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ORIGINAL ARTICLE

Investigation of Coxsackie Virus Type B in Grave's Thyroiditis

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ABSTRACT

Thyroiditis is a broad term that refers to a variety of clinical conditions marked by inflammation and damage to thyroid cells. These disorders can be chronic or acute, include lymphocytic infiltration, have a microbial cause, or be linked to autoimmune thyroid disease, such as in Graves' disease (GD). This cross-sectional study aimed to detect anti-TSH antibodies and investigate Coxsackie virus B (CVB) IgM and IgG antibodies in the serum of patients to know their role in the pathogenesis of thyroiditis. The study included 91 patients suffering from thyroid dysfunction through thyroid hormone tests (FT3, FT4 and TSH) by Snibe Maglumi-800 Chemiluminescence Immunoassay, where they were divided into three groups (subclinical thyroiditis, hypothyroidism and hyperthyroidism). To determine which of them has Graves' disease, detection of anti-TSH Ab have been performed then detection of antibodies to specific CVB IgM and IgG in the sera of patients by ELISA. The results of serology showed that the percentage of anti-TSH antibodies in the three groups was (68.13%), and the percentage was the highest in the hyperthyroidism group, which amounted to (85%). The serological diagnosis also showed that the presence of antibodies to CVB IgM was (65.93%) while, IgG levels were (20.87%). Conclusions: Coxsackie virus B infection is commonly seen in patients with thyroiditis and may have a role in the its pathogenesis.

Keywords: Coxsackie virus B, Graves' disease, Thyroiditis.

INTRODUCTION

The term "thyroiditis" refers to inflammation of the thyroid gland. It is caused by trauma, radiation, drugs or environmental factors such as viruses [1]. The thyroid dysfunction is classified into three groups: Firstly, subclinical thyroiditis is an asymptomatic condition associated with increased or decreased thyroid-stimulating hormone (TSH) with normal thyroxine (T4) and triiodothyronine (T3) levels [2]. Secondly, hypothyroidism results from low levels of thyroid hormone and untreated it increases morbidity and mortality. In the United States, autoimmune thyroid disease Hashimoto thyroiditis (HT) is the most common cause of hypothyroidism, but globally lack of iodine in the diet is the most common cause. Thirdly, hyperthyroidism defines a syndrome associated with excess thyroid hormone production. In the United States and most western countries, Graves' disease (GD) is the most common cause of hyperthyroidism [3].

As GD is autoimmune thyroiditis, which is distinguished by stimulating antibodies against thyroid stimulating hormone (TSH) receptors. This stimulation increases the synthesis of thyroid hormones and leads to the enlargement of the thyroid gland [4].

Viral infections are frequently cited as a major environmental factor implicated in thyroid gland diseases [5]. Coxsackie virus B (CVB) as one of enteroviruses which have tropism to many and different cell types inside human body, have various strategies to induce thyroditis, including CVB-induced direct damage to host cells via host inflammatory response to CVB infection and cell death of super adjuvants, to promote target organ tissue injury and dysfunction of the thyroid gland [6,7,8,9].

SUBJECTS AND METHODS

The current study included 91 patients with thyroid dysfunction, divided into 20 males and 71 females, with ages ranging from 10 to 68 years old, at different private labs in Al-Nasiriyah city. Those patients were selected from October 2021 to December 2021. All patients were confirmed to have thyroid dysfunction by performing thyroid tests.

Five ml of venous blood samples were collected from patients and collected in gel tubes for serum separation then sera divided into 3 parts and stored at -20 °C until used. One part for measuring of thyroid hormones (FT3, FT4 and TSH). The second part used for detection of anti-TSH antibodies, the third part used for measuring antibodies against CVB.

Thyroid tests (FT3, FT4 and TSH) were performed on patients' samples by Snibe Maglumi-800 Chemiluminescence Immunoassay. However, the normal values for thyroid hormones are 2.0 - 4.2 pg/ml for FT3, 8.9 – 17.20 pg/ml for FT4 and 0.38 – 4.50 µlU/ml for TSH. Serological diagnosis include detection of anti-TSH antibodies and (anti-CVB) IgM and IgG antibodies by ELISA kit uses Sandwich-ELISA as the method.

This study is subjected to the qualifications of ethical considerations and according to the form prepared for this purpose by the Iraqi Ministry of Health. Also, the research got the agreement of the committee of ethical standards at the College of Science, Thi-Qar University, of the colleges belonging to the Ministry of Higher Education and Scientific Research, Iraq. In addition, informed consent was obtained from all patients before taking samples.

