Role of Epstein–Barr virus in Iraqi patients with Autoimmune Thyroditis

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Abstract
Background: Autoimmune thyroiditis has a multifactorial aetiology involving both genetic and environmental factors. Epstein–Barr virus (EBV) has been implicated in the pathogenesis of autoimmune thyroiditis. Aim: To investigate the seropositivty of VCA IgG and EA-D IgG antibodies of EBV in patients and comparison with healthy controls Methods: A total of 100 patients with newly diagnosed autoimmune thyroditis, (50 Graves' disease and 50 Hashimotoes thyroditis) in addition to 50 apparently healthy individuals as control group were included in this study. All patients were collected by Endocrinology and diabetes consultant of Al-Imamain Al-Kadhymain Medical City in Baghdad and Specialized Center for Endocrinology and Diabetes of Thi-Qar health directorate from November 2020 to December 2021. Enzyme-Linked Immunosorbent Assay was used in present study. Result: Current study showed that EBV antibodies (VCA IgG & EA IgG) were significantly higher in the Autoimmune thyroiditis than healthy control (p=<0.001). Conclusion: Elevted EBV and EA-D antibodies suggest a possible association between EBV and Autoimmune thyroiditis.
Keywords: EBV, VCA IgG, EA-D IgG

Introduction

Graves' disease and Hashimoto's thyroiditis are included in the category of diseases known as autoimmune thyroiditis (AIT). It is a chronic disease characterized by tolerance loss to self-thyroid antigens (1). Which are characterized by the infiltration of the thyroid by B and T cells that react to thyroid antigens, as well as the generation of autoantibodies specific to the thyroid. Included among the primary thyroid autoantibodies are thyrotropin receptor antibodies (TRABs), thyroid peroxidase (anti-TPO), and thyroglobulin (anti-TG). Immunological injury to the gland leads in clinical and biochemical alterations (hyperthyroidism in GD and hypothyroidism in HT) (2).

It is yet unknown what causes HT, however genetic and environmental factors are commonly believed to have a role in their development. Viruses are usually regarded as a major environmental element in the development of autoimmune diseases (3). Thyroid cell destruction in HT is associated with a number of cellular and antibody-mediated immunological responses, including thyroid autoantibodies (TAbs) against TPO and thyroglobulin (Tg).

Epstein-Barr virus is thought to be an aetiological factor of autoimmune diseases because it is a common pathogen responsible for the global prevalence of autoimmune diseases, EBV remains in the body throughout life, explaining the chronic course of autoimmune diseases with frequent exacerbations of symptoms, and the virus modifies the host immune system.(4).

Materials and Methods

This case-control study was undertaken between November 2020 and December 2021 on 100 patients with newly diagnosed autoimmune thyroditis (50 Graves' disease and 50 Hashimotoes thyroditis) and 50 apparently healthy people as a control group. Each participant had 2 mL of whole blood drawn and collected in gel tubes for serum separation. Patients newly diagnosed with an autoimmune thyroid disease meet the inclusion criteria. Exclusion criteria: patients on therapy or with another autoimmune disorder; patients with negative anti-TSH Receptor antibody for Graves' disease and negative anti-thyroid peroxidase for Hashimoto's thyroditis; patients with radioactive iodine or thyroidectomy.

Statistical Analysis

For statistical analysis, version 26 of the Statistical Package for the Social Sciences (SPSS) was used, categorical data were expressed as counts and percentages, and the Chi-square test was employed to describe the association between these data. A statistically significant difference is acceptable if it is less than or equal to 0.05.

Result

The mean age of Graves' disease patients, Hashimotoes thyroditis patients and controls was 35.50±12.80, 42.80±12.73 and 38.16 ±11.69 respectively.

There were no statistically significant differences

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between the mean age of the Graves' disease patients and the control group (P = 0.286). Also, there were no statistically significant differences between the mean age of Hashimotoes thyroditis patients and the control group (p=0.064) but there was a statistically significant difference between the mean age of the Graves' disease and Hashimotoes thyroditis (p=0.004). (Table 1)

Table (1): Mean age of patients and controls

Age	Study groups		
	Graves	Hashimotoes	Control
Mean	35.50	42.80	38.16
Standard Deviation	12.80	12.73	11.69
Median	35.00	42.50	39.50
Percentile 50	14.65	20.75	18.00
Percentile 95	61.35	63.90	58.45
Graves vs Control /P value	.286 NS 0		
Hashimotoes vs Control/P value	0.064 NS		
Hashimotoes vs Graves/P value	0.004 *		

Most Graves disease patients were between the ages of 20-40 years old (56.0%), while Hashimoto's thyroditis patients were over 40 years old (60.0%), with the lowest age group being <20 years old for both patients group. Also, there was no statistically significant difference between the age of the autoimmune thyroditis patients and controls for the different age groups, indicating that they were of a comparable age (P=0.118), (Table 2).

