THE CORRELATION BETWEEN INFLAMMATORY AND METABOLIC PARAMETERS WITH THYROID FUNCTION IN PATIENTS WITH HASHIMOTO'S THYROIDITIS: THE POTENTIAL ROLE OF INTERLEUKIN 23 (IL-23) AND VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) – 1

M. Abbasalizad Farhangi^{1,2,*}, S. Tajmiri³

¹Nutrition Research Center, Department of Community Nutrition, School of Nutrition, Tabriz University of Medical Sciences, ²Drug Applied Research Center, Tabriz University of Medical Sciences, ³Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran

Abstract

Context. Hashimoto's thyroiditis is the most common autoimmune disorder as cause of secondary hypothyroidism. The disease is associated with several metabolic disturbances and inflammatory disorders.

Objectives. The aim of the current report was to evaluate several inflammatory and metabolic predictors of Hashimoto's thyroiditis.

Subjects and Methods. In the current study, forty patients with Hashimoto's thyroiditis participated in the current study. They were aged between 20 to 50 years old. Anthropometric and nutritional measurements were assessed and biochemical factors including serum VEGF, IL-23, Nesfatin-1 and serum lipids were measured.

Results. Waist circumference was higher among patients with lower serum TSH concentrations. Serum HDL and T4 concentrations were lower and serum IL-23 was higher among patients with higher TSH concentrations. BMI, WC and serum HDL were negative predictors of serum TSH while IL-23 was positively associated with TSH concentrations. Serum lipids including TC, TG and LDL were also negatively associated with T3 and T4 concentrations.

Conclusions. According to our findings, VEGF and serum IL-23 were potent predictors of Hashimoto's thyroiditis. However, further studies are warranted to better clarify these associations and underlying pathologic mechanisms.

Key words: Hashimoto's thyroiditis, IL-23, TGF- β , VEGF, Nesfatin-1.

INTRODUCTION

In Hashimoto's thyroiditis, the accumulation of lymphocytes in the thyroid gland occurs which eventually leads to thyroid fibrosis and gradual tissue destruction (1, 2). The disease affects 2% of general

population with the growing trend of its prevalence while women are five to ten times more likely to be affected (3, 4). It is also one of the leading causes of high blood pressure, cardiovascular disease and dyslipidemia (5). The presence of thyroid auto-antibodies including anti-thyroid peroxidase (TPO-Ab) and anti-thyroglobulin (TG-Ab) antibodies lead to deterioration of thyroid cells (1, 6). Thyroid failure and goiter develop in Hashimoto's thyroiditis and leads to papillary thyroid cancer and thyroid carcinoma (7, 8).

Increased blood flow and increased vascularization in the thyroid gland as a result of enlarged thyroid gland and its excessive function in Hashimoto's thyroiditis are caused by numerous vasoactive molecules potentially responsible for these changes (9). Vascular endothelial growth factor (VEGF) as an angiogenic factor and enhancer of vascular permeability is a kind of glycoprotein (10). The receptors of VEGF are present in epithelial cells of the thyroid and are a key regulator of thyroid epithelial (10). VEGF has unique potentials compared with other angiogenic factors because of its mitogenic activity (11). It has been proposed in Hashimoto's thyroiditis, increased thyroid stimulating hormone (TSH) concentrations enhance VEGF secretion from thyroid cancerous cells (11). Several studies had introduced VEGF as a potential target for ameliorating Hashimoto's thyroiditis sign and symptoms by showing the association of VEGF with pathogenic factors of the disease. VEGF induces the migration and proliferation of endothelial cells, stimulates leukocyte adhesion to the endothelium, and initiates chemotaxis and angiogenesis (12). Another important growth factor involved in the Hashimoto's thyroiditis is TGF- β 1. Suppressed TGF- β 1 concentrations lead to reduced