Data Analysis: The study variables are described with descriptive statistics, like frequency, percentage, and mean with standard deviation (SD) and statistical package for the social (SPSS).

RESULTS

Patients groups: The current study included 91 patients with a thyroid dysfunction. Patients were divided into three groups according to the level of thyroid hormones (FT3, FT4 and TSH). The percentage of patients among there groups compared 15.4% of hypothyroidism, 21.97% hyperthyroidism and 62.63% subclinical thyroiditis, as showed in table (3-1).

Table 3-1: Distribution of patients according to the type of thyroid dysfunction

Thyroid dysfunction	No. of patients (%)	Thyroid Hormones (Mean ± SD)		
		FT3 (pg/m)	FT4 (pg/m)	TSH (µIU/ml)
Hypothyroidism	14 (15.4%)	2.5 ± 1.3	6.8 ± 2.8	43.5 ± 34.3
Hyperthyroidism	20 (21.97%)	6.5 ± 3.2	21.5 ± 8.4	0.02 ± 0.025
Subclinical thyroiditis	57 (62.63%)	3.3 ± 0.6	12.3 ± 2.6	7.2 ± 14.3

On the other hand, the detection of anti-TSH antibody was performed to investigate the patients who have Graves' disease. The results indicate to the presence of anti-TSH antibody in 62 of 91 patients (68.13%) and in high percentage (85%) in hyperthyroidism, table (3-2).

Table 3-2: Detection of anti-TSH antibodies in thyroid dysfunction patients

Parameter		Study groups			p value
		Hypothyroidism	Hyperthyroidism	Subclinical thyroiditis	
Anti-TSH	Negative	5	3	21	0.63^{NS}
		35.70%	15%	36.80%	
	Positive	9	17	36	
		64.30%	85%	63.20%	

Age and sex data: The median of age for patient's groups (hypothyroidism, hyperthyroidism and subclinical thyroiditis) were 35, 46 and 37 years old, respectively. Statistically, the results showed significant difference among those groups regarding the

median of age (P=0.015)

The patients of this study divided according to the sex as 4 males and 10 females for hypothyroidism, 3 males and 17 females for hyperthyroidism and 13 males with 44 females in subclinical thyroiditis, table (3-3).

Table 3-3: Distribution of thyroid dysfunction patients according to age and sex

Parameter		Study Groups			P value
		Hypothyroidism	Hyperthyroidism	Subclinical thyroiditis	
Age	Median	35	46	37	0.015*
	Percentile 05	24	29	15	
	Percentile 95	52	62	55	
Sex	Female	10	17	44	0.623^{NS}
		71.40%	85.00%	77.20%	
	Male	4	3	13	
		28.60%	15.00%	22.80%	

Detection of anti-CVB IgM and IgG antibodies: Detection of anti-CVB IgM antibody was higher than IgG in all patient's groups. However, hypothyroidism group showed highest group in the presence of anti-CVB antibody, 78.60% for IgM and 28.60% for IgG, followed by hyperthyroidism group 70% for IgM and 25% for IgG then subclinical thyroiditis group 61.40% for IgM and 17.60% for IgG, table (3-4).

Table 3-4: Detection of anti-CVB IgM and IgG antibodies in patients with thyroid dysfunction

Type of antibody against CVB		Study groups			P value
		Hypothyroidism	Hyperthyroidism	Subclinical thyroiditis	
CVB-IgM	Positive	11	14	35	0.09 NS
		78.60%	70%	61.40%	
	Negative	3	6	22	
		21.40%	30%	38.60%	
CVB-IgG	Positive	4	5	10	0.063 NS
		28.60%	25%	17.60%	
	Negative	10	15	47	
		71.40%	75%	82.40%	

DISCUSSION

Distribution of patients according to the type of thyroid dysfunction: The present study revealed that the distribution of thyroid dysfunction was uneven across the groups, with the most common group being the subclinical thyroiditis group. It is a common problem, and the majority of patients remain asymptomatic [10]. Where the total percentage was 62.63%, which is divided into subclinical hypothyroidism and subclinical hyperthyroidism at 61.40% and 38.60%, respectively. In agreement with the cross-sectional study conducted in Iraqi Kurdistan where the subclinical hypothyroidism was 94.85% and the subclinical hyperthyroidism 2.20% [11]. This study also showed that the presence of hyperthyroidism was 21.97% and the percentage of hypothyroidism was 15.4%. These results are similar to the study in Nigeria which showed hyperthyroidism at 58%, hypothyroidism at 39% and euthyroid sick syndrome at 3.9 [12]. Similar to the study in the United States, 4.6% of the population had hypothyroidism (0.3% overt and 4.3% subclinical) and 1.3% had hyperthyroidism (0.5% overt and 0.7% subclinical) [13]. While the study contrasts with that of Turkestan in Turkey, where the most common form of hypothyroidism was 10.0%, while that of hyperthyroidism was 3.3% [14].

