



## Study of Epstein–Barr virus serological profile in Egyptian patients with Hashimoto's thyroiditis: A case-control study

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### ABSTRACT

**Background:** Hashimoto's thyroiditis (HT) is now considered one of the most prevalent autoimmune diseases. The aim of the present study was to determine the prevalence of different types of EBV antibodies in patients with HT in comparison to healthy controls, and to detect any correlation between EBV serological markers and different laboratory findings in HT patients.

**Subjects & methods:** This case-control study was conducted on 120 subjects divided into two groups: Sixty patients with HT (patients group), and sixty age and sex matched healthy volunteers (control group). All the participants were subjected to: Thyroid ultrasound, laboratory assessment including: Serum thyroid -stimulating hormone (TSH), free tetraiodothyronine (FT4), free triiodothyronine (FT3), anti-thyroid peroxidase antibody (anti-TPO Ab) and anti-thyroglobulin antibody (anti-TG Ab). Four types of EBV antibodies (VCA IgM, VCA IgG, EA IgG, and EBNA-1IgG) were measured in serum using ELISA.

**Results:** The mean serum levels of EBV VCA IgG and EA IgG were significantly higher in HT patients group in comparison to control group. In euthyroid HT patients, a significant positive correlation was observed between the age and EBV EA IgG. While in hypothyroid HT patients, a significant positive correlation between thyroid isthmus and EBNA-1IgG was observed. A significant negative correlation was found between the serum FT3 and EBNA-1IgG and a significant positive correlation was observed between serum TSH and EBV VCA IgG.

**Conclusions:** The high serum levels of EBV VCA IgG and EBV EA IgG in patients with HT suggest a possible association between EBV and HT.

### Introduction

Hashimoto's thyroiditis (HT) is now considered one of the most prevalent autoimmune diseases, as well as the most common endocrine disorder [1,2]. Also known as chronic lymphocytic thyroiditis, was firstly described by Hakaru Hashimoto in 1912 [3].

Autoimmune thyroid diseases affect up to 10% of the world population [4]. HT affects about 5% of the population at some point in their life [5]. It typically begins between the ages of 30 and 50 and is much more common in women than men [6]. Rates of disease appear to be increasing [7]. Patients with Hashimoto's disease may have other autoimmune diseases, and often have family history of thyroid or other

autoimmune diseases [8].

The etiology of HT remains unclear at present, but it is generally believed that both genetic and environmental factors contribute to their development. Viral infections are frequently cited as a major environmental factor involved in the pathogenesis of autoimmune processes [9,10].

The Epstein-Barr virus (EBV) is a double-stranded DNA virus, a member of the herpes virus family [11]. Approximately 95% of the world's adult population is infected during life and become lifelong carriers. Primary infection by EBV causes quite a different course of the disease depending on the age of the patient [12].

EBV infection is associated with the development of a variety of

**Abbreviations:** Anti TGAb, Anti- thyroglobulin antibody; Anti TPO Ab, Anti-thyroid peroxidase antibody; EBNA-1 IgG, Epstein–Barr nuclear antigen (EBNA-1) IgG; EBV, Epstein–Barr virus; EBV EA IgG, EBV early antigen (EA) IgG; FT3, Free Triiodothyronine; FT4, Free tetraiodothyronine; HT, Hashimoto's thyroiditis; TSH, Thyroid-stimulating hormone; VCA, Viral capsid antigen

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lymphoid and epithelial tumors and post-transplant lymphoproliferative disorders [13]. EBV infection is also known to be involved in the development of different autoimmune diseases. Several studies brought evidence for the connection of EBV infection and multiple sclerosis (MS) [14], and even suggested a prognostic role of elevated anti-EBV antibody titres in MS development [15].

Like other herpes viruses, EBV has a productive lytic cycle and a latent phase. The reactivated virus has the potential to induce the production of thyroid antibodies and has been implicated in many debilitating autoimmune symptoms [16].

The aim of the present study was to determine the prevalence of different types of EBV antibodies in patients with HT in comparison to healthy controls, and to detect any correlation between EBV serological markers and different laboratory findings in HT patients.

## Subjects and methods

A case-control study was carried out on 120 subjects divided into 2 groups: Group I (patients group): Included 60 newly diagnosed untreated HT patients enrolled from the Endocrinology Unit, Department of Internal Medicine, Faculty of Medicine, Alexandria University, Egypt. Their mean age was  $40.40 \pm 12.06$  years. The patients group was subdivided into two subgroups according to their thyroid function tests; Subgroup IA: It included 31 euthyroid HT patients. The euthyroid HT was defined as patients with serum thyroid hormones (TSH, FT3 & FT4) within the normal reference level, while, their thyroid antibodies were higher than the normal reference levels. Subgroup IB: It included 29 hypothyroid HT patients. The hypothyroid HT was defined as patients with high serum TSH and low or normal FT3 /FT4, while, their thyroid antibodies were higher than the normal reference levels. Enrolment started in January 2018 and ended in December 2018.