*Correspondence to: Mahdich Abbasalizad Farhangi MD, Nutrition Research Center, Department of Community Nutrition, School of Nutrition, Tabriz University of Medical Sciences, Tabriz, Iran, E-mail: abbasalizad_m@yahoo.com

immunosuppressive actions and higher autoimmunity (13). Moreover, recently, it has been proposed that Th3 and Th17 cells are responsible in the pathogenesis of chronic inflammatory disease, including Hashimoto's thyroiditis, asthma, psoriasis and rheumatoid arthritis (14, 15). Interleukin (IL)-23 is also a cytokine from the IL-12 family which is produced by macrophages and dendritic cells. Increased IL-23 concentrations in Hashimoto's thyroiditis leads to prolonged and elevated differentiation and proliferation of Th17 cells and increased inflammation (16). Another important peptide, involved in thyroid dysfunction and its related metabolic disorders including dyslipidemia and disturbed glucose homeostasis is Nesfatin-1 (17, 18). Liu *et al.* (19) reported that plasma Nesfatin-1 concentration is associated with serum TSH concentrations in diabetic patients. It has been proposed that Nesfatin-1 is involved in the regulation of thyroid hormone function; this arises from the fact that several immuno-positive neurons of Nesfatin-1 are located near the Thyrotropin-Releasing Hormone (TRH) neurons in the Periventricular Nucleus (PVN) of hypothalamus (20); it has been suggested that the central Nesfatin-1 administration has several impacts on the membrane potential of TRH neurons (20). Therefore, it will be worthwhile to study the association of these peptides with thyroid hormones in patients with Hashimoto's thyroiditis which has not been studied extensively before. Therefore the current study was aimed to test this hypothesis.

PATIENTS AND METHODS

Patients

Forty physician - diagnosed Hashimoto's thyroiditis patients were recruited from endocrinology and metabolism clinics. The patients were aged between 20-50 years. As exclusion criteria the patients should not take any nutritional supplements for at least 3 months prior participation in the study and they should not have any history of thyroid abnormalities including Graves' disease, autoimmune disorders, cardiovascular disease, pregnancy or lactation. Any history of thyroid surgeries or being on dietary restrictions three months prior participation in the study led to exclusion of individuals from the study. Participants were treated with the dose of 1.7 mcg/kg/day levothyroxine sodium.

Anthropometric parameters

Weight and height were measured with a digital scale and a stadiometer respectively. Body mass

index (BMI) was calculated as weight (kg) / height (m²). Waist circumference (WC) was also measured.

Dietary assessments

A 3-day food record with two weekdays and one weekend day was used to estimate energy, macronutrients and antioxidant vitamins intake. Nutrient analysis was done by the Nutritionist IV software (N-squared Computing, Salem, OR, USA).

Biochemical measurements

Fasting blood samples were obtained and sera were extracted and stored at -70°C immediately after centrifugation until their assays. Serum TSH, total triiodothyronine (T3) and total thyroxine (T4) were analyzed by enzyme linked immunosorbent assay (ELISA Kit, Pishtaz Tebe Co., Tehran, Iran). Anti-TPO antibodies was measured by the commercially solid-phase ELISA kit (Aeskulisa, Wendelsheim, Germany) with the normal range of less than 20 IU/mL. Serum concentrations of VEGF, TGF-β, IL-23 and Nesfatin-1 was measured by commercial ELISA kits (Hangzhou East biopharm Co, USA). The assay sensitivity was 10.42, 5.11, 1.52 ng/L and 0.15 ng/mL respectively. Serum lipids were also analyzed by enzymatic colorimetric method (Pars – Azmoon, Tehran – Iran). Serum insulin was analyzed with ELISA- Monobind Insulin kit (AccuBind, CA 92630, USA) with the sensitivity of 0.75 μIU/mL and mean inter and intra assay coefficient of variations (CV) less than 9.8% and less than 8% respectively. Atherogenic Index of Plasma (AIP) was calculated as $\log \text{ TG} / \text{ HDL-C}$ (21). Homeostasis model assessment of insulin resistance (HOMA-IR) was based on fasting glucose and insulin measurements as follows: $\text{HOMA-IR} = (\text{glucose (mg/dL)} \times \text{insulin (mU/L)}) / 405$. High HOMA-IR scores denote low insulin sensitivity.

