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Investigation of anti-EBV-EA IgG and EBNA IgG antibody profile in Iraqi patients with Hashimoto's Thyroiditis

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Abstract

Background: Hashimoto's Thyroiditis (HT) is a leading cause of hypothyroidism worldwide. Its pathogenesis is multifactorial, involving a complex interplay where genetic susceptibility plays a significant role and environmental triggers, particularly chronic viral infection, are increasingly recognized as critical factors in initiating the autoimmune response. Among these, the Epstein-Barr Virus (EBV) a ubiquitous double-stranded DNA (ds DNA) herpesvirus that establishes lifelong latency in host B-lymphocyte, has emerged as a primary candidate. Elevated levels of EBV-Early-antigen-immunoglobulin-G (EBV-EA-IgG) generally signify either an acute primary infection or an active phase of viral reactivation. While EBV-nuclear antigen IgG (EBNA IgG) detection characteristically rules out acute mononucleosis, confirming that the patient has established lifelong viral latency. **Aims of the study:** To investigate the association of anti-EBV-EA IgG and anti-EBNA IgG levels in patients with HT. **Methods:** The current case-control study was conducted on the Iraqi population. The 100 individuals in this study were divided into two groups: the Patient group (50 subjects) newly diagnosed with HT, and control group include healthy people (50 subjects). ELISA used for serological test to assess anti-EBV-EA IgG, anti-EBV-EBNA IgG and anti-TPO**. Results:** In the current study, the seroprevalence of anti-EBNA IgG in patient group was (94%), while in control group was (90%); there was no statistically significant association between the two groups (p-value > 0.46). the seroprevalence of anti-EBV-EA-IgG in patient group was (72%), compared to only (9%) in the control group, demonstrating a highly statistically significant association between the groups (p-value < 0.0001). **Conclusions:** The present of EBV-EA IgG in patients with HT compared to the healthy group suggests a possible association between EBV-reactivation and HT progression. In contrast, no significant association was found between EBNA IgG and HT.

Keywords: HT, EBV-EA IgG, EBNA-IgG.

Introduction

Hashimoto's thyroiditis (HT), is an autoimmune disease that effects the thyroid gland and is a leading cause of hypothyroidism in developed countries **[1].** The HT is considered by inflammatory distraction to the thyroid gland, that involving both cellular immune response and humoral response. The humeral response is mediated by the production of an (immunoglobulin-G) IgG autoantibody against thyroid peroxidase (TPO) and thyroglobulin (Tg), while the cellular response is characterized by lymphocytic infiltration of the thyroid, leading to thyroid tissue destruction and permanent hypothyroidism **[2].** HT has a multifactorial etiology involving both genetic and environmental factors and may develop at any stage of life, affecting both females and males **[1,3].** Many studies indicate that viral infection may act as a potential trigger for HT **[1,4].** The Epstein-Barr Virus (EBV) is one of the most common viruses in humans, and humans are the only known host. The EBV is transmitted mainly through saliva and other body fluids **[5].** Most people are infected with EBV during early childhood. The virus is associated with several diseases, most notably infectious mononucleosis (IM), and has also been implicated in the development of various cancers and autoimmune diseases. Among the autoimmune diseases that have been linked to EBV is HT **[5,6].** The mechanism linking EBV to HT is molecular mimicry, where EBV viral proteins structurally resemble host proteins, leading to cross-reactive immune responses targeting both viral and self-antigens. This process can trigger or exacerbate autoimmune reactions in susceptible individuals **[1,7].** Furthermore, EBV infection may dysregulate the immune system, impairing its ability to distinguish between self and non-self-antigens, thereby contributing to the development of autoimmune diseases **[1,8].**

Study design and population

The current case-control study was conducted on the Iraqi population. A total of 100 blood samples were collected in the period from June 2025 until January 2026 at different hospitals in Iraq. The 100 individuals in this study were divided into two groups: the patient group (50 subjects) newly diagnosed with HT, and the control group, which include healthy people (50 subjects).

Exclusion criteria: The following criteria were excluded (pregnant women, patients with other autoimmune, those previously diagnosed with HT and patients undergoing treatment).

Methods

A (3-mL) venous blood sample was collected from each participant in both studied groups. The blood samples were placed into gel tubes and left at room temperature for 15 minutes to clot. Subsequently, they were centrifuged at 4000-rpm for 5-minutes. The separated serum was then distributed into three aliquots in Eppendorf tubes and stored frozen at -20°C until used for serological testing. Enzyme-linked immune sorbent assay (ELISA) was performed to assess anti-EBV-EA IgG (Human Epstein-Barr Virus Early Antigen IgG kit; Abbexa/USA), anti-EBNA IgG (Human Anti-Epstein Barr Virus Nuclear Antigen IgG kit; Abcam/UK) and anti-TPO (Human Anti-Thyroid Peroxidase Antibody kit; Abbexa/USA).