Patients with past history of other immune system endocrinopathies, those with past history of drugs known to affect thyroid functions and/or thyroid immunity (interferon or glucocorticoids) were excluded from the study.

The control group (Group II) consisted of 60 age and sex matched healthy volunteers, recruited from the community via advertisement in the same regions where patients were recruited, with mean age of  $38.20 \pm 12.28$  years. They had TSH, free T4, free T3, anti TPO, anti TG within normal range. They also had normal thyroid ultrasound.

All participants signed a written informed consent prior to providing blood samples. The study was approved by the Ethical Committee of the Faculty of Medicine, Alexandria University, Egypt.

*All participants were subjected to the following:*

### Anti-EBV serological testing

EBV antibodies were determined in the sera of all studied subjects using commercial quantitative ELISA kits (IBL International, Germany). The following antibodies were assessed: Viral capsid antigen (VCA) antibodies (both IgM&IgG), EBV nuclear antigen (EBNA-1) IgG and EBV early antigen (EA) IgG. The analytical sensitivity of the performed assays mean was: 1.29 U/mL [17]. Absence of all antibodies (VCA IgM, VCA IgG, EBNA-1 IgG, EA IgG) indicated absence of EBV infection. The presence of VCA IgM  $\pm$  VCA IgG in the absence of EBNA-1 IgG denoted acute infection. The presence of VCA IgG and EBNA-1.

IgG in the absence of VCA IgM denoted past infection. The presence of VCA IgG and EBNA-1 IgG together with EA IgG antibodies marked a reactivation [17].

### Thyroid laboratory investigations

Thyroid function tests including: serum Free tetraiodothyronine (FT4), serum Free triiodothyronine (FT3), serum thyroid stimulating hormone (TSH) and thyroid autoantibodies including: serum anti-thyroid peroxidase antibody (anti-TPO Ab) and serum anti-thyroglobulin antibody (anti-TG Ab) were performed. The normal reference

levels were set according to the manufacturer's instructions as follows: Free T4 (0.93–1.7 ng/ml), Free T3 (2.0–4.4 pg/mL), TSH (0.27–4.8  $\mu$ IU/ml), Anti-TPO antibodies (Up to 34 IU/ml), Anti-TG antibodies (Up to 115 IU/ml).

### Ultrasonographic assessment of the thyroid gland

Ultrasound assessment of the thyroid organ was done utilizing an economically accessible continuous instrument (Kontron-imagic Agile) utilizing a 7.5 MHz direct transducer in cross transverse & longitudinal planes. Patients were lying prostrate with their neck marginally hyper stretched out by putting a cushion underneath their shoulders, with full remark on the accompanying focuses:

- Length, width and profundity of every projection and the isthmus.
- Calculation of the volume of the thyroid organ: Volume was the aggregate of the two flaps each determined by utilizing the prolate ellipsoid strategy (volume = length  $\times$  expansiveness  $\times$  profundity  $\times \pi/6$ ). Thyroid extension was characterized as thyroid volume surpassing 9.5 ml in females, as indicated by the mean glandular thyroid volume for females of our nearby district of our unpublished information.
- Echogenicity of the organ. The echogenicity of the thyroid was additionally inspected and characterized in examination with the anatomic structures that are isoechoic (submandibular organs) or hypoechoic (neck muscles) as for the ordinary thyroid tissue
- Vascularity of the organ by the shading Doppler.
- The nearness of knobs which are additionally inspected: size, shape, echogenicity, outskirt, edge, radiance, vascularity and calcification.
- Cervical lymph hubs: shape, size and nearness of hilum.

### Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. The significance of the obtained results was judged at the 5% level.

The used tests were:

1: Chi-square test:

For categorical variables, to compare between different groups.

2: Fisher's Exact:

Correction for Chi-square when > 20% of the cells have expected count less than 5.

3: Student *t*-test:

For normally quantitative variables, to compare between two studied groups.

4: Mann Whitney test:

For abnormally quantitative variables, to compare between two studied groups.

5: Binary logistic regression.

The covariate included were: Total volume (ml), Isthmus (cm), Vascularity (peak velocity) (ml/sec), TSH ( $\mu$ IU/ml), FT3 (pg/ml), FT4 (ng/ml), Anti-thyroglobulin Ab (IU/ml), EBV VCA IgG (U/ml), EBV EA IgG (U/ml). These covariates were selected because they were significant with Hashimoto's thyroiditis in univariate analysis. EA IgG was

**Table 1**

Comparison between patients with Hashimoto's thyroiditis and healthy controls according to Anti-EBV serological profile.

