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Serological Detection of Epstein-Barr Virus among Hashimoto's Thyroiditis Patients in Thi-Qar Province

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Abstract:

Background: Hashimoto's thyroiditis (HT), an autoimmune disease of the thyroid gland that is the leading cause of hypothyroidism in developed countries, HT may develop at any stage of life, especially between the ages of 30 and 50 years, and affects both male and female. However, it is usually found in female within a female-to-male ratio of 10:1. The risk of HT is higher in areas where there is adequate iodine intake, especially among individuals with genetic predispositions. The prevalence of HT is approximately 2 percent across all age groups, with an annual incidence of 0.3 to 1.5 cases per 1,000 individuals. HT causes permanent hypothyroidism, such that the thyroid gland becomes unable to produce hormones, which requires therapeutic replacement. The TH etiology is multifactorial, incorporating both genetic and environmental factors. Currently, there are no cures and a limited understanding of the disease mechanisms. However, emerging evidence suggests that viral infections may serve as potential triggers for HT. The Epstein-Barr virus (EBV) is a double-stranded DNA virus that demonstrates a distinctive tropism towards B lymphocytes and epithelial tissue. Many studies have linked the EBV infection to the onset or development of autoimmune diseases, since EBV possesses the ability to modulate and evade the immune system. A hypothesis suggests that genetically vulnerable B lymphocytes during the EBV lytic infection infiltrate the thyroid, producing autoantibodies and activating autoreactive T cells. These autoantibodies then contribute to the autoimmune response that ultimately leads to HT.

Objective: The current study aims to explore the affiliation between HT and EBV infection by comparing the positive proportions of anti-Epstein-Barr virus viral capsid antigen antibodies (Anti-EBV-VCA) between patients newly diagnosed by the specialist physician as HT patients and healthy individuals.

Methods: Sixty serum samples from newly diagnosed by the specialist physician as HT patients, 51 females and 9 males, and sixty healthy controls, 48 females and 12 males, were obtained and tested by the indirect Chemiluminescence Immunoassay (CLIA) for both Anti-EBV-VCA IgG and Anti-EBV-VCA IgM.

Results: The results did not show any significant difference in the Anti-EBV-VCA IgG positive proportions between the patients and the healthy groups (P. value= 1), while a significant difference was noticed in the positive proportions of Anti-EBV-VCA IgM (P. value< 0.001).

Conclusion: Increased positive proportions of VCA-IgM for EBV in the patients with HT compared to the healthy controls suggest that active EBV infection may have a role in the HT onset or progression.

Keywords: Hashimoto's Thyroiditis (HT), Epstein-Barr Virus (EBV), Anti-Epstein-Barr Virus Viral Capsid Antigen (Anti-EBV-VCA), Chemiluminescence Immunoassay (CLIA).

Introduction: The HT is a predominant autoimmune endocrine disorder responsible for most hypothyroidism cases in regions with adequate iodine intake (1). The HT is characterized by distractive inflammation of the thyroid gland, which combines both the humoral response, mediated by the generation of an IgG-class autoantibody against thyroglobulin (Tg) and thyroid peroxidase (TPO), and the cellular response, represented by lymphocyte infiltration of the thyroid, which results in thyroid tissue distraction and permanent hypothyroidism (2). Unidentified stimuli activate the CD4+ T cells, which target the thyroid antigen and convey signals to recruit lymphocytes and macrophages to the thyroid gland, resulting in the formation of the germinal centers, the production of plasma cells, and the death of thyroid cells (3). Multiple studies indicate that viral infection may serve as a potential trigger for HT (4). The EBV is one of the most common viruses among humans, as humans are considered the only host of the virus. The EBV is dsDNA, which belongs to the gamma-1 herpes family and is transmitted primarily through saliva and other bodily fluids (5). Most people are infected with the virus in the early stages of life. The EBV causes many diseases, the most famous of which is infectious mononucleosis (IM), and it can cause many cancers and autoimmune diseases. Among the autoimmune diseases that have been linked to EBV is HT (5,6). One proposed mechanism is molecular mimicry, where EBV viral proteins structurally resemble host proteins, leading to cross-reactive immune responses targeting both the virus and self-antigens. This phenomenon can trigger or exacerbate autoimmune reactions in susceptible individuals (7). Furthermore, EBV infection can dysregulate the immune system, impairing its ability to differentiate between self and non-self antigens, thus contributing to the development of autoimmune diseases (8). EBV can also manipulate various immune cells and signaling pathways, leading to immune dysfunction and chronic inflammation, which are hallmarks of autoimmune disorders (5,8). The EBV creates a latent infection in B cells that lasts a person's whole life, stays dormant in most people but can sometimes become active again, and expresses over 80 genes. According to a concept, B-cells infected with EBV infiltrate the thyroid gland in genetically vulnerable individuals. These B-cells then create autoantibodies and convey signals to autoreactive T-cells, which further contribute to the autoimmune response (9,10). Cytotoxic CD8+ T-cells typically regulate the EBV infection by eliminating actively dividing and lytically infected B-cells. A reduced EBV-specific CD8+ T-cell count can lead to impaired regulation of EBV. Autoimmune disorders are typically associated with an elevated CD4/CD8 ratio (10).

