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Detection of Anti-Thyroglobulin and Anti-Thyroid Peroxidase Autoantibodies Related with Epstein-Barr Virus Infection in Thyroiditis Patients

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ABSTRACT

Background: *Thyroiditis is a comprehensive term that includes many clinical diseases marked by inflammation and harm to cells in the thyroid gland. These anomalies can arise due to a range of reasons, including radiation exposure, microbial infection, or the occurrence of autoimmune thyroid diseases (AITDs) such as Hashimoto's thyroiditis (HT) and Graves' disease (GD). The cross-sectional study aimed to investigate the relationship between autoantibodies (anti-thyroglobulin and anti-thyroid peroxidase) and Epstein-Barr virus (EBV) infection. The study involved 100 patients afflicted with Thyroid dysfunction can be detected through a physical examination and certain tests. The concentrations of thyroid hormones (3,5,3'-triiodothyronine (T3) and thyroxine (T4)) and thyroid-stimulating hormone (TSH) were measured to determine the patients who have hypothyroidism or hyperthyroidism. Then the patients determined which of them had AITDs by the detection estimation of anti-thyroid antibodies (anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin (anti-TG). The tests were separated into two groups (Graves and Hashimoto). Additionally, EBV was investigated by the detection of Epstein Barr nuclear antigen-1 (EBNA-1) IgG in the sera of patients by Enzyme-Linked Immunosorbent Assay (ELISA). The present results show the mean level of serum Total EBNA-1 IgG is 9.78 ± 3.35 and 9.93 ± 4.33 in patients with HT and patients with GD, with no significant difference at ($P < 0.877$). The findings demonstrate a significant difference and positive association between EBNA-1 IgG and anti-TPO ($r= 0.454$, $p= 0.001$), as well as between EBNA-1 IgG and anti-TG ($r= 0.279$, $p= 0.039$) in patients diagnosed with HT. In patients with GD, there is a strong positive association between EBNA-1 IgG and anti-TPO level ($r= 0.436$, $p= 0.001$), as well as between EBNA-1 IgG and anti-TG level ($r= 0.322$, $p= 0.031$).*
Conclusion*: Detecting anti-viral (EBNA-1 IgG) antibodies in serum samples from patients provides proof of the virus being present in the patient's tissue, which could contribute to the development of thyroiditis. There is a significant difference and positive association relationship between the levels of anti-viral (EBNA-1 IgG) antibodies and the levels of autoantibodies (anti-TPO and anti-TG) in HT, and GD.*

Keywords: AITDs, HT, GD, EBV, EBNA-1 IgG, anti-TPO, anti-TG.

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INTRODUCTION

Autoimmune thyroiditis, often known as Hashimoto's disease, is the prevailing autoimmune disease that specifically targets the thyroid gland. It affects around 5% of the population and shows notable gender disparities, with women being affected at a rate of 5-15% and men at a rate of 5% (1). Both GD and HD were classified as autoimmune thyroiditis. Hypothyroidism and hyperthyroidism were mostly caused by HT and GD. Both GD and HT exhibit a cellular and humoral immune response to antigens in the thyroid gland. This response involves the infiltration of T and B cells, Autoantibodies production, and the subsequent development of clinical symptoms, which indicate a

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breakdown in immunological tolerance (2). Lymphocytic infiltration alters the functionality of the thyroid gland and causes harm to the surrounding tissue. Autoantibodies or sensitized T lymphocytes cause harm to thyroid cells by interacting with them, leading to an inflammatory reaction and sometimes cell lysis. The reference is from (3). The presence of a substantial number of patients with autoimmune thyroiditis is suggested by the identification of anti-TPO and anti-TSH antibodies in their blood serum.

GD is a medical condition characterized by hyperthyroidism, goiter, ophthalmopathy, and dermopathy. It was named after Robert Graves, who first identified the association in 1835 (4)(5). GD is responsible for up to 80% of cases of hyperthyroidism, making it the most common cause (6). It impacts women ten times more than men. There has been evidence of a high frequency between the ages of 40 and 60 (5). There is a chance of having GD and experiencing signs at a younger age if there is a history of thyroid sickness in the family, especially among maternal relatives (7). The presence of circulating TSH receptor Ab leads to the development of GD by binding to and activating the TSH receptor. This results in the expansion and hyperplasia of follicles. In addition, there is an augmentation in the synthesis of thyroid hormones and an alteration in the T3 to T4 ratio in the circulatory system (8).