	HT Patients group (n = 60)		Control group(n = 60)		Test of Sig.	p
	No.	%	No.	%		
<b>EBV VCA IgM (U/ml)</b>						
Negative (< 0.8)	0	0.0	0	0.0	–	–
Equivocal (0.8–1.2)	0	0.0	0	0.0		
Positive (> 1.2)	60	100.0	60	100.0		
Mean ± SD.	7.86 ± 4.43		11.72 ± 14.59		U = 1682.0	0.536
Median (Min.–Max.)	7.10 (2.20–24.90)		7.25 (1.30–76.50)			
<b>EBV VCA IgG (U/ml)</b>						
Negative (< 0.8)	0	0.0	0	0.0	–	–
Equivocal (0.8–1.2)	0	0.0	0	0.0		
Positive (> 1.2)	60	100.0	60	100.0		
Mean ± SD.	135.1 ± 68.93		98.63 ± 61.69		U = 1217.0*	0.002*
Median (Min.–Max.)	116.8 (19.50–272.6)		83.55 (1.80–262.4)			
<b>EBNA-1 IgG (U/ml)</b>						
Negative (< 0.8)	0	0.0	0	0.0	–	–
Equivocal (0.8–1.2)	0	0.0	0	0.0		
Positive (> 1.2)	60	100.0	60	100.0		
Mean ± SD.	53.59 ± 41.87		42.02 ± 25.60		U = 1568.5	0.224
Median (Min.–Max.)	45.50 (4.70–228.1)		41.15 (1.80–101.2)			
<b>EBV EA IgG (U/ml)</b>						
Negative (< 0.8)	40	66.7	55	91.7	$\chi^2 = 14.388^*$	$MC_p = 0.001^*$
Equivocal (0.8–1.2)	0	0.0	1	1.7		
Positive (> 1.2)	20	33.3	4	6.7		
Mean ± SD.	6.66 ± 16.43		3.16 ± 16.60		U = 1335.0*	0.001*
Median (Min.–Max.)	0.0 (0.0–75.20)		0.0 (0.0–92.10)			

 $\chi^2$ :Chi square test.

MC: Monte Carlo.

U: Mann Whitney test.

p: p value for comparing between the studied groups.

\*: Statistically significant at  $p \leq 0.05$ .

EBV VCA IgM: Epstein-Barr virus viral capsid Antigen IgM.

EBV VCA IgG: Epstein-Barr virus viral capsid antigen IgG.

EBNA-1 IgG: Epstein-Barr nuclear antigen-1 IgG.

EBV EA IgG: Epstein-Barr virus Early Antigen IgG.

not normally distributed. So, we categorized the EA IgG into 2 categories (less than or equal 1.2 and more than 1.2 to overcome the non-normalization see [table 5](#).

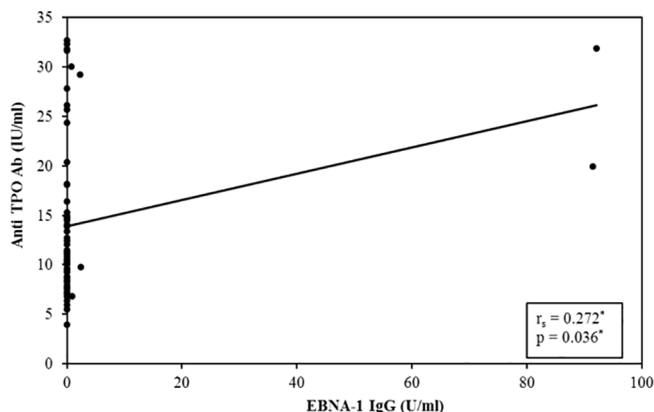
## Results

### Anti-EBV serological testing

[Table 1](#) demonstrates that all tested antibodies were positive in both cases and controls except EA IgG, which was positive in 20 HT cases compared to only four controls ( $p = 0.001$ ). Additionally, the mean serum level of EBV VCA IgG and EBV EA IgG were statistically significantly higher in HT patients group in comparison to control group ( $p = 0.002$ ,  $p = 0.001$  respectively). When comparing the two patients' subgroups (euthyroid and hypothyroid HT), there was no statistically significant difference in EBV serologic profile between the two subgroups.

A significant positive correlation between EBNA-1 IgG and serum Anti TPO Ab (IU/ml) was found in healthy controls,  $r = 0.272$ ,  $p = 0.036$  ([Fig. 1](#)).

