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Herpes virus antibodies seroprevalence in children with autoimmune thyroid disease

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Abstract *Background* Elevated titers of antibodies against different herpes virus antigens have been reported in some immunodeficient and systemic autoimmune disorders. *Objective* To examine if Herpes Simplex Virus (HSV), Epstein-Barr virus (EBV), and cytomegalovirus (CMV) IgG and IgM antibodies are detected more frequently in children with autoimmune thyroid disease (AITD) compared to controls. *Subjects and methods* Thirty-four children with AITD, aged 9.62 ± 2.35 years, and 31 matched controls, aged 9.24 ± 2.98 years, were studied. *Results* The percentage of EBV IgG+ children with AITD was statistically higher than the percentage of EBV IgG+ controls (82.35% versus 51.61%, $P = 0.008$). The percentage of EBV IgG+ children with AITD and hypothyroidism was statistically higher than the percentage of EBV IgG+ children with AITD, without hypothyroidism (100% versus 70%, $P = 0.024$). No other statistically significant differences were observed in HSV−1+2, and CMV IgG or IgM antibodies between the subgroups of children studied. *Conclusions* EBV seroprevalence is higher in children with AITD compared to controls and the underlying pathology remains to be elucidated.

Keywords Autoimmune thyroiditis · Herpes virus · Seroprevalence

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Introduction

Autoimmune thyroid disease (AITD) is the most common cause of thyroid dysfunction in children and adolescents and is also the most common cause of acquired hypothyroidism with or without goiter [1]. The underlying pathology, which appears to involve several genetic and environmental factors, is mainly focused on thyrocyte and its ability to express a wide array of immunologically active "self" and "non-self" molecules, which may exacerbate or diminish the autoimmune response. From the limited data available, it is not clearly known whether the autoimmune phenomena induced by viruses represent a non-specific response to the inflammatory release of thyroid antigens or constitute a specific autoimmune disease triggered by the viral infection itself.

Although herpes virus infection has been implicated to the pathophysiology of several chronic autoimmune inflammatory processes in adults [2], few reports relate the presence of *Herpesviridae* to AITD. Cytomegalovirus (CMV) infection of primary cultures of thyroid cells results in induction of HLA-DR expression on thyroid follicular cells [3], which act as antigen presenting cells and may be involved in the induction of AITD. Probing the B-cell repertoire of patients with Hashimoto's disease using Epstein-Barr virus (EBV)-infection led not only to the production of polyreactive low affinity antibodies against thyroglobulin (Tg), and thyroid peroxidase (TPO) antigen,

but also to high frequencies of cell producing monoreactive IgG autoantibodies to thyroid antigen. The latter were significantly higher compared to healthy adult subjects [4]. EBV infection has been linked to the development of Hashimoto's disease and the appearance of TPO antibodies in some patients [5]; however, a direct cause–effect relationship has not been proved. Furthermore, a higher frequency of EBV infected, B-lymphocytes secreting antibody to TSH-R has been reported to be present in the peripheral blood of Graves' disease (GD) patients compared to control individuals [6]. Elevated titers of antibodies against different antigens of EBV have been measured in adult patients with autoimmune thyroiditis as compared to the healthy volunteers [7]. In contrast, there are no data relating herpes virus infection to children with AITD.

Purpose of this study was to examine if herpes simplex virus (HSV), EBV, and CMV IgG and IgM antibodies are detected more frequently in children with AITD compared to controls and to evaluate possible factors for the different seroprevalence.

Materials and methods

Patients

In total 34 children (26 female and 8 male), aged 9.62 ± 2.35 years, with AITD type 1 (euthyroid children, with or without goiter) or 2 (hypothyroid children, with or without goiter), and 31 controls (25 female and 6 male), aged 9.24 ± 2.98 years, were studied. The diagnosis was based on the clinical symptoms and signs, confirmed by laboratory tests [TSH, fT4, thyroid peroxidase autoantibodies (anti-TPO), thyroglobulin autoantibodies (anti-Tg)], ultrasound examination and in a few cases by thyroid ^{131}I scanning.