HT is the primary cause of hypothyroidism in regions with high levels of iodine (9). Hakaru Hashimoto was the first to diagnose it in 1912 when he characterized a disease in four women, which he termed Struma Lymphomatosa (10). The estimated incidence rate is 0.8 per 1000 per year in men and 3.5 per 1000 per year in women. The incidence of thyroid disease generally rises with advancing age (11). The cause of Hashimoto's disease is currently not well known. The majority of patients produce antibodies against various thyroid antigens, with the most prevalent being anti-TPO Abs. In addition, many individuals also produce anti-TG and TSH receptor-blocking antibodies (TBII). These antibodies target the thyroid tissue, ultimately resulting in insufficient synthesis of thyroid hormones (12)(13).

Patients with HT exhibit abnormal thyroid function test findings, characterized by high serum TSH levels, while maintaining normal or low serum levels of T4 and T3 (14). Patients diagnosed with GD frequently exhibit an enlarged thyroid gland compared to individuals who do not have any thyroid-related conditions. Additionally, these patients exhibit heightened levels of T3 and T4 hormones, accompanied by a decrease in TSH (15). Elevated levels of thyroid autoantibodies (anti-TPO and anti-TG), have been linked to the diagnosis of AITDs (16). Epstein-Barr Virus (EBV), commonly known as human herpesvirus 4, is the microorganism responsible for causing infectious mononucleosis (IM). It was acknowledged that the prevalence of this issue is widespread globally (17). EBV can be transmitted from children who show no symptoms to adults through activities such as kissing, as well as through the exchange of blood, semen, and donated organs. According to (18), EBV has been found in patients who have AITDs.

PATIENTS AND MATERIALS

The population of Najaf city in Iraq was the subject of this hospital-based nested a cross-sectional study. A total of almost 400 patients provided blood samples between July 2023 and November 2023. A total of 100 patients, comprising both ill were chosen. The present

study involved a total of 100 volunteers, who were separated into three groups as follows: The user did not provide any text. Group 1. HT, identified as 55 cases. Group 2. GD, identified as 45 cases.

Approval of the Ethical Committee

The Medical Ethics Committee authorized the research of the College of Medicine, University of Kufa, following a review process. The study received research ethical approval from the Iraqi Ministry of Health, as well as from the Environment/Oncology Hospital, Al-Sadr Teaching Hospital, and the Endocrine Centre.

Evaluation using Statistical Methods

The data were gathered, condensed, examined, and shown using the Statistical Package for Social Sciences (SPSS) version 26 and Microsoft Office Excel 2010. The numeric data were reported as the mean and standard deviation after doing the Kolmogorov-Smirnov normality test and determining whether the variables were normally or non-normally distributed. The independent sample t-test was employed to examine the disparity in means between two groups, assuming that the variable follows a normal distribution. The chi-square test was employed to examine the relationship between two categorical variables. The level of significance was set at a P-value of less than 0.05, with a highly significant level at 0.01 or below (19).

RESULTS

The distribution of AITDs patients based on diagnostic antibody parameters are shown in table (1). The present results show the mean level of serum Total EBNA-1 IgG is 9.78 ± 3.35 and 9.93 ± 4.33 in patients with HT and patients with GD respectively, with no significant difference at (P < 0.877).

The present results show there is a significant difference and positive association relationship between EBNA-1 IgG level and anti-TPO Abs (r= 0.454, p= 0.001), as well as between EBNA-1 IgG level and anti-TG Abs (r= 0.279, p= 0.039) in patients with HT. Similarly, there is a considerably significant difference and positive association between EBNA-1 IgG level and anti-TPO level Abs (r= 0.436, p= 0.001), and between EBNA-1 IgG level and anti-TG Abs level (r= 0.322, p= 0.031) in patients with GD.