In euthyroid HT patients, a significant positive correlation was observed between the age and EBV EA IgG ( $r = 0.378$ ,  $p = 0.036$ ), ([Fig. 2](#)). While, in hypothyroid HT patients, a significant positive correlation was observed between thyroid isthmus size and EBNA-1 IgG ( $r = 0.374$ ,  $p = 0.045$ ), ([Fig. 3](#)). On the other hand, a significant negative correlation was found between the serum FT3 and EBNA-1 IgG ( $r = -0.402$ ,  $p = 0.031$ ), ([Fig. 4](#)). A significant positive correlation was also observed between serum TSH and EBV VCA IgG ( $r = 0.367$ ,  $p = 0.049$ ). ([Fig. 5](#))



**Fig. 1.** Correlation between EBNA-1 IgG (U/ml) with serum Anti TPO Ab (IU/ml) in healthy control group (n = 60).

### Measurement of thyroid hormones and thyroid auto-antibodies

The mean values of serum TSH ( $\mu$ IU/ml), anti-TPO Ab (IU/ml) and anti-TG Ab (IU/ml) were statistically significantly higher in patients group in comparison to control group ( $p < 0.001$ ). While, the mean serum FT3 (pg/ml) and FT4 (ng/ml) values were statistically significantly lower in patients group in comparison to control group ( $p < 0.001$ ,  $p = 0.003$  respectively). ([Table 2](#))

Concerning the two HT subgroups, the mean serum levels of TSH ( $\mu$ IU/ml) and Anti TPO Ab (IU/ml) were significantly higher in hypothyroid HT in comparison to euthyroid HT, ( $p < 0.001$ ,  $p = 0.008$

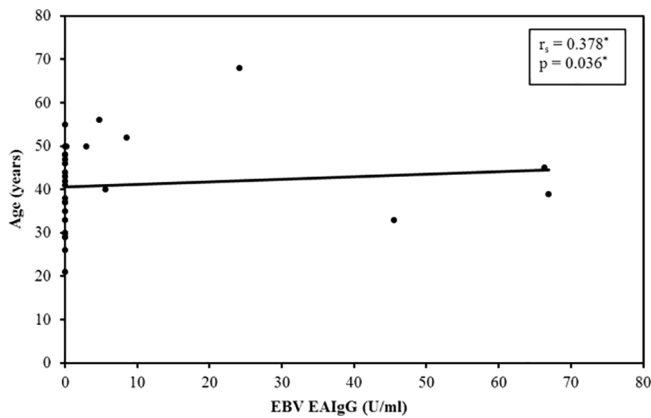


Fig. 2. Correlation between EBV EA IgG (U/ml) with age (years) in euthyroid HT patients subgroup (TSH  $\leq$  4.8, n = 31).

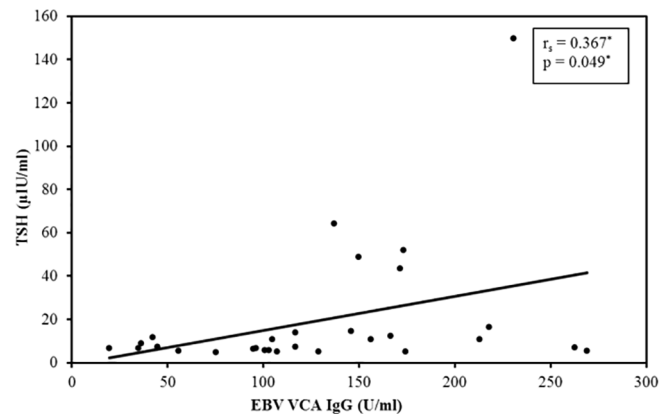


Fig. 5. Correlation between EBV VCA IgG (U/ml) with TSH ( $\mu$ IU/ml) in hypothyroid HT patients subgroup (TSH > 4.8, n = 29).

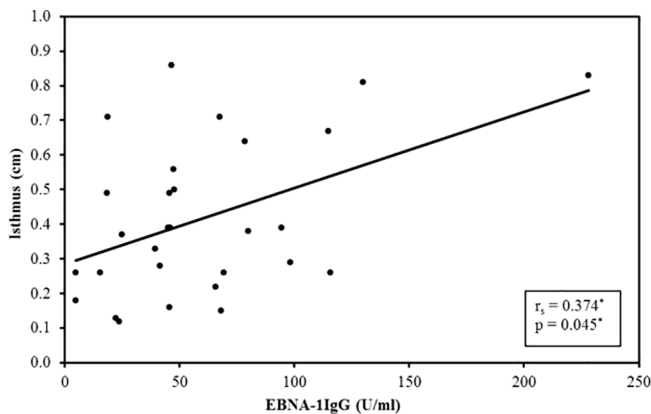


Fig. 3. Correlation between EBNA-1 IgG (U/ml) with Isthmus size (cm) by sonography in hypothyroid HT patients subgroup (TSH > 4.8, n = 29).