Aims of the Study: The current study aims to explore the hypothesis that EBV infection is a candidate as one of the environmental factors that can trigger HT by estimating positive proportions of antibodies against the EBV-VCA in the serum of patients newly diagnosed by the specialist physician as HT patients and comparing them with the EBV-VCA positive proportions in healthy individuals.

Methods:

Subjects: We conducted the current case-control study on the Iraqi population in the Thi-Qar Provinces. A total of 120 blood samples were collected in the period from August 2023 until February 2024 at Al-Nasiriyah Teaching Hospital and Souq Al-Shuyoukh General Hospital in Thi-Qar Provinces. The 120 individuals in this study were divided into two groups: the Patients Group,

which consists of 60 patients, 51 females and 9 males, with an age range of 11–67 years, newly diagnosed with HT by the specialist physician, and the Control Group, which includes 60 healthy individuals, 48 females and 12 males, with an age range of 12-63 years, without symptoms or chronic disease. The control group volunteers were medical staff, blood donors, and visitors to the premarital screening unit.

Inclusion criteria: All patients who were newly diagnosed by the specialist physician as suffering from HT were included in the current study. The specialist physician used the American Thyroid Association's (ATA) diagnostic criteria for diagnosing patients with HT.

Exclusion criteria: The following criteria were excluded:

- previously diagnosed and under treatment for HT
- Non-autoimmune hypothyroidism.
- Patients with other autoimmune disorders.
- Patients with genetic disorders.
- Nonthyroidal systemic disorders.
- History of thyroidectomy.
- Benign and malignant patients.
- Ageing stage patients
- pregnant woman's

Sample Collection: A five-milliliter venous blood sample was drawn from both studied groups of individuals. Blood was placed in a clot activator tube, left to clot in a 37 °C water bath, centrifuged for 10 minutes at 3000 rpm, and separated into equal aliquots. All samples were identified by ID numbers and stored at -20 °C until use.

Detection of Anti-EBV-VCA IgG and IgM: The indirect CLIA technique was used to quantitatively estimate the levels of Anti-EBV-VCA IgM and Anti-EBV-VCA IgG using a fully automated instrument, the Maglumi 800. All manufacturer's instructions were followed during the current study. The instrument and the kits were provided by Snibe Co., Ltd., China.

Ethical Approval: The medical ethics committees of Jabir Ibn Hayyan University for Medical and Pharmaceutical Sciences, Medical College, approved this study protocol. Also, the study was approved by the Iraqi Ministry of Health, Thi-Qar Health Directorate, under the document number [153/2023, 24/7/2023]. Also, we informed all participants about the study's objectives, and they verbally agreed.

Statistical Analysis: The data for the current study was statistically analyzed using SPSS 29 and Microsoft Excel 2021. The chi-square test was employed to compare the categorical variables at a p-value < 0.05, while the independent-samples t-test was used to compare the means of the numerical variables at a P. value < 0.05 as well. Also, we used the degree of freedom (DF), Odds Ratio (OR), and 95% CI for result interpretation and statistical inference.