Table (1): Diagnostic EBV by EBNA-1 IgG level.

Characteristic	**Patients with HT** ***n*** **= 55**	**Patients with GD** ***n*** **= 45**	***P***
EBNA-1 IgG (ng/l)			
Mean ±SD	**9.78 ± 3.35**	**9.93 ± 4.33**	**0.877** † **NS**
Range	**2.85 –30.11**	**2.39– 25.05**	

SD: standard deviation; †: independent samples t-test; test; **NS**: not significant at P > 0.05; S: significant at P <0.05.

Table (2): Correlation between EBNA-1 IgG level and anti-TPO and anti-TG Abs level patients with HT and patients with GD.

Parameters	EBNA-1 IgG level			
	Patients with HT		Patients with GD	
	r	*P*	*r*	*P*
Anti-TPO Abs	0.454	0.001**	0.436	0.001**
Anti-TG Abs	0.279	0.039*	0.322	0.031*

***r*:** correlation coefficient; **NS**: not significant at P > 0.05; **S:** significant at P <0.05.

DISCUSSION

The present results show that the mean level of serum total EBNA-1 IgG was significantly higher in HT patients than GD patients. Furthermore, the present results show a significant differences and positive correlation between EBNA-1 IgG and anti-thyroid antibodies (anti-TPO and anti-TG) in patients with HT and patients with GD. The expression of EBNA-1 let EBV genome to persist inside B cell during cell division and transformation into a memory cell (20)(21).

There is the idea suggesting that in individuals with a genetic susceptibility, EBV infects B-cells that respond against the body's own tissues, specifically the thyroid gland. Subsequently, these B-cells that are affected by infection produce autoantibodies and activate auto-reactive T-cells by means of co-stimulatory signals. Usually, the infection by EBV is effectively managed, particularly by cytotoxic CD8+ T-cells that destroy rapidly proliferating and aggressively infected B-cells. A reduction in the quantity of EBV-specific CD8+ T-cells can result in compromised control of EBV. Autoimmune diseases are commonly linked to an increased ratio of CD4 to CD8 cells (18).

The previous studies demonstrated patients with systemic lupus erythematosus (SLE) exhibit elevated levels of antibodies against EBV. This led to the suspicion that the EBV may be involved in the progression of autoimmune disorders. Furthermore, there is now substantial indication suggesting that EBV infection can function as a causal role in the onset of autoimmune illnesses, such as SLE (22). Furthermore, numerous investigations have confirmed the presence of elevated levels of EBV DNA in the bloodstream of patients with rheumatoid arthritis (23). Various mechanisms of autoimmunity have been demonstrated to be implicated in the development of various disorders (18). EBV is widely recognized for its ability to freeze memory B-cells and employ various mechanisms to regulate and evade the body's immune responses. This could explain the connection between EBV and the onset and worsening of autoimmune disease (24).

The recent results show that the percentage of patients with EBNA-1 IgG antibodies in HT and GD is 9.78 ± 3.35 and 9.93 ± 4.33, respectively, this results are similar to many other studies, like the one conducted in Egypt to reveal the role of EBV in Hashimoto patients, where EBV-IgG were 100% positive

in both HT patients and controls (25). (26) conducted a study to identify the occurrence of EBV infection in AITDs. Their findings revealed that 62.5% of GD cases and 80.7% of HD cases tested positive for EBV. Children with AITDs had a greater seroprevalence of EBV infection compared to control groups. The study also demonstrated a statistically significant increase in the percentage of EBV IgG in children with AITDs compared to the percentage of EBV IgG in the control group (27). The present findings contradict a study that failed to detect EBV IgG antibodies in patients with AITDs (28). A cohort research conducted in southern China found no association between EBV and thyroid disorders (29).

CONCLUSIONS

1- Detecting anti-viral (EBNA-1 IgG) antibodies in serum samples from patients provides proof of the virus being present in the patient's tissue, which could contribute to the development of thyroiditis.

2- There is a significant difference and positive association relationship between the levels of anti-viral (EBNA-1 IgG) antibodies and the levels of autoantibodies (anti-TPO and anti-TG) in HT and GD.

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