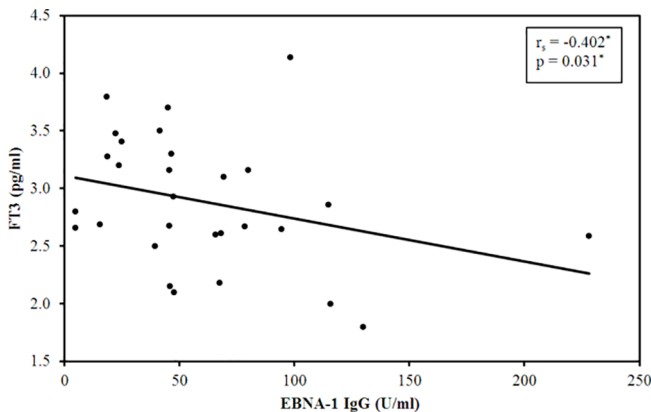


Fig. 4. Correlation between EBNA-1 IgG (U/ml) with serum FT3 (pg/ml) in hypothyroid HT patients subgroup (TSH > 4.8, n = 29).

respectively). While, the mean serum FT4 (ng/ml) value was statistically significantly lower in hypothyroid HT in comparison to euthyroid group ( $p = < 0.001$ ). (Table 3)

#### Ultrasonographic assessment of the thyroid gland

Table 4 shows that total thyroid volume, the isthmus measurement and the vascularity (peak velocity) were statistically significantly higher in patients group in comparison to the control group ( $p = < 0.001$ ).

Thyroid nodule was present in 15% (n = 9), and pseudonodular

appearance and fibrous septa was found in 85% (n = 51) of HT patients. While, thyroid nodules was detected in 5% (n = 3) of healthy controls. Hypo echogenicity was present in all HT patients, on the other hand, normal echogenicity was reported in all controls.

On comparing the two patients subgroups (euthyroid and hypothyroid), there was no statistically significant difference in their sonographic findings.

Univariate and multivariate analysis for the parameters, which may be associated with HT, revealed that the most independent factors were thyroid vascularity (peak velocity) (ml/sec), TSH ( $\mu$ IU/ml), FT3 (pg/ml) and anti-TG Ab (IU/ml). While EA IgG was not significant with binary logistic regression (Table 5).

#### Discussion

HT is the most common cause of hypothyroidism in iodine-sufficient areas of the world [18]. The exact factors that can lead to the onset of HT have not yet been sufficiently clarified. In addition to a family (genetic) pre-stress, there are also stress, ongoing viral diseases (such as mononucleosis, herpes zoster), dysfunction of the adrenal cortex, microchimerism and environmental effects [19].

EBV is considered to be an etiological factor of autoimmune diseases because of the following: The virus is a common pathogen responsible for the worldwide prevalence of autoimmune diseases. EBV stays in the body throughout life, which explains the chronic course of autoimmune diseases that are often accompanied by exacerbations of symptoms [20].

The aim of the present study was to determine the prevalence of different types of EBV antibodies in patients with HT in comparison to healthy controls, and to detect any correlation between EBV serological markers and different laboratory findings in HT patients.

The present study demonstrated that all tested EBV antibodies were positive in all cases and controls except EA IgG, which was positive in 20 HT cases compared to only four controls. Since EA IgG is considered a marker of virus reactivation, it may play a role in the pathogenesis of HT. Additionally, EBV VCA IgG mean level was significantly higher in cases ( $135.1 \pm 68.93$ ) than in controls ( $98.63 \pm 61.69$ ). The mean serum level of EBV EA IgG, was also statistically significantly higher in HT cases ( $p = 0.001$ ). The positivity of most tested antibodies in cases and controls is not surprising considering the endemicity of EBV in Egypt, however, the higher number of cases positive for EA IgG antibodies compared to controls, should draw the attention to the possible role of virus reaction in disease pathogenesis and/or progression. Further studies targeting latent viral proteins and DNA in the thyroid tissue itself should be addressed to prove a causal relationship. However, the comparison of EBV serological profile between euthyroid and hypothyroid groups revealed no significant difference in the