Results: The results showed that the mean ± Standard Deviation (SD) of the age was 37.0 ± 12.9 years in the patient group. While the mean ± SD of age was 37.5 ± 12.9 years in controls, there were no significant differences in the mean ages of the patients and controls (P. value = 0.566), as shown in Figure 1.

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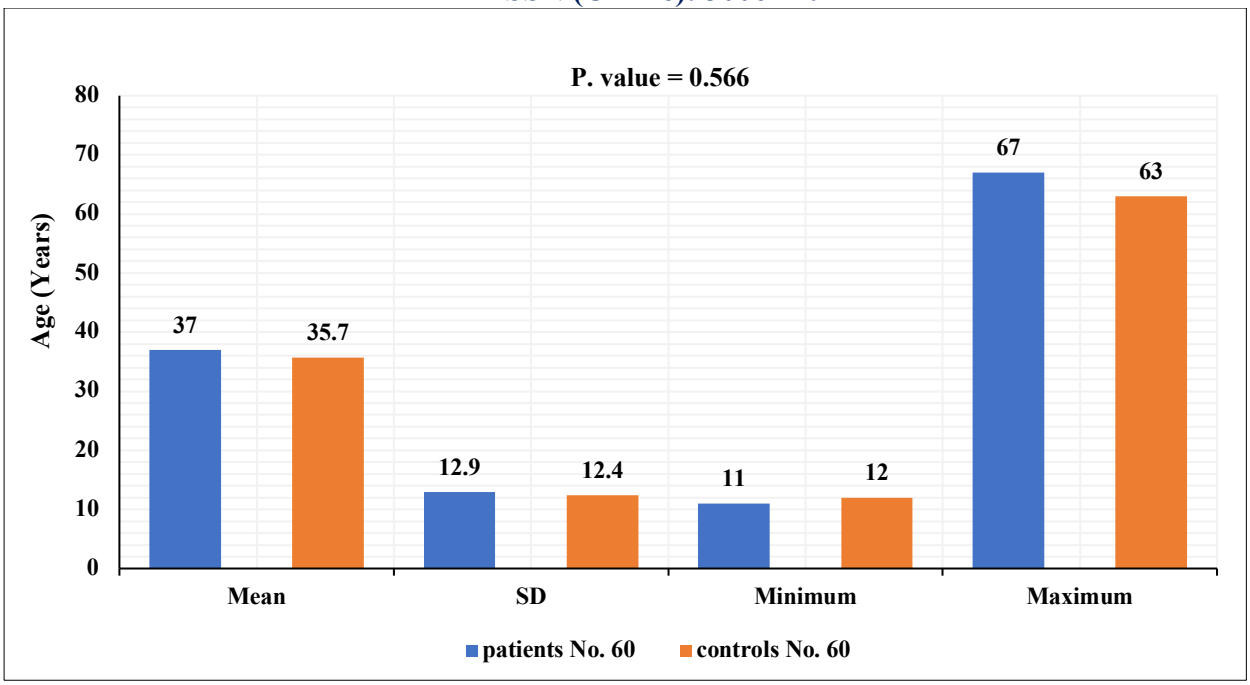

Figure (1): Distribution of Participants According to Age.

The participants were classified into five categories based on their age. The study setting ensured that the patients' ages were similar to those of the controls since there was no statistically significant difference in their ages (P. value = 0.234) or between patients' age groups (P. value = 0.215), Table 1.

Table (1): Distribution of Participants According to Age Groups.

Age Groups	Patients		Controls		Total	
	No.	%	No.	%	No.	%
11 – 20 Years	7	11.67	5	8.33	12	10.0
21 – 30 Years	15	25.0	17	28.33	32	26.67
31 – 40 Years	14	23.33	20	33.33	34	28.33
41 – 50 Years	14	23.33	8	13.33	22	18.33
51 – 67 Years	10	16.67	10	16.67	20	16.67
Total	60	50.0	60	50.0	120	100
Patients Vs. Controls: $Calx^2$= 5.52 $Tabx^2$= 9.48 Df=4 P. Value 0.234						
For Patients Only: $Calx^2$= 5.80 $Tabx^2$= 9.48 Df=4 P. Value 0.215						

In terms of the sex-based distribution of the research groups, the study group consisted of 51 females and 9 males, whereas the control group had 48 females and 12 males. Based on sex, the patient and control groups did not differ significantly (P. value= 0.352). whereas a significant difference was noticed in patent (P. value < 0.001), Table 2.