Statistical assays

Statistical analysis was performed by SPSS™ statistical software (SPSS Inc., Chicago, IL, USA). Mean ± standard deviation (SD) and frequency and percent were used to present quantitative and qualitative data respectively. The normality of data was checked with one-sample Kolmogorov-Smirnov test. The comparison of continuous variables between groups was performed by ANOVA and ANCOVA with adjusting for the confounders including age and gender. Association between variables was identified with Pearson correlation coefficient. P-values less than 0.05 were considered as statistically significant. Sample

size calculation was based on the serum concentrations of TSH by 80% power and an α -error of 5% according to the following equation:

$$n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 (S_1^2 + S_2^2)}{(\mu_1 - \mu_2)^2}$$

RESULTS

Table 1 presents the general characteristics of study participants. The percentage of females and married population was higher. WC was higher among patients with lower serum TSH concentrations (Table 2). There was no significant difference in other anthropometric and nutritional parameters between groups. Among biochemical variables (Table 3) serum HDL and T4 concentrations were lower and serum IL-23 was higher among patients with higher TSH concentrations. Table 4 presents the association between thyroid function tests with anthropometric and

biochemical variables among groups. BMI, WC and serum HDL were in negative association with serum TSH while IL-23 was positively associated with TSH concentrations. The graphic association between IL-23 and TSH is presented in Figure 1. Serum lipids including TC, TG and LDL were also negatively associated with T3 and T4 concentrations.

DISCUSSION

In the current study, we demonstrated that serum HDL was lower while serum IL-23 and VEGF were significantly higher among patients with higher TSH concentrations. Moreover, serum lipids were in negative association and IL-23 was in positive association with thyroid function tests.

Our finding was in consistent of previous study by Ruggeri *et al.* (16) reporting higher IL-23 concentrations in Hashimoto's thyroiditis and suggesting the potential role of T-helper 17 lymphocytes (Th17) and their secreting cytokine, IL-17, in the pathogenesis of Hashimoto's thyroiditis. IL-23, as member of IL-12 family conduces T-cells into the Th17 phenotype. Increased serum IL-23 concentrations and its elevated production by antigen presenting cells (APCs) in thyroid glands leads to increased autoimmune inflammation (16). In the current study, we demonstrated that serum lipids were in negative association with thyroid function tests. This is in contrast with the previous report by Tagami *et al.* indicating positive associations between serum TSH levels and lipid parameters (22). These inconsistent findings might be due to the difference between the patients' characteristics and the disease status; for example the patients in the Tagami's study were untreated patients with Hashimoto's Thyroiditis.

In the current study, serum VEGF concentrations were higher among patients with higher

Table 1. Subject characteristics

Characteristics	Value
Age (years)	34.83 $\pm$ 8.39
Female [n (%)]	34 (85)
Marital status [n (%)]	
Married	33 (82.5)
Single	7 (17.5)
Educational attainments (years)	
≤ 12	17 (42.5)
> 12	23 (57.5)
Occupation [n (%)]	
Employee	16 (40)
Non-employee	23 (57.5)
Physical activity (Met-min/day)	5.27 $\pm$ 0.47

Met, metabolic equivalent; The values are presented as Mean $\pm$ SD for continuous or number and percent for discrete variables.

Table 2. Anthropometric variables and nutritional intakes (mean $\pm$ SD) in patients according to TSH classification

Characteristics	Serum TSH concentrations			P
	< 4.5 (N=11)	4.5-6.5 (N=17)	> 6.5 (N=12)	
Weight (kg)	74.44 $\pm$ 12.89	69.16 $\pm$ 10.41	66.07 $\pm$ 11.34	0.19
BMI (kg/m ²)	27.92 $\pm$ 5.10	26.91 $\pm$ 3.90	24.43 $\pm$ 3.27	0.11
WC (cm)	91.84 $\pm$ 1.75	88.42 $\pm$ 6.49	85.12 $\pm$ 7.70	0.049
Energy (kcal/d)	2305.64 $\pm$ 324.03	2133.21 $\pm$ 312.15	2256.08 $\pm$ 373.07	0.38
Carbohydrate (%)	56.33 $\pm$ 2.88	57.81 $\pm$ 2.98	57.13 $\pm$ 4.20	0.51
Protein (%)	15.81 $\pm$ 1.86	15.21 $\pm$ 1.41	15.14 $\pm$ 1.17	0.45
Fat (%)	27.63 $\pm$ 2.50	26.85 $\pm$ 2.01	26.29 $\pm$ 2.13	0.31
Vitamin E (mg/d)	2.91 $\pm$ 0.84	3.65 $\pm$ 2.91	2.91 $\pm$ 1.21	0.24
Vitamin C (mg/d)	76.10 $\pm$ 21.89	79.51 $\pm$ 21.37	77.42 $\pm$ 17.42	0.90
Selenium (mg/d)	42.35 $\pm$ 4.73	54.21 $\pm$ 20.89	46.16 $\pm$ 20.17	0.25