Statistical analysis

The data for the current study were statistically analyzed using IBM SPSS software (version 28.0). The chi-square test was employed to compare the categorical variables between the study groups. Statistical significance was set at a p-value <0.05.

Results

Table-1 provides a summary of the participants' demographics, including age and sex distribution. Regarding sex distribution, females predominated in the patient group (80%) compared to the control group (64%), although this difference did not reach statistical significance ($p = 0.075$). The results showed no statistically significant difference in age between the patient group and the control group ($p = 0.78$).

The majority of participants in both groups were between 18 and 38 years old, representing 50% of the patient group and 54% of the control group.

Table-1: Distribution of Age and Sex among patients and control groups.

Distribution		Hashimoto's group (n=50)	Control group (n=50)	Total	P-value
Sex	Female	40 (80%)	32 (64%)	72 (72%)	**0.075**^{NS*}
	Male	10 (20%)	18 (36%)	28 (28%)	
	Total	50 (100.0%)	50 (100.0%)	100 (100%)	
Age	<18	2 (4.0%)	3 (6.0%)	5 (5%)	**0.78** ^{NS*}
	18-38	25 (50.0%)	27 (54.0%)	52 (52%)	
	38-58	23 (46.0%)	20 (40.0%)	43 (43%)	
	Total	**50 (100.0%)**	**50 (100.0%)**	**100 (100%)**	

*NS= non-significant

The results presented in Table-2 indicate that the prevalence of anti-EBNA IgG was 94% in the patient group and 90% in the control group, with no statistically significant association observed between the two groups ($p > 0.46$). In contrast, the prevalence of anti-EBV-EA-IgG was 72% in the patient group compared to 6% in the control group, demonstrating a highly statistically significant association between the groups ($p < 0.0001$). All HT patients (100%) were positive for anti-TPO antibodies, while all individuals in the control group were negative ($p < 0.0001$).

Table-2: Serological Profile of EBV and Anti-TPO Antibodies in study groups.

Variables	Status	Hashimoto's Group (n=50)	Control Group (n=50)	P value
		No. (%)	No. (%)	
Anti-EBNA-IgG	**positive**	47 (94%)	45 (90%)	**0.46** ^{NS*}
	negative	3 (6%)	5 (10%)	
Anti-EBV-EA-IgG	**positive**	36 (72%)	**3 (6%)**	**0.0001****
	negative	14 (28%)	**47(94%)**	
Anti-TPO	**positive**	50 (100%)	0 (0%)	**0.0001****
	negative	0 (0%)	50 (100%)	
Total		**50 (100.0%)**	**50 (100.0%)**	

*NS= non-significant, **0.0001= highly significant

Table-3, reveals a highly significant association between sex and the presence of all tested antibodies within the Hashimoto's Thyroiditis (HT) patient group ($p < 0.0001$). Females showed a clear predominance, representing the majority of seropositive cases for Anti-TPO, Anti-EBV-EA IgG, and Anti-EBNA-IgG (80%, 91.7%, and 80.9%, respectively) compared to males.

Table-3: Association between the serological profile of EBV and Anti-TPO antibodies and Sex in HT patients.

Variables		Anti-TPO	Anti-EBV-EA IgG	Anti-EBNA-IgG
		No. (%)	No. (%)	No. (%)
Sex	Female	40 (80.0%)	33 (91.70%)	38 (80.90%)
	Male	10 (20.0%)	3 (8.30%)	9 (19.10%)
Total		50 (100.0%)	36 (100.0%)	47 (100.0%)
P-Value		0.0001**	0.0001**	0.0001**

**0.0001= highly significant

As shown in the organized tables-4, a highly significant difference was observed in the prevalence of anti-EBV-EA antibodies among HT patients according to age groups. While anti-TPO and anti-EBNA-IgG showed no significant variation across age groups ($p > 0.05$), anti-EBV-EA-IgG displayed a highly significant age-dependent increase, rising from 48% in the 18-38 group to 95.7% in the 38-58 group ($p < 0.0001$). Positivity remained consistently high for anti-EBNA-IgG in both the 18–38 group (96%) and the 38–58 group (95.7%), with an overall prevalence of 94%. Anti-TPO antibodies showed a 100% positivity rate across all age groups.

Table-4: Association between the serological profile of EBV and Anti-TPO antibodies and age groups in HT patients.