**Table 2**  
Comparison of thyroid markers and auto-antibodies between Hashimoto's thyroiditis patients and controls.

	HT Patients (n = 60)		Controls (n = 60)		Test of Sig.	p
	No.	%	No.	%		
<i>TSH (μIU/ml)</i>						
Euthyroid Hashimoto (≤ 4.8)	31	51.7	60	100.0	$\chi^2 = 38.242^*$	< 0.001*
Hypothyroid Hashimoto (> 4.8)	29	48.3	0	0.0		
Mean ± SD.	10.56 ± 22.24		2.10 ± 1.07		U = 861.0*	< 0.001*
Median (Min.–Max.)	4.40 (0.32–150.0)		1.96 (0.42–4.70)			
<i>FT3 (pg/ml)</i>						
Normal (2–4.4)	59	98.3	60	100.0	$\chi^2 = 1.008$	FE <sub>p</sub> = 1.000
Abnormal	1	1.7	0	0.0		
Mean ± SD.	2.95 ± 0.47		3.27 ± 0.34		U = 1084.0*	< 0.001*
Median (Min.–Max.)	3.05 (1.80–4.14)		3.23 (2.52–4.16)			
<i>FT4 (ng/ml)</i>						
Normal (0.93–1.7)	50	83.3	60	100.0	$\chi^2 = 10.909^*$	0.001*
Abnormal	10	16.7	0	0.0		
Mean ± SD.	1.14 ± 0.29		1.27 ± 0.16		t = 3.097*	0.003*
Median (Min.–Max.)	1.16 (0.38–1.68)		1.27 (0.94–1.69)			
<i>Anti TPOAb (IU/ml)</i>						
Normal (up to 34)	3	5.0	60	100.0	$\chi^2 = 108.571^*$	< 0.001*
Abnormal	57	95.0	0	0.0		
Mean ± SD.	406.3 ± 376.4		14.30 ± 8.08		U = 47.0*	< 0.001*
Median (Min.–Max.)	321(11.60–2141.3)		11.20 (4.0–32.70)			
<i>Anti-thyroglobulin Ab (IU/ml)</i>						
Normal (up to 115)	21	35.0	60	100.0	$\chi^2 = 57.778^*$	< 0.001*
Abnormal	39	65.0	0	0.0		
Mean ± SD.	479.8 ± 758.6		32.80 ± 31.88		U = 437.50*	< 0.001*
Median (Min.–Max.)	182(2.66–3200.0)		14.65 (9.0–112.3)			

$\chi^2$ : Chi square test.

FE: Fisher Exact U: Mann Whitney test.

t: Student *t*-test.

p: p value for comparing between the studied groups.

\*: Statistically significant at  $p \leq 0.05$ .

present work, which could explain that virus reactivation may have a possible role in triggering disease pathogenesis more than progression.

In 1971, Evans et al. [18] demonstrated raised antibody titers to EBV in patients with systemic lupus erythematosus (SLE). This was the reason to suspect that the EBV might participate in the development of autoimmune diseases.

In agreement with our study, Thomas et al. [21] observed in 2008 a significantly higher level of anti-EBV IgG antibodies in children with autoimmune thyroid disease (study group) (n = 34) than in a control group (P = 0.008), and formulated a hypothesis that EBV infection might play a role in the pathogenesis of autoimmune thyroid disease in children.

In later years, Akahoriet al. [22] further investigated this phenomenon and showed three cases of women with newly diagnosed GD and acute primary EBV infection in the form of infectious mononucleosis.

In keeping with our results, Vrbikova et al. [23] demonstrated that there was elevated titers of antibodies against different antigens of EBV in some immunodeficient states, malignancies or in autoimmune disorders. They examined EBV serology in the group of 22 patients with autoimmune thyroiditis as compared with the group of 35 healthy volunteers. Titers of antibodies against viral capsid antigen (VCA IgG) were more often found in the group of patients than in the control group (p = 0.00035 for younger than 40 years and p = 0.00115 for older than 40 years) and the positivity of antibodies against early antigen (Ig-G– EA–D/DR) was also significantly more often found in the group of patients (p = 0.0031 and p = 0.0019 respectively) than in the control group.

Janegova et al. [24] conducted a study on patients with HT (n = 26), and Graves' disease (n = 8), and nodular goitres (n = 8) as controls. They observed that in none of the Graves' disease specimens but in 34.5% of HT cases the cytoplasmic expression of LMP1 was

detected in follicular epithelial cells and in infiltrating lymphocytes. They reported that evaluation of viral nuclear RNA (EBER) positivity pointed to a high prevalence of EBV in cases of HT (80.7%) as well as in Grave's disease (62.5%). On the contrary, none of the control goitres demonstrated EBER expression. The question is whether this high occurrence of EBV in thyroid gland samples is associated directly with autoimmune disease development or if it is the result of the high EBV carrier rate in the population. The absence of EBV in all control goitres observed in their study favours the first possibility. An analogical attempt to map the herpes simplex 1 and 2, CMV and human herpes virus type 6 and 7 infection in tissue samples of AITDs did not provide any conclusive connections.

Ling W et al. [25] reported a 51-year-old married Chinese woman, with a history of enlargement of the right lobe of thyroid for 8 months, diagnosed as Rosai-Dorfman disease which was induced by EBV, the patient underwent a total thyroidectomy and lymph node dissection. During surgery, multiple enlarged nodes and abnormal thyroid tissue were found. The excised thyroid specimen was grayish in color. Surgical histopathology of the thyroid and lymph nodes revealed sinus histiocytosis with massive lymphadenopathy along with HT. In their case, ultrasonography revealed enlargement of the thyroid glands with heterogeneous hypoechogenicity.

A significant positive correlation was observed between serum TSH and EBV VCA IgG (r = 0.367, p = 0.049). A significant negative relation was also found between the serum FT3 and EBNA-1 IgG (r = -0.402, p = 0.031) in HT subjects with hypothyroidism. This would suggest a relation between EBV and hypothyroid state. Absence of these correlations in the control group despite the presence of similar antibodies may be due to the higher levels of VCA IgG and EBNA IgG in patients than in control group.