BMI, body mass index; WC, waist circumference. The comparisons were performed by ANCOVA after adjusting for the confounder variables.

Table 3. Biochemical variables (mean $\pm$ SD) in patients according to TSH classification

Characteristics	Serum TSH concentrations (m IU/L)			P
	< 4.5 (N=11)	4.5-6.5 (N=17)	> 6.5 (N=12)	
FSG (mg/dL)	88.64 $\pm$ 9.14	86.42 $\pm$ 10.21	86.91 $\pm$ 5.19	0.78
TC (mg/dL)	180.35 $\pm$ 44.60	177.21 $\pm$ 41.38	187.50 $\pm$ 49.84	0.84
TG (mg/dL)	184.50 $\pm$ 54.80	183.42 $\pm$ 42.36	175.91 $\pm$ 63.83	0.90
LDL-C (mg/dL)	119.92 $\pm$ 25.23	114.78 $\pm$ 37.36	118.91 $\pm$ 33.78	0.92
HDL-C (mg/dL)	41.78 $\pm$ 6.36	43.78 $\pm$ 4.31	38.91 $\pm$ 5.19	0.03
AIP	0.63 $\pm$ 0.14	0.61 $\pm$ 0.11	0.63 $\pm$ 0.14	0.88
HOMA-IR	2.11 $\pm$ 1.64	1.71 $\pm$ 0.93	2.19 $\pm$ 1.60	0.64
T3 (mmol/L)	1.01 $\pm$ 0.34	1.02 $\pm$ 0.30	1.14 $\pm$ 0.39	0.59
T4 (mmol/L)	9.28 $\pm$ 2.76	8.30 $\pm$ 1.21	6.23 $\pm$ 3.42	0.016
Anti-TPO (IU/mL)	274.68 $\pm$ 49.13	246.08 $\pm$ 52.48	346.15 $\pm$ 54.06	0.39
VEGF (ng/L)	2835.34 $\pm$ 1734.34	2245.67 $\pm$ 1709.20	3443.57 $\pm$ 1988.35	0.01
IL-23 (ng/mL)	326.90 $\pm$ 166.84	310.47 $\pm$ 764.45	488.52 $\pm$ 165.86	0.023
TGF β (ng/mL)	1618.22 $\pm$ 738.46	1362.77 $\pm$ 764.45	1785.75 $\pm$ 765.17	0.36
Nesfatin-1(ng/mL)	34.12 $\pm$ 17.07	25.57 $\pm$ 23.53	43.12 $\pm$ 26.62	0.23

FSG, fasting serum glucose; TC, total cholesterol; TG, triglyceride; LDL-C, low density lipoprotein cholesterol; HDL-C, HDL-cholesterol; AIP, atherogenic index plasma; HOMA-IR, VEGF, vascular endothelial growth factor;. Values are presented as Mean $\pm$ SD. The comparisons were performed by ANCOVA after adjusting for the confounder variables.

