23	25
		%	8.0%	92.0%	100.0%

Studied group		Value	df	P-value
Hyperthyroidi	Chi-Square	12.092	2	0.002 HS

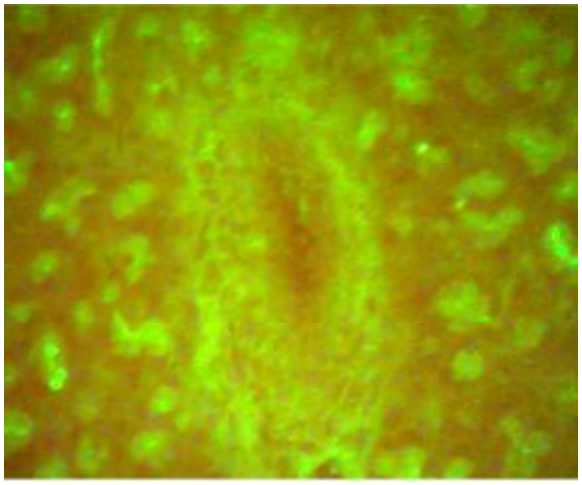


Fig. (3): Immunofluorescent of Anti-islets cells Abs by IFAT (Dark green staining identified positively labeled Anti-islets Ab) magnification power (100 x).

Discussion

Autoimmune endocrine diseases are disorders in which deregulation of the immune system results in immune attack of endocrine glands as a result, these endocrine gland usually are destroyed, resulting in deficiency in the hormones secretion (15-20). While autoimmune disorders can affect many endocrine glands by far, the most common endocrine autoimmune endocrine disorders are type1 DM, celiac disease and thyroid diseases are regarded circulated auto–antibodies (21, 22). These diseases could be explained by molecular mimicry by which gliadin or tissue transglutaminase activates T cells that are cross-reactive with various self antigen .Such inflammatory responses may have the capacity to persist in genetically susceptible host and lead to chronic organ–specific autoimmune diseases via epitopes spreading (23,24). The infiltrated of lymphocytes T cells and large numbers of mononuclear cells typically occurs around or within individual islets to the pancreatic gland resulting in dysfunction of the gland (10-26). The study confirmed there was relationship between thyroid disease and type1 DM especially in women with hyper thyroidism (2, 8%) cases had positive autoimmune islets cells ,and these women had thyroid dysfunction and had symptoms for diabetic disease (11-15).Whereas in patients with hypothyroidism had negative autoimmune islets cells and the results disagreement with other studies which had been showed that there was a relationship between autoimmune islets cells and thyroid dysfunction(hypothyroidism), this may be due to diabetes onset in these patients is too late (27) or most patients with hashimotos disease administered steroids or immunosuppressant treatment, the researchers concluded that there are variety of finding that can be associated (28). They may be the number of samples included in the study were insufficient, the disease stages, regional differences situation and the sensitivity and specificity of the test that used ELISA method is more specific than IFAT and all studies carried out previously by ELISA tests. Type1 DM results from a cell–mediated autoimmune attack against pancreatic beta cells, which secret insulin, as the disease progresses the pancreatic release insulin to stop (29). Diabetic patients have a higher prevalence of thyroid disorders compared with normal population. (24, 30). Many factors may be contributes in the development in type1 diabetes.One,genetic factors the most important genes contributing to the development of HLA-DR3.Another important immune regulatory gene to both type1 diabetes and autoimmune thyroiditis is CTLA-4 gene (26,31,32) and as explained above about infiltrated T lymphocytes within islets cells with circulated antibody. Early reports showed a powerful diabetogenic effect. Cytokines derived from Th1 cells such as IFN-gamma (33) and IFN-gamma has been implicated with contradictory results in the pathogenesis process of autoimmune thyroiditis and thyroid dysfunction (34). The association of juvenile diabetes and autoimmune thyroiditis is also seen with families (26, 35, 36). These finding imply that the association between DM and autoimmune thyroiditis may be even stronger in cases of familial diabetes (12, 26).It has been found that these auto antibodies precede the development of diabetes by many months or years allowing prediction overt disease and identification of subjects at high risk of developing (37). Two or more glands are affected in patients with insulin dependent type1, Hashimotos thyroiditis and Graves disease (38).

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الخلاصة

إن الهدف من الدراسة هو التحري عن المرض المناعي الذاتي لداء السكر من النوع الأول في المرضى المصابين باعتلال الغدة الدرقية (المفرط والمنقوص). تضمنت الدراسة (50) مريضا يعانون من اعتلال الغدة الدرقية, تتراوح أعمارهم من (1-30) سنة. تم اختيار المرضى بصورة عشوائية من مركز الكندي للغدد الصم والعقم. و تضمنت الدراسة مجموعة من السيطرة (20) شخص من الأصحاء وتمت المقارنة مع العمر والجنس مع بقية مجاميع الدراسة. تضمنت اختبارات الغدة الدرقية (الهرمون المحفز للغدة الدرقية TSH, وهرمون الثايروكسين T4 وهورهمون الثايرونين T3) ,تم قياسها باستخدام جهاز المنيفايدز وتم قياس الأضداد الذاتية لخلايا بيتا في غدة البنكرياس في نفس المرضى باستعمال تقنية التفلور المناعي غير المباشر بطريقة تدعى تبرقش البايوجبس. أظهرت نتائج وظائف الغدة الدرقية أن (25) مريض مصاب بالغدة الدرقية من النوع المفرط و(25) مريض مصاب بالغدة الدرقية من النوع المنقوص.وكشفت نتائج طريقة البايوجبس أن مجموعة الدراسة للمرضى المصابين بالغدة الدرقية المفرط يمتلكون أضداد ذاتية نوعية لخلايا بيتا (خلايا الجزر) بنسبة 8% (2 من 25) مريض بينما لا توجد علامات عن هذه الأضداد الذاتية لخلايا الجزر في مجموعة الدراسة لمرضى الغدة الدرقية المنقوص. وقد كشفت الدراسة بانتشار اعتلالات الغدة الدرقية في النساء أكثر منه في الرجال.