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Table (2): Distribution of Participants According to Sex.

Sex	Patients		Controls		Total	
	No.	%	No.	%	No.	%
Male	9	15.0	12	20.0	21	17.5
Female	51	85.0	48	80.0	99	82.5
Total	60	50.0	60	50.0	120	100
Patients Vs. Controls:	$Calx^2$= 0.86	$Tabx^2$= 3.84	DF=1	P. Value 0.352		
For Patients Only:	$Calx^2$= 49.0	$Tabx^2$= 3.84	DF=1	P. Value <0.001		

Quantitative observations of Anti-EBV-VCA IgG showed that the positive proportions are approximately equal between the patient and control groups (95% and 96.7%), respectively. On the other hand, the study showed that the Anti-EBV-VCA IgM positive proportions of patients were higher than those of the control group (45% and 6.7%), respectively. According to the OR result, Anti-EBV-VCA IgM can be considered a risk factor for HT development, Table 3.

Table (3): Proportions of Anti-EBV-VCA Antibodies in the Study Groups.

Parameters		Patients		Controls		OR	95% CI	P. Value
		No.	%	No.	%			
VCA-IgG	Positive	57	95	58	96.7	0.826	0.39 – 1.73	0.999
	Negative	3	5	2	3.3			
VCA-IgM	Positive	27	45	4	6.7	2.349	1.73 – 3.19	<0.001
	Negative	33	55	56	93.3			

Table 4 showed that the Anti-EBV-VCA IgG positive proportions were significantly different (P. value < 0.001) according to the patient age groups.

Table (4): Age Groups Based Distribution of Anti-EBV-VCA IgG in Patients.

VCA-Igg	Positive		Negative		Total	
	No.	%	No.	%	No.	%
11 – 20 Years	7	100	0	0.0	7	11.67
21 – 30 Years	15	100	0	0.0	15	25.0
31 – 40 Years	13	92.86	1	7.14	14	23.33
41 – 50 Years	14	100	0	0.0	14	23.33
51 - 67 Years	8	80.0	2	20.0	10	16.67
Total	57	95.0	3	5.0	60	50.0
$Calx^2$= 59.3	$Tabx^2$= 9.48	DF=4	P. Value < 0.001			

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Regarding the Anti-EBV-VCA IgG proportion distribution in the patients according to sex, there was no significant difference (P. value = 0.013) between males and females, Figure 2.