Thyroid peroxidase and thyroglobulin autoantibodies were measured in the same laboratory using the same commercial chemiluminescence assay (Diasorin, Advantage analyser). Only individuals with high titers of anti-Tg and/or anti-TPO, if confirmed in a second test, were considered eligible for the AITD group. Patients with borderline thyroid autoantibodies levels were excluded from the study in order to avoid both false positive and false negative results. TSH and fT4 were measured by electrochemiluminescence assay (Roche, Elecsys 2010).

Ultrasound examination was considered positive for Hashimoto's thyroiditis (HT) in the presence of inhomogeneous enlarged thyroid gland, heterogeneous and hypoechoic pattern, fine micronodular appearance, diaphragms, alteration in mean peak systolic velocity of intrathyroidal blood flow, or focal thyroiditis with solid, hyperechoic nodules, with ill-defined margins and sometimes with calcifications and cystic change. For the identification of goiter, thyroid volume upper normal limits were considered according to the reference values for iodine-sufficient schoolchildren, from both WHO [8] and Greek population [9] data.

The control group consisted of children with TSH, fT4, anti-TPO, and anti-Tg levels within normal range, without symptoms or signs diagnostic for AITD, and normal thyroid ultrasound (thyroid volume within normal range for sex, age, and BMI). A written consent was obtained from the parents of all participants, following the guidelines of Greek Bioethical Committee. The clinical characteristics of the studied children are shown in Table 1.

Herpes virus antibody assays

Blood samples from children enrolled in the study were obtained at the first visit. For the detection of HSV−1+2, EBV, and CMV IgG and IgM, an enzyme immunoassay

Table 1 The clinical characteristics of the children participated in the study

Children studied	*n*	Age	Height (cm) (±SD)	Weight (kg) (±SD)	BMI (kg/m^2) (±SD)
AITD					
Type 1A[a]	4	10.3 ± 1.3	131.7 ± 3.9	29.1 ± 6.8	16.8 ± 2.4
Type 1B[b]	14	9.9 ± 2.5	139.9 ± 14.8	44.7 ± 16.1	22.9 ± 9.1
Type 2A[c]	8	9.5 ± 2.7	135.4 ± 14.5	37.9 ± 9.7	20.7 ± 7.4
Type 2B[d]	8	9.1 ± 2.5	136.5 ± 14.9	39.7 ± 23.1	21.3 ± 13.5
Controls	31	9.3 ± 3.0	131.1 ± 20.1	40.6 ± 21.7	23.6 ± 9.2
		$P = 0.86$	$P = 0.75$	$P = 0.753$	$P = 0.557$

[a] Euthyroid children with positive thyroid autoantibodies and goiter

[b] Euthyroid children with positive thyroid autoantibodies, without goiter

[c] Hypothyroid children with positive thyroid autoantibodies and goiter

[d] Hypothyroid children with positive thyroid autoantibodies, without goiter

(ELISA) (Dia. Pro., Milano, Italy) was applied, according to the manufacturer's instructions.

Statistical analysis

Following the application of Levene's test for homogeneity of variances, non-parametric Kruskal–Wallis one way analysis of variance (ANOVA) was used to confirm the absence of differences in mean values of age, height, weight, and BMI between children with AITD types 1A, 1B, 2A, 2B, and controls. Post hoc comparisons in mean values were made using Mann–Whitney U test with a downward adjustment of the α level to compensate for multiple comparisons. Chi-square test (when needed, Yate's correction was applied) was used to compare the percentage of herpes IgG+ and IgM+ between children with AITD and controls or between children with AITD, with or without goiter or with or without hypothyroidism. Calculations were performed using the Number Cruncher Statistical System and Power Analysis System (NCSS/PASS 2004, Dawson Edition) and Statistical Package for Social Sciences (SPSS V.10). $P < 0.05$ was considered statistically significant.

Results

Herpes virus seroepidemiology in children of the study

No statistically significant differences were observed in the percentage of HSV IgG+ and CMV IgG+ subjects between the group of children with AITD and control group. In contrast, the percentage of EBV IgG+ children with AITD was statistically higher than the percentage of EBV IgG+ controls (Fig. 1). Two EBNA+ children were observed in the AITD group (one with AITD type 1 and one with AITD type 2) and 2 EBNA+ children in the control group. No statistically significant differences were observed in the percentage of CMV IgM+ and EBV IgM+ children between group of patients with AITD and control group. No HSV IgM+ children were observed (Fig. 1).