Table 4. Pearson correlation coefficients of thyroid function tests with anthropometric and metabolic parameters among participants

	TSH		T3		T4	
	r	P	r	P	r	P
BMI	-0.35	0.02	-0.04	0.76	0.17	0.27
WC	-0.29	0.05	-0.19	0.23	0.03	0.84
FSG	-0.05	0.73	0.16	0.32	0.05	0.72
TC	0.08	0.59	-0.31	0.05	-0.08	0.6
TG	0.09	0.54	-0.41	0.009	-0.28	0.05
LDL	-0.08	0.59	-0.34	0.03	0.02	0.89
HDL	-0.34	0.011	0.07	0.67	0.13	0.42
VEGF	-0.008	0.96	0.019	0.91	0.03	0.83
TGF β	-0.07	0.65	0.08	0.59	0.16	0.32
IL-23	0.37	0.02	-0.03	0.84	-0.18	0.26
Nesfatin-1	-0.007	0.96	0.06	0.71	0.03	0.81

BMI, body mass index; WC, waist circumference; TC, total cholesterol; TG, triglyceride; LDL-C, low density lipoprotein cholesterol; HDL, high density lipoprotein; VEGF, vascular endothelial growth factor; TGF β , transforming growth factor β ; IL-23, interleukin 23.

Figure 1. Association between serum IL-23 and TSH concentrations in patients ($r = 0.37$, $P = 0.02$).

TSH concentrations ($P < 0.05$). In normal human thyroid cells, VEGF is minimally expressed (23); however in pathological situations, enhanced TSH concentration is a potent stimulator of VEGF secretion (11). TGF- β 1 may suppress indirectly VEGF production. TGF- β 1 deficiency impairs the VEGF mRNA expression and suppresses the tissue macrophage activation by reducing nitric oxide synthase activity (NOS); in the study by Vural P *et al.* (24), similar to our results, VEGF and TGF- β have been identified as potent predictors of Hashimoto's thyroiditis. Reduction in serum thyrotropin Receptor Anti-bodies (TR-Ab) and TSH concentrations contribute to the reductions in serum VEGF levels, intrathyroidal vascular area, and thyroid volume (25). VEGF is involved in the regulation of thyroid epithelial cells' function via its receptors localized in epithelial cells (10). Elevated

VEGF concentrations are associated with increased risk of mortality and reduced survival in thyroid cancer (26) and its strong expression is reported in thyroiditis and thyroid carcinomas (27).

In the recent findings of Rancier *et al.* study (28), the relationship between five isoforms of VEGF mRNA and their plasma levels in individuals treated for Hashimoto's thyroiditis have been assessed. The results showed that different isoforms of VEGF might have different roles in pathogenesis of autoimmune thyroid disease; their study showed decreased levels of VEGF-189 mRNA and increased levels of VEGF-165 mRNA in patients with Hashimoto's thyroiditis. The authors suggested that interventional trials are needed to better clarify the role of VEGF and its isoforms in therapeutic approaches of Hashimoto's thyroiditis.

In the current study, serum Nesfatin-1 concentrations were not significantly different between patients with different values of TSH concentrations and also its concentrations were not correlated with study anthropometric and biochemical parameters. The studies evaluating the association between serum Nesfatin-1 and thyroid abnormalities are very scarce. In one study recruited in children, reduced Nesfatin-1 concentration in children with untreated subclinical hypothyroidism was reported (19). Other study reported no association between serum Nesfatin-1 concentrations and thyroid dysfunction (29). Further studies should be applied to better clarify the possible role of Nesfatin-1 in thyroid abnormalities.

The prevalence of Hashimoto's thyroiditis is much higher among women compared with men to the extent that women are up to 10 times more likely to develop the disease (30). Although the exact underlying mechanism is unknown, one possible explanation is the key role of sex steroids as revealed by several experimental models (31). Also, it has been suggested that skewed X chromosome inactivation and fetal microchimerism are other possible reasons (31). Accordingly, the number of female participants in the current study was more than of men, however, the comparisons between all of the study parameters are adjusted for the possible confounding effects of age and gender by analysis of covariance.

In conclusion, we have demonstrated that higher serum IL-23 and VEGF could play an important role in the pathogenesis of Hashimoto's thyroiditis.

Conflict of interest

The authors declare that there is no conflict of interest.

Ethical approval and consent to participate

All participants signed a written informed consent approved by the Institutional Review Board of Tabriz University of Medical Sciences. The study design and protocol was approved by the ethical committee of Tabriz University of Medical Sciences (Project number: 93173).

Authors' contributions

MAF conceived and designed the project and wrote the manuscript and performed the statistical analysis and revised the manuscript, ST performed the sampling and data collection.

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