Article

a special issue for the scientific conference held by the Department of Chemistry- College of Education for Girls/University of Kufa,under the title: (6'th Postgraduate Students Annual Conference) (PSAC2025). which held for Tuesday, 15/4/2025.

The Role of Vascular Endothelial Growth Factor VEGF-A Levels in Thyroid Vascular changes in Patients with Hashimoto's Thyroiditis

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Abstract

Background: Tissue damage is a common and characteristic feature of autoimmune diseases. Tissue damage has been significantly associated with blood vessels, the expression of vascular endothelial growth factor (VEGF- A) it has great importance of angiogenesis in organs, tissues, and glands. aimed the current study to measure the levels of VEGF- A and its relationship to clinical manifestations of thyroid tissue damage and follicle enlargement in patients with autoimmune Hashimoto's disease (HI). Evaluated demographic factors (Age, BMI, systolic pressure, diastolic pressure) for the two study groups and functional thyroid hormone levels (TSH, T4, T3, FT4, FT3) Also assessing the levels of diagnostic antibodies to the disease (Anti-TPO, Anti-TG), The current study was conducted on 30 Iraqi female patients in Najaf Governorate in Iraq diagnosed with Hashimoto's disease and 30 healthy women. The

ages of the study groups ranged from 25 to 50 years. methods used electro chemiluminescence immune assay ECLIA, enzyme-linked immunosorbent assay Sandwich -ELISA, (Competitive- ELISA) in determining the levels of Study criteria. The findings indicated that the Hashimoto's group's BMI was higher than that of the health group., while the levels of functional hormones T3, FT3, FT4 decreased, the study also showed an increase in levels of (TSH, Anti-TPO, Anti-TG), the study showed VEGF-A levels in the serum of women in the HI group were significantly higher than those in the health group. The study revealed the involvement of VEGF-A in the tissue damage that occurs to the thyroid gland in the autoimmune disease Hashimoto's

Key words: VEGF-A, Endothelial cells, Hashimoto's disease, Autoimmune diseases, Anti-TPO

Introduction

HI is an autoimmune disease that is considered one of the most common thyroid diseases [1], the disease is directly linked to the immune system, which is considered the most important system in the body after the nervous system [2]. The main manifestations of HI are elevated levels of thyroid stimulating hormone (TSH) and abnormal levels of hormonesT4, T3, FT4, FT3 Also high levels of antibodies to Anti-TPO, Anti-TG [3,4]. Antithyroid immunity arises from a defect in the function of regulatory immune cells Treg Because it is the main responsible for maintaining the anti-inflammatory response, and in its absence, the balance turns into an inflammatory response [5]. Anti-thyroid immunity develops for several reasons, including genetic predisposition, environmental factors [6], and a deficiency of nutrients such as zinc, magnesium, selenium, and vitamin D. The most important reasons are digestive and intestinal disorders (leaky gut) and an unhealthy lifestyle [7,8] Hashimoto's disease is diagnosed by measuring elevated levels of thyroid peroxidase antibodies Anti-TPO, which is the main test for the disease [9]. Treatment is with hormone replacement thyroxine and Levo thyroxine to compensate for the severely deficient levels of thyroid

hormone [10] Thyroid dysfunction is one of the main causes of metabolic syndrome [11], which are metabolic disorders that cause the risk of cardiovascular diseases, and one of the most dangerous manifestations of these disorders is obesity [12].

Autoimmune diseases are associated with tissue damage and are often accompanied by a disruption in the regulation of blood vessels in the damaged area, whether an organ or gland [13]. VEGF-A is a cytokine that regulates the migration and division and differentiation of endothelial cells EC in vascular tissues Secreted by immune cells Macrophages, platelets and many other cells [14], the process of angiogenesis takes place under very strict and regulated mechanisms. The process usually occurs for reasons such as the growth and development of organs, lack of oxygen and lack of perfusion in the tissues, in the case of wound repair, and in cases of cancer and fibroids [15]