Variables	Status	Age Groups (n=50)				
		<18	18-38	38-58	Total	P-value
		No. (%)	No. (%)	No. (%)		
Anti-EBV-EA IgG	Positive	2 (100.0%)	12 (48.0%)	22 (95.70%)	36 (72.0%)	0.0001**
	Negative	0 (0.0%)	13 (52.0%)	1 (4.30%)	14 (28.0%)	
Total		2 (4.0%)	25 (50.0%)	23 (46.0%)	50 (100.0%)	
Anti-EBNA-IgG	Positive	2 (100.0%)	24 (96.0%)	22 (95.70%)	47 (94.0%)	0.5 NS*
	Negative	0 (0.0%)	1 (4.0%)	1 (4.30%)	3 (6.0%)	
Total		2 (4.0%)	25 (50.0%)	23 (46.0%)	50 (100.0%)	
Anti-TPO	Positive	2 (100.0%)	25 (100.0%)	23 (100.0%)	50 (100.0%)	1.0NS*
	Negative	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Total		2 (4%)	25 (50.0%)	23 (46.0%)	50 (100.0%)	

*NS= non-significant, **0.0001= highly significant

Discussion

The development of HT is thought to be significantly influenced by a variety of factors, such as genetics, viral illnesses, the environment, and others **[9].** The current study demonstrated that all subjects in the patient group were positive for anti-TPO, while all subjects control group were negative for anti-TPO. In contrast, a study conducted by **[10]** reported that only (40%) of patients were positive and (60%) were negative, whereas the results for all controls were negative. The variance observed between this study and those reported by **[10]** may originate from differences in patient selection and the specific clinical phases of HT investigated. The complete (100%) seropositivity of anti-TPO in our patient cohort highlights its definitive status as a primary diagnostic indicator for HT, particularly during the early stages of diagnosis when anti-thyroid immune activity peaks. Conversely, the total absence of anti-TPO in the control group demonstrates the exceptional specificity of the ELISA testing and solidifies the strong association between anti-TPO seropositivity and clinical HT **[11][12]**. In this study female more susceptible to HT than men. There were no credible studies contradicting this outcome, with prevalence increasing significantly in middle-aged and older females. Every major epidemiological study confirms that women are significantly more susceptible to HT than men. The discrepancy lies only in the ratio ranging from (4:1 to 10:1) depending on geographic region, age, and dietary iodine intake **[13][14].** Women are more likely to get HT due to a complex interplay of genetics, sex hormones and reproductive physiology **[15]**. In recent years, increasing attention has been directed toward the role of viral infections in the pathogenesis of HT. Particular focus has been placed on viruses that exhibit latency and the capacity for reactivation, given their potential to disrupt immune tolerance and trigger autoimmune responses **[16].** EBV is the most prevalent of the viruses associated with the onset of numerous autoimmune illnesses. Once a patient is infected with the virus, the virus can stay dormant within B-cells until it finds an appropriate activation trigger, which makes the infection permanent **[9].** Accumulating evidence indicates elevated levels of EBV-related latent transcripts, viral antigens, and specific antibodies in both the peripheral blood and thyroid tissues of HT patients **[17].** The present investigation demonstrated a substantially higher sero-positivity rats of anti-EBV-EA IgG Abs in the HT group compared to the healthy group. Additionally, the current study revealed highly significant differences in the prevalence of anti-EBV-EA antibodies among HT patients across different age groups. Similarly, a study conducted in Egypt **[18]** reported that the mean serum levels of EBV-EA IgG were significantly higher in-patient group in comparison to control group. Furthermore, among HT patients, a significant positive correlation was observed between the age and EBV-EA IgG levels. This increased early antigen antibody positivity suggests a potential reactivation of latent EBV infection, further supporting the hypothesis that EBV contributes to both the initiation and progression of HT **[19].** In contrast, the current study demonstrated no significant association of EBNA IgG Abs in the HT group compared with the healthy group. Conversely, the study by [16] observed a significant positive correlation between thyroid isthmus and EBNA-1IgG. In addition, an elevated serum level of EBV-EBNA was observed in patients with Hashimoto's thyroiditis in another study **[20].** Detection of EBNA IgG antibodies in patients confirms a past, resolved infection. This antibody appears weeks to months after primary infection and persists for life **[21].** The variations between our outcomes and those of studies **[17]** and **[20]** may originate from differences in cohort demographics or the nature of the evaluated samples. Specifically, while study **[17]** documented a localized correlation tied to thyroid tissue and isthmus involvement, our study examined peripheral serum. This indicates that a localized viral presence within the thyroid may not invariably manifest as an elevated systemic anti-EBNA IgG response **[22].** Eventually, this highlights that active viral reactivation marked by anti-EBV-EA IgG is a more reasonable contributor to HT development than a simple state of chronic latency marked by anti-EBNA IgG **[23].**

Conclusions

The present of EBV-EA IgG in patients with HT compared to the healthy group suggests a possible association between EBV-reactivation and HT progression. This increased early antigen antibody positivity suggests a potential reactivation of latent EBV infection. In contrast, no significant association was found between EBNA IgG and HT. Detection of EBNA IgG antibodies in patients confirms a past, resolved infection. This antibody appears weeks to months after primary infection and persists for life.

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