Herpes virus seroepidemiology in children with AITD and goiter

No statistically significant differences were observed in the percentage of HSV IgG+, CMV IgG+, and EBV IgG+ children between patients with AITD and goiter and patients with AITD, without goiter (Fig. 2). No statistically significant differences were observed in the percentage of EBV IgM+ children between patients with AITD and goiter and patients with AITD, without goiter. No HSV IgM+ or CMV IgM+ children with AITD (with or without goiter) were observed (Fig. 2).

Herpes virus seroepidemiology in children with AITD and hypothyroidism

No statistically significant differences were observed in the percentage of HSV IgG+ and CMV IgG+ children between patients with AITD and hypothyroidism and patients with AITD, without hypothyroidism (Fig. 3). In contrast, the percentage of EBV IgG+ children with AITD and hypothyroidism was statistically higher than the percentage of EBV IgG+ children with AITD, without hypothyroidism (Fig. 3). No statistically significant differences were observed in the percentage of EBV IgM+ children between patients with AITD and hypothyroidism and patients with AITD, without hypothyroidism. No HSV IgM+ or CMV IgM+ children with AITD (with or without hypothyroidism) were observed (Fig. 3).

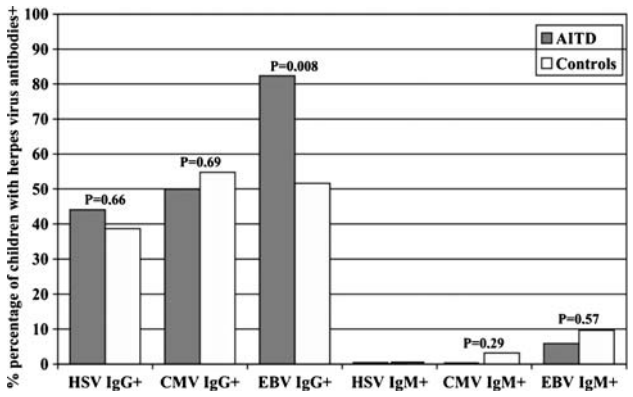

Fig. 1 Histogram indicating the prevalence of HSV IgG, CMV IgG, EBV IgG, HSV IgM, CMV IgM, and EBV IgM between children with autoimmune thyroid disease and control group

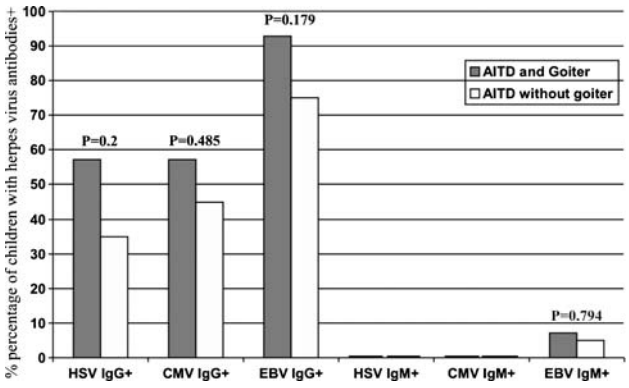

Fig. 2 Histogram indicating the prevalence of HSV IgG, CMV IgG, EBV IgG, HSV IgM, CMV IgM, and EBV IgM between children with autoimmune thyroid disease with goiter and children with AITD, without goiter

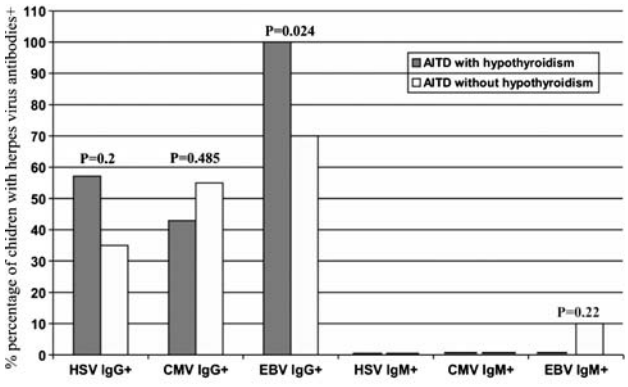

Fig. 3 Histogram indicating the prevalence of HSV IgG, CMV IgG, EBV IgG, HSV IgM, CMV IgM, and EBV IgM between children with AITD with hypothyroidism and children with AITD, without hypothyroidism