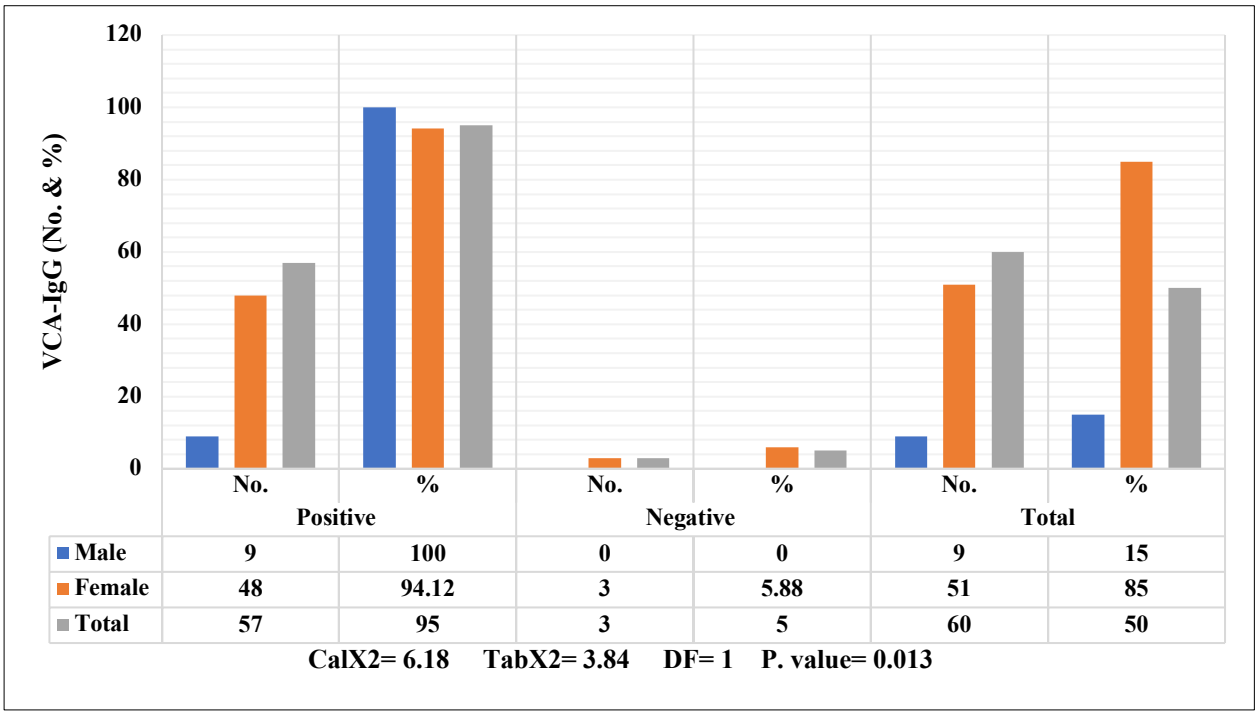


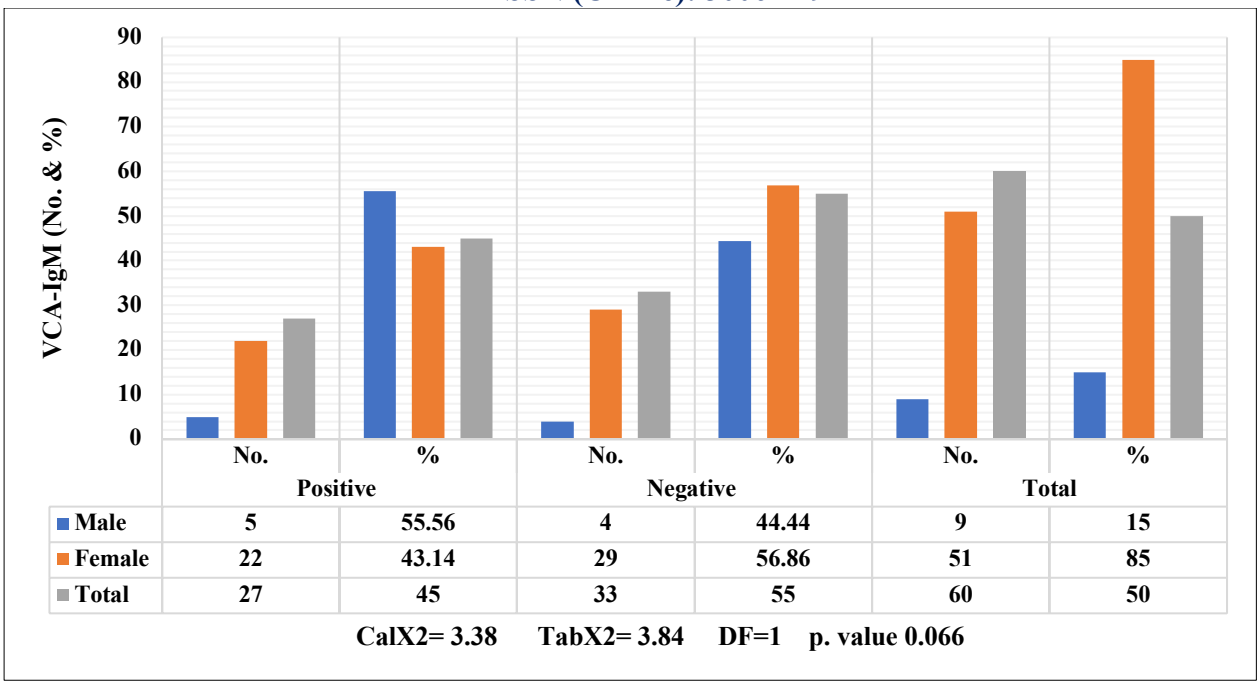
Figure (2): Sex-Based Distribution of Anti-EBV-VCA IgG in Patients.

