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RESEARCH ARTICLE

Association between Hypothyroidism and Herpes Simplex Virus with Interleukin-23

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Abstract

Background: Hashimoto's thyroiditis and Hypothyroidism play a distinct role in the suppression and reactivation of herpes viruses. Herpes simplex virus infections have been assumed as one of the factors that may trigger autoimmune thyroid diseases; however, IL-23 has an important role in the pathogenesis of several inflammatory and autoimmune conditions. Objective: This study aimed to investigate the association between thyroid hormones in hypothyroid state and the Herpes simplex virus status in such patients; besides detecting the role of IL-23 in these cases. Materials and Methods: The present study included 65 Iraqi female hypothyroid patients with age ranged between 26-54 years and 25 healthy controls with age ranged between 25-50 years. Serum samples were collected from the study groups. Levels of thyroid hormones (TSH, T4, and T3) were determined by using automated Chemiluminescence Immunoassay (CLIA) analysis system. The detection of herpes simplex virus IgG and IL-23 levels in the serum samples were determined by an enzyme linked immunosorbent assay (ELISA) kits. Results: The current study results revealed that serum IL-23 and HSV levels are increased significantly ($P<0.001$) in the hypothyroid patients group as compared with control. There was a significant positive correlation between TSH and (IL-23, HSV); also between IL-23 and HSV ($P<0.01$). Moreover, there was a significant negative correlations ($P<0.01$) between IL-23 with (T4, T3) and between HSV with (T4, T3). Conclusion: The present study pointed to the association between thyroid hormones deficiency in Hashimoto thyroiditis cases and IL-23 levels through disruption of the patient's immune system; and with Herpes simplex viruse-1by their role in the replication.

Keywords: *Hashimoto Thyroiditis, Hypothyroidism, Herpes Simplex Virus, IL-23.*

Introduction

Thyroid hormones, Triiodothyronin [T3], Thyroxin [T4] and Thyroid Stimulating Hormone [TSH] are effective stimulators of metabolism; they play an important role in regulating energy expending and other physiological mechanisms such as development and growth [1]. Deficiency of thyroid hormones activity that results from either insufficient production or action of thyroid hormones leads to Hypothyroidism; a total decrease of metabolic processes in the human's body [2].

Autoimmune thyroid diseases (AITD) like Hashimoto thyroiditis (HT) are complex diseases in which autoimmunity act against thyroid auto-antigens, provoked by exposure to environmental factors. It's the most common cause of goiter and hypothyroidism [3]. They occur due to loss of tolerance to thyroid peroxidase auto-antigens (TPO) and thyroglobulin (Tg) which leads to the infiltration of the gland [4]. Hashimoto's thyroiditis is the most frequent autoimmune disease with genetic and environmental etiologies. Viral infections have been assumed as one of the factors that trigger autoimmune diseases. Many studies suggest that Herpes simplex virus (HSV) infections are involved in a variety of autoimmune diseases, including thyroid autoimmunity [5].

Conversely, thyroid hormone (T3) plays a distinct role in the suppression and reactivation of herpes virus type-1 (HSV-1) through its role in virus replication [6, 7].Herpes simplex virus type-1 (HSV-1) cause's diseases ranging from mild oral lesions to severe keratitis and lethal encephalitis.

HSV-1 infection is composed of four phases: acute infection, establishment of latency, maintenance of latency, and reactivation from latency [8, 9]. HSV-1 and HSV-2 symptoms occur sporadically throughout the patient's lifetime with little predictability. The infection has an alternating duality between symptomatic, lytic, and asymptomatic latent periods [10, 7]. IL-23 is a heterodimeric cytokine, belonging to the IL-12 family, it is composed of a unique p19 subunit and a common p40 subunit shared with IL-12 [11, 12]. Both cytokines are produced by dendritic cells and macrophages, namely (APC) [11].

IL-23 also stimulates dendritic cells, and possibly NK cells, to produce IFN_Y, in addition an enhanced production of IL-23 by the antigen presenting cells (APCs) may drive the immune response towards a Th17 phenotype and contribute to promote the autoimmune inflammation of thyroid gland [13, 14].IL-23 plays an important role in the pathogenesis of several inflammatory and autoimmune conditions, mostly through the expansion of the Th17 cells [14]. Despite evidence demonstrating that Th17 lymphocytes play a major role in AITD, very few studies have focused their attention on IL-23 levels in these patients [15].

Data suggest that IL-23 is involved in the development of HT disease; it acts on memory CD4+ T cells to promote their proliferation and support their differentiation into T helper1 effectors during a secondary response [13].Some researchers interpret these data to mean that IL-23 primary role is to promote sustained cell-mediated responses designated to clear persistent intracellular infections [16].The present study aimed to investigate the association between thyroid hormone levels in patients with Herpes simplex virus infection-1 in relation to IL-23 levels.