Materials and Methods

Iraqi women from Najaf Governorate participated in the current study, which is a case-control study., for the period from October 2024 to January 2025, From Sadr Medical City- Diabetes and Endocrinology Center, and Al-Hakeem General Hospital in Najaf Governorate after obtaining official approvals, blood samples were collected. The study groups were between the ages of 25 and 50. Thyroid functional variables (TSH, T4, and T3) were measured in the subjects' serum using the ECLIA method. The diagnostic variables Anti-TPO, Anti-TG, and FT3 levels were estimated using sandwich ELISA. Free Thyroxin FT4 levels were estimated using competitive ELISA. Sandwich -ELISA was applied to estimate the levels of VEGF-A

SPSS version 27 was used to process and analyze the data of the current study. and the Excel statistical analysis system. The mean, standard deviation, and correlation coefficient of the variables were calculated, the probability of deviation from the controls is statistically significant if p-value <0.05

Results and discussion

Explain Table 1 displays the statistical findings, which demonstrated distinct variations in the research groups' demographic attributes. Significant but negligible age differences were between the two study groups (p=0.040). In contrast to the healthy control group, the disease group's BMI, systolic and diastolic blood pressures rose (P<0.00). According to the results, the Hashimoto's disease group's blood serum had significantly higher levels of anti-TPO antibodies than the control group (P=0.001). The primary diagnostic measure for Hashimoto's disease is the Anti-TPO test. Levels of Anti-TG were elevated in the disease group P= 0.001. The patient group's serum TSH levels increased, and the study revealed significant differences in TSH levels (P<0.001), while T3, FT4, and FT3 levels decreased in comparison to the healthy control group (P=0.027, P<0.001, and P=0.042, respectively). Table 2 shows that there were no significant differences between the two study groups for T4 levels (P=0.425). According to study results, the Hashimoto's disease group had higher levels of VEGF-A than the healthy control group (P=0.003), as indicated in Table 3.

Vascular permeability factor (VPF), or VEGF. They are dynamic signaling proteins released to stimulate blood vessel growth, since evolution and in different stages of life [16], A previous study has shown that there is an association between autoimmune diseases and the expression of the general level of VEGF-A. A previous study showed high concentrations of VEGF-A in the serum of patients with rheumatoid arthritis autoimmune disease [17], and another study showed an increase in the same factor in the blood serum of children with type 1 diabetes [18]. Inflammation and oxidative stress are significant factors in Hashimoto's autoimmune thyroid diseases. which leading to elevate inflammatory cytokines such as IL-6 and TNF-α which induced high VEGF production which can stimulate vascularization in response to thyroid tissue damage, as well as in response to proceed tissue damage the thyroid tissues attempt to repair itself and since VEGF plays role in remodeling and fibrosis for this show high levels of VEGF. Additionally, the increase of inflammation status may cause localized hypoxia

which can enhance VEGF expression to elevate percentage of O2 uptake then reduced hypoxia. oxidative stress causes cell enlargement and damage to endothelial tissue in the thyroid gland [19,20]. Finally, VEGF production affected by thyroid hormones, and the disturbances of T3 and T4 levels due to Hashimoto's disease may be leading to high expression of VEGF [21,22] A recent study evaluating the relationship between thyroid disorders and treatment with VEGF-A receptor inhibitors found that the positive results for hypothyroidism were much stronger than for hyperthyroidism [23]. Since HT is characterized by persistent thyroid gland inflammation, obesity is recognized as a significant risk factor for an inflammatory response, present data showed an association between VEGF-A levels in serum of HT women and BMI, which is consistent with previous studies [24,25]