Shimon I et al. [26] reported a unique 16-year old female patient

**Table 3**

Comparison of thyroid markers and auto-antibodies between euthyroid and hypothyroid Hashimoto's thyroiditis patients.

	Euthyroid Hashimoto's (TSH ≤ 4.8) (n = 31)		Hypothyroid Hashimoto's (TSH > 4.8) (n = 29)		Test of sig.	p
	No.	%	No.	%		
<i>TSH (μIU/ml)</i>						
Euthyroid Hashimoto (≤ 4.8)	31	100.0	0	0.0	-	-
Hypothyroid Hashimoto (> 4.8)	0	0.0	29	100.0		
Mean ± SD.	2.27 ± 1.28		19.42 ± 29.72			
Median (Min.-Max.)	2.39 (0.32-4.61)		7.6 (4.97-150.0)			
<i>FT3 (pg/ml)</i>						
Normal (2-4.4)	31	100.0	28	96.6	$\chi^2 = 1.087$	<sup>FE</sup> p = 0.483
Abnormal	0	0.0	1	3.4		
Mean ± SD.	3.02 ± 0.36		2.89 ± 0.57		U = 377.50	0.287
Median (Min.-Max.)	3.11 (2.26-3.59)		2.80 (1.80-4.14)			
<i>FT4 (ng/ml)</i>						
Normal (0.93-1.7)	31	100.0	19	65.5	$\chi^2 = 12.828^*$	< 0.001*
Abnormal	0	0.0	10	34.5		
Mean ± SD.	1.28 ± 0.17		1.0 ± 0.31		t = 4.240*	< 0.001*
Median (Min.-Max.)	1.30 (0.95-1.58)		0.97 (0.38-1.68)			
<i>Anti TPO Ab (IU/ml)</i>						
Normal (up to 34)	3	9.7	0	0.0	$\chi^2 = 2.954$	<sup>FE</sup> p = 0.238
Abnormal	28	90.3	29	100.0		
Mean ± SD.	296.7 ± 294.5		523.5 ± 421.9		U = 271.50*	0.008*
Median (Min.-Max.)	198(12-1353.1)		499(42.1-2141)			
<i>Anti-thyroglobulin Ab (IU/ml)</i>						
Normal (up to 115)	10	32.3	11	37.9	$\chi^2 = 0.212$	0.645
Abnormal	21	67.7	18	62.1		
Mean ± SD.	384.0 ± 474.5		582.3 ± 974.8		U = 424.50	0.712
Median (Min.-Max.)	192.2(11.5-2182)		173.2 (2.66-3200)			

 $\chi^2$ :Chi square test.

FE: Fisher Exact U: Mann Whitney test.

t: Student t-test.

p: p value for comparing between the studied groups.

\*: Statistically significant at p ≤ 0.05.

who presented after acute EBV infection with severe primary hypothyroidism. Her thyroid test results were level TSH of 198 mU/L (normal, 0.4-4 mU/L), FT4 2.5 pmol/L (normal, 10-25 pmol/L), TT3 > 19.5 nmol/L (normal, 1.3-2.7 nmol/L), and free FT3 0.77 pmol/L (normal, 3.3-6.3 pmol/L). She had high titers of anti TPO and anti TG autoantibodies. In vitro triiodothyronine (T3)-binding measured by radioimmunoprecipitation was 86% (normal, up to 8.5%) and thyroxine (T4)-binding 8.2% (normal, 6.4%). Serum immunoglobulin G (IgG) absorption, achieved by protein-G Sepharose

beads, decreased TT3 toward normal. Levothyroxine treatment normalized the low baseline FT4 and FT3 values, and suppressed TSH to normal. However, TT3 remained highly elevated and returned to normal after 20 months, while T3 binding gradually decreased. Thus, her severe hypothyroidism was masked by this unusual phenomenon. Thirty-four patients with EBV infection (15 with acute disease and 19 with previous infection) were tested by the same authors for thyroid hormone levels. EBV antibodies (early antigen immunoglobulin M (IgM) and IgG and anti-Epstein-Barr virus nuclear antigen (EBNA) IgG)

**Table 4**

Comparison between patients with Hashimoto's thyroiditis and healthy controls according to ultrasound findings of the thyroid gland.