In addition, the proportion of patients carrying anti-TSH antibodies was higher in the hyperthyroidism group at 85%. This indicates a high probability of developing Graves' disease, which is the most common cause of hyperthyroidism and is responsible for 60% to 80% of hyperthyroidism cases [15]. GD is distinguished by stimulating antibodies against thyroid stimulating hormone (TSH) receptors. This stimulation increases the synthesis of thyroid hormones and leads to the enlargement of the thyroid gland [4]. The cause of Graves' disease is also thought to be multifactorial, arising from the loss of immunotolerance and the development of autoantibodies that stimulate thyroid follicular cells by binding to the TSH receptor [16].

Distribution of thyroid dysfunction patients according to age and sex: The current study showed that the mean age in the three groups was 35, 46 and 37 years old. In agreement with the study conducted in Iraq in Dohuk governorate, where the majority of thyroid disorders were in adults aged 30-50 years [17]. The cross-sectional study conducted in Egypt found that the thyroid dysfunction was in the age group (49.5 ± 15.2) [18].

It appeared in our study that the number of females was higher than that of males, as the percentage of females was 78.02%, while in males it was 21.97%. Most studies indicate a female predominance over males in thyroid diseases, such as the study conducted in Iraq's Diyala Governorate, where the incidence of thyroid diseases in females (86.57%) was found to be higher than in males (13.43%) [19]. Because thyroid disorders are among the most common pathological conditions, especially in females, although the reasons for the prevalence in females are still unknown, a possible explanation can be found in the role of female sex hormones [20].

Detection of anti-CVB IgM and IgG antibodies: Enteroviruses, such as Coxsackie virus B, have been implicated in the past as pathogens associated with subacute thyroiditis. Less commonly, viruses are associated with autoimmune thyroid diseases such as HT and GD [21]. Our study showed that the percentage of patients with antibodies against CVB IgM antibodies in hypothyroidism, hyperthyroidism and subclinical thyroiditis was 78.60%, 70% and 61.40%, respectively. Compared to CVB-IgG, the proportion of those carrying anti-CVB-IgG antibodies was lower in hypothyroidism, hyperthyroidism and subclinical thyroiditis, it was 28.60%, 25% and 17.60%, respectively. Since CVB IgG is considered a marker of viral activation, it may play a role in the pathogenesis of thyroiditis. These results are consistent with the study of Volpe et al. (1967) who found antibodies against Coxsackie virus by the neutralization test in 14- 21% of patients with subacute thyroiditis [22]. However, inconsistent with the study that showed no relationship between subacute thyroiditis and the Coxsackie virus (IgM and IgG) [23].

A PubMed search from 1950 to the present using the terms "Coxsackie virus", "enterovirus", "Graves' disease", "thyrotoxicosis", and "hyperthyroidism" was performed and found limited literature and no case reports. Kraemer and colleagues explored the possibility of a connection between Coxsackie virus B infection and GD [24]. They determined that GD is associated with the human leukocyte antigen HLA-DR3. They further demonstrated a significant association between HLA-DR3 antigen and lymphocytotoxic antibodies, i.e., IgG antibodies from GD patients that were cytotoxic to normal B cells. Following infection with Coxsackie virus B, lymphocytotoxic reactions against HLA-DR3+ B cells were completely inhibited [24]. Also, a study strongly suggested a mechanism that HLA-DR3-reactive antibodies to Coxsackie virus could contribute to the pathogenesis of GD [21]. On the other hand, Desailud and colleagues sought to determine whether enterovirus genome sequences could be isolated in postoperative thyroid tissue from GD patients [25].

CONCLUSION

Thyroid tests indicate the division of patients into 3 main groups, subclinical thyroiditis, was the most common group followed by hyperthyroidism and hypothyroidism. Detection of anti-TSH antibodies in the serum of patients conclude the presence of high percentage of Graves' disease among patient's group. Detection of (anti-CVB) antibodies in patient's serum samples give an evidence to presence of the virus in patient's tissue and this may be contribute in the pathogenesis of thyroiditis.

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