Table (2): Distribution of age groups among patients and controls

Age groups	Study groups				
	Graves	Hashimotoes	Control	Total	P value
<20	5	2	5	12	NS0.118
%	10.0%	4.0%	10.0%	8.0%	
20-40	28	18	22	68	
%	56.0%	36.0%	44.0%	45.3%	
>40	17	30	23	70	
%	34.0%	60.0%	46.0%	46.7%	
Total	50	50	50	150	
%	100.0%	100.0%	100.0%	100.0%	

Regarding the sex distribution, among the Graves' disease and Hashimoto's thyroditis patients there were 37 (74.0%) and 43 (86.0%) females, while 13 (26.0%) and 7 (14.0%) were males, respectively. Also, there was no significant difference between autoimmune thyroditis groups and controls (P=0.144) (Table 3

Table (3): Distribution of Sex among patients and controls

Sex group	Study groups			
	Graves	Hashimotoes	Control	Total
Female	37	43	35	115
%	74.0%	86.0%	70.0%	76.7%
Male	13	7	15	35
%	26.0%	14.0%	30.0%	23.3%
Total	50	50	50	150
%	100.0%	100.0%	100.0%	100.0%
P value	0.144 NS			
Graves vs Control	.636 NS 0			
Hashimotoes vs Control	.060 NS 0			
Hashimotoes vs Graves	.157 NS 0			

The Anti VCA IgG antibody was positive in 35 (70.0%), 41 (82.0%), and 2 (4.0%) of the Graves' disease, Hashimoto's thyroditis, and control groups, respectively. The seropositivity of anti VCA IgG was significantly higher in the Graves' disease and Hashimotoes thyroditis in comparison with control group (P<0.001). (Table 4)

Table(4): VCA antibody status in Autoimmune thyroditis patients and controls					
VCA	Study groups				
	Graves	Hashimotoes	Control	Total	P value
Positive	35	41	2	78	<0.001**
%	70.0%	82.0%	4.0%	52.0%	
Negative	15	9	48	72	
%	30.0%	18.0%	96.0%	48.0%	
Total	50	50	50	150	
%	100.0%	100.0%	100.0%	100.0%	

In addition, the current study showed that the anti-EA-D IgG antibody was positive in 25 (50.0%) and 8 (16.0%) of patients with Graves disease and Hashimotoes thyroditis respectively. The seropositivity of anti-EA-D IgG was significantly different between autoimmune thyroditis patients and the control group (P=<0.001) (Table 5).

Table (5): EA-D antibody status in Autoimmune thyroditis patients and controls					
EA-D	Study groups				
	Graves	Hashimotoes	Control	Total	P value
Positive	25	8	0	33	<0.001**
%	50.0%	16.0%	0.0%	22.0%	
Negative	25	42	50	117	
%	50.0%	84.0%	100.0%	78.0%	
Total	50	50	50	150	
%	100.0%	100.0%	100.0%	100.0%	

Discussion

In the current study, most of the Graves disease (GD) patients were 20–40 years old, and the mean age was 35.50±12.80. Peter Laurberg *et al*. showed that the disease is most prevalent between the ages of 20 and 50, and the majority of hyperthyroidism patients <40 years old may be presumed to have Graves' disease (5).

The mean age of Hashimotoes thyroditis (HT) was 42.80±12.73, this result is agreement with other study by Assaad *et al*. who found that the mean age was 40.40±12.06 years (3) and near from another study by Yuji Hiromatsu *et al* (6), that considered the more ages of disease, it usually starts between 30 and 50 years of age. This may return to the thyroid gland, undergo important functional changes during aging. (7), and this is associated with an increased risk of developing autoimmune disease. When compared to younger people, the elderly demonstrates a higher proliferation of naive T lymphocytes in the periphery. Furthermore, aging is related to higher levels of DNA in the circulation, which is also linked to autoimmunity. Wang et al. found that DNA accumulated in the cytoplasm of elderly human CD4+ T lymphocytes, leading to increased cellular activation and proliferation. (8).Females have more autoimmune thyroid diseases than males. As with many auto immune diseases, autoimmune thyroid problems affect females more than males (9). Males have immune suppression compared to females, which is related to male sexual activity (10),(11).

The reactivated virus has the ability to trigger thyroid antibody production and has been associated with a variety of debilitating autoimmune symptoms (12). Previous research hypothesized that the high prevalence of EBV infection in instances of Hashimoto's and Graves' diseases suggests a possible role for EBV in the pathogenesis of autoimmune thyroiditis (13). EBV infection is also implicated in the development of several autoimmune disorders (14). The current study showed the seropositivity of Anti VCA IgG and Anti EA-D IgG antibodies was significantly higher in the Graves disease (GD) and Hashimotoes thyroditis (HD) in comparison with control group. Barzilai *et al*. observed several prevalent connections between EBV and autoimmune disease, supporting the idea that EBV is a notable environmental component in the development of autoimmune diseases (15). The present results are in agreement with Vrbikova *et al*. who found that the IgG antibody titers against viral capsid antigen (VCA) and the presence of antibodies against EA were considerably greater in patients with autoimmune thyroiditis compared to the control group (16), This may because IgG antibodies of VCA and EA-D produced during the acute phase of the infection. Also in agreement with Thomas *et al*. who showed a significantly elevated level of anti-EBV IgG antibodies in children with autoimmune thyroid disease compared to a control group (p=0.008) (17).

Hiroshi Akahori *et al* discovered that the IgM and IgG antibodies to EBV viral capsid antigen (VCA) were detected in GD patients (18). Also, Kannangai R. *et al*. discovered a statistically significant difference (P=0.011) in IgM reactivity to VCA protein between HT patients and controls (19), this explain an increased EBV activation among the autoimmune patient groups compared to the normal healthy controls. Rachel and Didier describe direct evidence of Epstein Barr virus or its components in the HT patients (20). Also, Assaad *et al*. discovered that the

mean serum levels of EBV VCA IgG and EA IgG in HT patients were significantly greater than in the control group (3). So this suggest EBV can play a pathogenic role in the development of autoimmune thyroiditis.

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