	HT Patients group (n = 60)		Control group (n = 60)		Test of sig.	p
	No.	%	No.	%		
<i>Total volume (ml)</i>						
Mean ± SD.	7.35 ± 5.82		4.53 ± 2.28		U = 1125.0*	< 0.001*
Median (Min.-Max.)	6.0 (0.96-32.60)		4.15 (1.65-12.90)			
<i>Isthmus (cm)</i>						
Mean ± SD.	0.39 ± 0.22		0.28 ± 0.31		U = 867.50*	< 0.001*
Median (Min.-Max.)	0.34 (0.07-1.34)		0.21 (0.10-2.4)			
<i>Vascularity (peak velocity) (ml/sec)</i>						
Mean ± SD.	9.54 ± 4.47		5.13 ± 1.91		U = 634.5*	< 0.001*
Median (Min.-Max.)	8.17 (3.29-20.0)		4.75 (1.60-10.36)			
<i>Nodules</i>						
No nodules	0	0.0	57	95.0	$\chi^2 = 111.0^*$	< 0.001*
Pseudonodular appearance and fibrous septa	51	85.0	0	0.0		
Nodular	9	15.0	3	5.0		
<i>Echogenicity</i>						
Normal echogenicity	0	0.0	60	100.0	$\chi^2 = 120.0^*$	< 0.001*
Hypo echogenicity	60	100.0	0	0.0		

 $\chi^2$ :Chi square test.

U: Mann Whitney test.

p: p value for comparing between the studied groups.

\*: Statistically significant at p ≤ 0.05.

**Table 5**  
Univariate and multivariate analysis for the parameters associated with Hashimoto's thyroiditis.

	Univariate		#Multivariate	
	p	OR (95%CI)	p	OR (95%CI)
Total volume (ml)	0.002*	1.243* (1.082–1.427)	0.194	1.195 (0.913–1.56)
Isthmus (cm)	0.030*	11.61* (1.26–106.40)	0.740	1.750 (0.064–47.62)
Vascularity (peak velocity) (ml/sec)	< 0.001*	1.638* (1.33–2.014)	0.031*	1.397* (1.032–1.89)
TSH (μU/ml)	< 0.001*	1.71* (1.32–2.225)	0.005*	2.454* (1.32–4.56)
FT3 (pg/ml)	< 0.001*	0.150*(0.054–0.418)	0.023*	0.062* (0.006–0.684)
Anti-thyroglobulin Ab (IU/ml)	< 0.001*	1.024* (1.014–1.035)	0.002*	1.031* (1.012–1.051)
EBV VCA IgG (U/ml)	0.004*	1.009* (1.003–1.015)	0.253	1.009 (0.994–1.024)
@EBV EA IgG (U/ml) (> 1.2)	0.001*	7.0* (2.22–22.055)	0.315	3.284 (0.323–33.348)

OR: Odd's ratio, C.I: Confidence interval.

#: All variables with  $p < 0.05$  was included in the multivariate.

\*: Statistically significant at  $p \leq 0.05$ .

@: Categories.

were measured by enzyme-linked immunosorbent assay (ELISA). In 15 patients with acute EBV the mean TT3 level was  $2.47 \pm 0.39$  nmol/L (5 had TT3 values above normal) compared to a mean TT3 of  $1.70 \pm 0.53$  nmol/L in 19 subjects with previous infection ( $p < 0.0005$ ; only 1 had a TT3 result above normal), with no differences in FT4 and TSH concentrations between the two groups. Acute EBV infection may be associated with transient mild to severe TT3 elevation as a result of assay interference by anti-T3 autoantibodies.

In contrast to the high echodensity of the normal thyroid parenchyma due to the follicle structure, a reduction in thyroid echogenicity by US is commonly encountered in autoimmune thyroid diseases, being probably attributed to both lymphocytic infiltration and disruption of normal tissue architecture. Currently, thyroid hypoechogenicity is viewed as an early sign of thyroid autoimmunity, which may even precede the clinical suspicion of thyroid disorder [27].

Using a subjective measure of thyroid echogenicity, previous studies demonstrated that in patients with thyroid autoimmune diseases, the presence of circulating thyroid antibodies (Abs) as well as the development of hypothyroidism was closely correlated with the degree of thyroid hypoechogenicity [28].

Limitations of the current study include the fact that all subjects (HT and controls) had evidence of EBV exposure and temporality between EBV exposure and development of HT cannot be determined. Therefore, it can only suggest association, but cannot confirm or determine causation. However, the strength of the present work comes from the fact that, to the best of our knowledge, this is the first study in Egypt and in the Middle East to explore the different serological profile markers of EBV in HT patients.

## Conclusion

The high serum levels of EBV VCA IgG and EBV EA IgG in patients with HT point to a possible association between EBV and HT. However, the EBV serological status did not differ between euthyroid and hypothyroid HT. This might suggest the absence of a relation between EBV and the severity of thyroid affection.

The direct relation between serum TSH and EBV VCA IgG, and the negative relation between serum FT3 and EBNA-1IgG would however suggest that EBV status may be associated with the degree of thyroid hormonal dysfunction.

The significant positive correlation between serum EBNA-1 IgG levels and anti TPO titer in healthy control subjects may suggest a role of EBV in triggering thyroid autoimmunity, early before true development of HT.

Further studies with larger sample size to prove the causal relationship between EBV and HT are mandatory.

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