Materials &Methods

Study Population

Sixty five Iraqi female hypothyroid patients were rounded up from The National Center for Endocrine Disease and Diabetes; their ages ranged between (26- 54) years. Beside 25volunteers female subjects who were considered as healthy control, their ages and gender were matched with patients; their ages ranged between (25-50) years. Blood samples were collected from the study groups. Four milliliter of venous blood was collected from patient and control groups under aseptic conditions. The blood samples were placed in clot activator tubes, centrifuged at 3000 rpm for 5 minutes and the serum was immediately separated into small equal parts and stored in deep freeze at (-20 °C) till used. Throughout selection of patients and controls, certain exception criteria were followed to exclude unsuitable subjects (All patients and control had no other chronic or systemic diseases, and lactating female patients and pregnant women were excluded).

Thyroid hormones Level Estimation

The diagnosis of hypothyroidism was based on the clinical features and biochemical tests that depended mainly on low or normal T_3, low T_4 and elevated levels of TSH in the serum by using automated Chemiluminescence Immunoassay (CLIA) analysis system produced by Shenzhen New Industries Biomedical Engineering Co., Ltd (SNIBE). Besides measuring thyroid hormone for diagnosis, thyroid antibodies (TPO-Abs and Tg-Abs) were measured to confirm the presence of Hashimoto Thyroiditis in those hypothyroid patients.

Interleukin-23 level

Detection of IL-23 level in serum was determined by using commercially available Human Interleukin-23(IL-23) ELISA Kit from (MyBiosource- MBS265395, USA).

Herpes Simplex Virus

Detection of IgG antibodies against Herpes simplex virus type-1in human serum was performed by using commercially available ELISA Kit from (Human com.-51216, Germany).

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to identify the effect of different factors in study parameters. The variables were described by mean± standard error (SE) and tested for statistical significance by t-test**.** Correlations between all the studied variables were evaluated using Pearson's correlation coefficient(r) and linear regression analyses were used for the evaluation of data.

Results & Discussion

The present study was based on the analysis of 65 hypothyroid female patients in comparison with 25 volunteers as apparently healthy control. The results of the current study showed significant increase($p<0.001$) in serum levels of thyroid stimulating hormone (TSH) in the hypothyroid patients group (11.04 ± 0.33 µIU/ml) in comparison to with healthy control group (4.83 ± 0.26 µIU/ ml). On the other hand, the level of tri-iodothyronine (T3) and thyroxin (T4) (0.512 ± 0.017ng/ml and 3.17 ± 0.08) were decreased significantly ($P<0.001$) in patients group as compared to controls level of T3 and T4 (1.011± 0.087 and 6.06 ± 0.35 ng/ ml) respectively table-1; these results confirm the diagnosis of hypothyroidism which is characterized by elevated TSH and decreasing T3 and T4 hormone levels in comparison with control. The result could be explained by that hypothyroidism is a condition in which the thyroid gland is underactive and produces too little thyroid hormone. Nevertheless in HT autoimmune disorder the body's immune system attacks the thyroid gland and destroys thyroid cells [17]. Thyrocytes will be destroyed by cells and antibody mediated immune processes. This will cause a decrease in thyroid function and eventually lead to the clinical disorder known as hypothyroidism [18, 19].

Table 1: Levels of TSH, T3and T4 in Hypothyroidism and Control groups

The Group		TSH (µIU/ ml)	T3 (ng/ml)	T4 (ng/ml)
Hypothyroidism (N= 65)	Range	5.34-15.80	0.10-1.10	1.06-5.17
	Mean ± SE	11.04 ± 0.33	0.612 ± 0.017	2.87 ± 0.08
	Median	11.59	0.510	3.17
	SD	2.67	0.141	0.71
Control (N= 25)	Range	2.71-8.80	0.16-0.83	2.42-9.10
	Mean ± SE	4.83 ± 0.26	1.011± 0.087	6.06 ± 0.35
	Median	4.86	0.830	6.70
	SD	1.41	0.480	1.92
P-value	--	0.001**	0.001**	0.001**

These results agree with El-Shenawy *et al.* [20]; Mincer and Jialal [17] that demonstrated the most common laboratory findings for hypothyroidism and HT defined with an elevated TSH and low T4 levels coupled with increased anti-thyroid peroxidase (TPO) antibodies in most cases. The results indicated that the mean serum levels of IL-23 were increased significantly ($P<0.0001$) in hypothyroid group (112.55 ± 11.00 pg/ml) in comparison with healthy control (43.53 ± 3.96 pg/ml) as shown in table-2. On the other hand, the level of HSV-IgG in hypothyroid patient group (1.353 ± 0.032 HU/ml) increased significantly ($P<0.0001$) in comparison with control (0.344 ± 0.051 HU/ml) as shown in table-3.