The Anti-EBV-VCA IgM proportions according to the patient age groups are shown in Table 5.

Table (5): Age Groups Based Distribution of Anti-EBV-VCA IgM in Patients.

VCA-IgM	Positive		Negative		Total	
	No.	%	No.	%	No.	%
11 – 20 years	3	42.86	4	57.14	7	11.67
21 – 30 years	5	33.33	10	66.67	15	25.0
31 – 40 years	8	57.14	6	42.86	14	23.33
41 – 50 years	6	42.86	8	57.14	14	23.33
51 - 67 years	5	50.0	5	50.0	10	16.67
Total	27	45.0	33	55.0	60	50.0
$CalX^2$= 12.95 $TabX^2$= 9.48 DF= 4 P. value= 0.012						

Regarding the distribution of Anti-EBV-VCA IgM proportion in the patients according to sex, there was no significant difference (p. value = 0.066) between males and females, Figure 3.



	Positive		Negative		Total	
	No.	%	No.	%	No.	%
Male	5	55.56	4	44.44	9	15
Female	22	43.14	29	56.86	51	85
Total	27	45	33	55	60	50

CalX2= 3.38 TabX2= 3.84 DF=1 p. value 0.066

Figure (3): Sex-Based Distribution of Anti-EBV-VCA IgM in Patients.

Discussion:

The EBV that has been reactivated possesses the capacity to stimulate the generation of thyroid antibodies and has been associated with a multitude of autoimmune symptoms (11). Previous research has suggested that the fact that EBV infections are more common in people with HT could mean that EBV is a possible cause of HT (12). EBV infection has been associated with the onset of certain autoimmune diseases (13). The present investigation demonstrated a substantially higher positive proportion of Anti-EBV-VCA IgM and IgG antibodies in the HT group compared to the healthy group. An Egyptian study showed elevated Anti-EBV-VCA IgM and IgG positive proportions in HT patients, which is consistent with our outcomes (14). Additionally, our findings did not indicate a substantial difference in the positive proportion of Anti-EBV-VCA IgG antibodies in the HT group compared to the healthy group. This observation contradicts the findings of Vrbikova et al. (15), who observed that individuals diagnosed with HT exhibited notably elevated levels of Anti-EBV-VCA IgG compared to the controls. Furthermore, these findings are in contrast to the observations made by Thomas et al. (16), who found that children diagnosed with autoimmune thyroid disease exhibited a significant elevation of Anti-EBV-EBV IgG compared to the controls (P. value= 0.008). Barzilai et al. (17) conducted a study that linked EBV to autoimmune diseases, supporting the notion that EBV is a well-known environmental component in the context of autoimmune disorders. EBV invades B cells and has the potential to permanently infect a small proportion of them in individuals who are otherwise in good health (15). We can employ two overarching pathways to elucidate the potential correlation between EBV infection and thyroid autoimmunity onset. There is empirical evidence suggesting that the virus has the potential to generate a deceptive antigenic stimulus, hence inducing the activation of autoreactive T cells. The second criterion is valid if there is evidence that the immune response to viruses gives the innate immune system a non-specific stimulus, which makes it easier for autoreactive T cells to become activated and multiply. The persistent innate immune response to

viral infection can also contribute to autoimmunity (18). Kannangai et al. (19) found notable differences in the IgM response to EBV-VCA between those suffering from HT versus control subjects, with a statistically significant result (P. value= 0.011). This discovery could elucidate the reason for the heightened EBV activity in autoimmune illness cohorts as compared to individuals without autoimmune diseases. Desailloud & Hober (20) conducted a study that provided definitive evidence of the presence of EBV or its components in individuals with HT. Therefore, it can be inferred that the EBV potentially has a detrimental effect on the development of HT.

Conclusion:

Increased positive proportions of VCA-IgM for EBV in the patients with Hashimoto's thyroiditis compared to the healthy controls suggest that active EBV infection may have a role in the disease's onset or progression.

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