Discussion

Seroepidemiological surveys have demonstrated that HSV−1+2 [10], CMV [11], and EBV [12, 13] are highly prevalent in the human population. This prevalence has been shown to be also increased in some immunodeficient states, and in some systemic autoimmune disorders [7, 14, 15]. Therefore, we examined the prevalence of herpes virus antibodies in children with AITD and we found EBV seroprevalence increased compared to control healthy children.

It is not known whether herpes viral infection and AITD could co-exist simply by chance or the former could trigger the latter. Furthermore, from our data it is not possible to conclude whether thyroid autoantibodies or herpes virus antibodies developed first and in each case the underlying pathology could only be explained hypothetically.

Two general mechanisms could be used to explain the possible association between herpes-viral infection and induction of thyroid autoimmunity. Although the first explanation depends on evidence that virus itself may provide a counterfeit antigenic stimulus [16] that provokes autoreactive T cells (cross-reactive T-cell activation, molecular mimicry), until now no published data could verify cross-reactivity between HSV−1+2, CMV, EBV and thyroid tissue elements.

The second mechanism depends on evidence that the immune response to viruses provides a non-specific stimulus (non-specific inflammatory mechanisms) of the innate immune system that promotes activation and expansion of autoreactive T cells [17]. Continued activation of innate immunity against herpes-viral infection can also potentiate autoimmune disorders through chronic immune-mediated tissue damage and autoantigen release without the need for specific activation of autoreactive T cells by herpes-viral mimic.

Several issues cannot be explained by the mechanisms mentioned above. For example, it cannot be explained why healthy children with previous exposure to herpes virus antigens do not develop AITD. Moreover, we observed that some children with AITD are seronegative for herpes virus antibodies. It is not known whether HSV−1+2, CMV, and EBV are capable of persisting in thyroid gland after primary infection. In addition, it cannot be inferred from this study or other experimental data that this possible persistence involves a true latent state, without production of infectious virus, a low-level chronic replication or both. Based on large epidemiological studies, it seems reasonable to presume that the main underlying pathology cannot be the differences in seroprevalence, but immune's system failure to encounter the infection instead; this hypothesis concerning the relation between EBV and AITD remains bibliographically unsupported and has to be proved.

Although herpes virus DNA has been detected in postoperative thyroid tissue specimens of adults with AITD [18], no similar data for EBV in children are available. Members of the subfamily *Gammaherpesvirinae* [EBV and human herpes virus type-8 (HHV-8)] replicate mainly in lymphoblastoid cells [19] and have been implicated in several autoimmune diseases such as rheumatoid arthritis [20], pediatric systemic lupus erythematosus [21], and multiple sclerosis [22]. Autoimmunity has been suggested in such cases to be induced by a variety of diverse mechanisms, such as inducing modifications of self antigens, mimicking self-molecules, inducing polyclonal T-cell activation, altering the idiotypic network, forming immune complexes, and inducing expression of human leukocyte antigen (HLA) molecules on epithelial cells. Such mechanisms could relate EBV to AITD, but this remains to be proved.

Autoimmune thyroiditis has been estimated to occur in as many as 3.2% of boys and 5.8% of girls in a study carried out in the Athens area [23] and is the most common cause of acquired hypothyroidism in the pediatric population. Most children who are euthyroid at presentation remain euthyroid, although a percentage of patients become hypothyroid gradually within months or years [24]. We observed a high percentage of EBV seropositive children with AITD and hypothyroidism compared to controls, but more data are needed to support the role of EBV aggravation in disease progression. As discussed above, there is no direct evidence that EBV infection precedes the onset of hypothyroidism.

Our findings leave more topics unanswered than we initially tried to resolve. More studies are necessary to find out the underlying mechanisms and to support the potential role of EBV in the development of hypothyroidism.

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