Table 2: the values of IL-23 in Hypothyroidism and Controls

The Group		IL-23 (pg/ml)
Hypothyroidism (N= 65)	Range	12.82-324.43
	Mean ± SE	112.55±11.00
	Median	72.52
	SD	88.69
Control (N= 25)	Range	12.72-82.72
	Mean ± SE	43.53 ± 3.96
	Median	32.28
	SD	21.70
P-value	---	0.0001**

Table 3: The values of HSV in Hypothyroidism and Controls

The Group		HSV (HU/ml)
Hypothyroidism (N= 65)	Range	0.840-1.970
	Mean ± SE	1.353 ±0.032
	Median	1.33
	SD	0.259
Control (N= 25)	Range	0.050-1.20
	Mean ± SE	0.344 ± 0.051
	Median	0.280
	SD	0.281
P-value	---	0.0001**

Furthermore, Pearson correlation applying on serum biomarkers, demonstrated in table-4 that there is significant positive correlation between HSV level and TSH level (r=0.71, p=0.0001), while there was significant negative correlation between levels of HSV and T4, T3(r= -0.62, p=0.0001; r= -0.44, p=0.0001) respectively. On the other hand there was significant positive correlation between IL-23 level and TSH level (r= 0.25, p= 0.0102); and significant negative correlation between IL-23 and T4, T3 (r= -0.30, p= 0.0033; r= -o.24, p= 0.0170). Moreover, significant positive correlation between HSV and IL-23 levels was noticed (r= 0.26, p= 0.0099).

Table 4: Correlation coefficient between parameters study

Serum Clinical Parameters		HSV	IL-23
TSH	**r=**	0.71	0.25
	P=	0.0001**	0.0102**
T4	**r=**	-0.62	-0.30
	P=	0.0001**	0.0033**
T3	**r=**	-0.44	-0.24
	P=	0.0001**	0.0170*
IL-23	**r=**	0.26	1
	P=	0.0099**	
HSV	**r=**	1	
	P=		
(P<0.05) *, (P<0.01) **			

The significant increase in the level of IL-23 in female patients agree with Ruggeri, *et al.* [21] whom founded that serum IL-23 concentrations increased significantly in HT patients in comparison with both Grave's disease patients and healthy subjects. As well as with Konca *et al.* [22] who indicated that there is an increased concentration of IL-23 level in HT patients in comparison with control. Nevertheless, Esfahanian and his assistants showed that HT patients have high levels of IL-23 in comparison with normal controls, but with no significant differences between them [23]. The study of Zheng *et al.* found that IL-23 was highly expressed in the thyroid follicular cells (TFCs) of HT patients and could be induced by the Th1 cytokines, such as IFN-γ.

They also found that expression IL-23 receptor (IL-23 R) in TFCs was induced under inflammatory conditions [24]. Collectively, these results suggested that IL-23 might function in an autocrine pattern to stimulate TFCs, which contributed to HT pathology. So, elevated IL-23 serum level may play a role in the pathogenesis of HT, guiding T cells towards the Th17 phenotype and promoting the autoimmune inflammation of the gland [25, 24]. The significant increase of HSV IgG titer in patients' serum among controls is corresponding with Thomas *et al.* who reported the detection of HSV- in 13 out of 18 (72.22%) in their patient group. Although no data are available relating the direct effect of herpes infection on thyroid epithelial cells, but a better explain how an aberrant immune response against the thyroid gland is initiated and propagated through herpes virus infection [26].

Likewise, Varedi *et al.* [27] demonstrated *in vitro* the presence of thyroxine levels in the culture media of Vero cells with decreased virus infectivity. Remarkably, hypothyroid animals showed a significant increase (10-fold) in spleen viral load as compared to that of their euthyroid counterparts.

These data clearly show that the HSV-1 infectivity is affected by THs. However, the results in table -3 (about HSV-1) were incompatible with Mousa and Abd, [28] results for HSV 1-specific IgM,, who stated that 50% of patients were found to have a positive antibody titer whereas the other 50% of them were negative; they explained that

there was no correlation between thyroid hormones and HSV-1. On the other hand; the majority of patients (92.8%) were having a negative titer for HSV-1 specific IgG with only 7.2% of them having a positive titer for the same antibody. Thyroid hormone (T3) has been suggested to participate in the regulation of herpes virus replication during reactivation via its nuclear receptors in differentiated cells.

Clinical observations and *in vivo* experiments suggest that T3 is involved in the suppression of herpes virus replication. *In vitro*, differentiated cells, human neuron-like cells, further resisted HSV-1 replication upon addition of T3 [7]. There was a suggestion that TR/TH inhibits the HSV1 key gene expression, leading to blockade of viral replication and α expression therefore favors maintenance of latency in neurons. Therefore, transient or chronic hypothyroidism decreases the TH level and affects viral replication, gene expression, release of infectious viruses, and viral reactivation [9, 29]. There are few studies about the link between IL-23 and HSV-1, however, Broberg and his colleagues reported that the expression of IL-23 was detected in the brain of mice, even though no significant changes were found during the acute HSV-1 infection [30].

The present study pointed to the association between hypothyroidism in Hashimoto Thyroiditis with IL-23 through the disruption of the patient's immune system; and Herpes simplex viruse-1by their role in the replication.

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