Age and VEGF-A had a weakly positive association, according to Pearson's correlation coefficient, also systolic and Diastolic pressure r=0.265, r= 0.026, r= 0.147 respectively, as shown in Table 4, VEGF-A highly positively correlated with BMI (r=0.591 at $p<0.001$). Thyroid stimulating hormone (TSH) and VEGF-A were shown to be strongly positively correlated (r=0.508 at p=0.004). Additionally, the correlation coefficients for T3 and T4 are weakly positive, FT3 is moderately positive, and F T3 is weakly negative. as indicated in Table 5. According to table 6, the study's findings indicated a poor link with thyroglobulin antibodies and a positive correlation between VEGF-A and anti-TPO. results of the current study, agreement with previous studies [26,27], demonstrated a strong favorable association with TSH levels, which is considered the most important thyroid hormone. These results prove the relationship between the expression of vascular endothelial growth factor levels and thyroid diseases in general, and in particular pathogenic thyroid tissue damage. Positive association of VEGF-A with anti- thyroperoxidase anti-TPO reveals involvement of VEGF-A expression in autoimmune thyroid disease and thyroid tissue damage in Hashimoto's disease patients.

Present study have good area under the curve for VEGF-A as illustrated in Figure:1 which were 0.818 in Hashimoto's disease, that is mean strong performance but many have some errors, also have high sensitivity (97%) and the specificity was moderate

which was (70%), as show in Table (7) for this may be consider VEGF-A as candidate diagnosis marker for Hashimoto's disease, also use as predictor of disease progression and surveillance marker of disease severity

Table 1: Demographic characteristics of the study group

Parameters	**Mean ± SD n=30 Healthy women**	**Mean ± SD n=30 Patient women**	p-value
(Age)	**33.40 ± 7.646**	**38.67 ± 8.343**	**0.040**
BMI *(*Kg/m$^{2)}$	**25.881 ± 1.794**	**30.702 ± 4.860**	**0.001**
Systolic blood pressure (mmHg)	**118.50 ± 5.538**	**137.33 ± 9.356**	**0.001**
***Dia*stolic blood pressure (mmHg)**	**78.77 ± 4.207**	**85.93 ± 7.483**	**0.001**

Table 2: levels of (Mean ± SD) Thyroid Function and Diagnostic tests in Sera of the Study Groups

Parameters	Mean ± SD n=30 Healthy women	Mean ± SD n=30 Patient women	p-value
TSH (µIU/ml)	**2.719 ± 0.928**	**6.065 ± 2.615**	**0.001**
T4 (nmol / l)	**124.500 ± 10.153**	**116.992 ± 24.309**	**0.425**
T3 (nmol / l)	**1.819 ± 0.348**	**1.392 ± 0.338**	**0.027**
FT4 (pmol / l)	**19.40 ± 4.245**	**9.388 ± 3.971**	**0.001**
FT3 (ng /l)	**75.040 ± 9.006**	**63.355 ± 10.726**	**0.042**
Anti-TPO (pg /ml)	**447.946 ± 91.780**	**829.836 ± 251.203**	**0.001**
Anti-TG (pg /ml)	**304.355 ± 70.924**	**681.237 ± 196.028**	**0.001**

Table 3: Levels of (Mean ± SD) For VEGF-A in Serum of the Study Groups

Subjects	***VEGF-A (pg /ml) (Mean ∓ SD)***	***(Minimum-Maximum)***	***p-value***

Control n = 30	27.243 ± 13.760	13.170 – 63.45	0.003
Hashimoto's n = 30	47.017 ± 19.799	29.17 – 96.85	

Table 4: Correlation of VEGF-A with Demographic Characteristics in Hashimoto's Patients

Parameters	Hashimoto Patients	
	Pearson coefficient (r)	p-value
Age	0.265	0.157
BMI	0.591	< 0.001
Systolic Pressure	0.026	0.892
Diastolic Pressure	0.147	0.438

Table 5: Relationship between Functional Hormones and VEGF-A in Patients with Hashimoto's

Parameters	Hashimoto Patients	
	Pearson coefficient r	(p-value)
TSH	0.508	0.004
T4	0.231	0.219
T3	0.111	0.560
FT4	-0.271	0.147
FT3	0.385	0.036

Table 6: Correlation of VEGF-A with Anti-body in Hashimoto's Patient

Parameters	Hashimoto Patients	
	Pearson coefficient (r)	p-value
Anti-TPO	0.401	0.028
Anti-TG	0.009	0.964

Figure:1 Curve of VEGF-A Receiver Operating Characteristic in Hashimoto disease

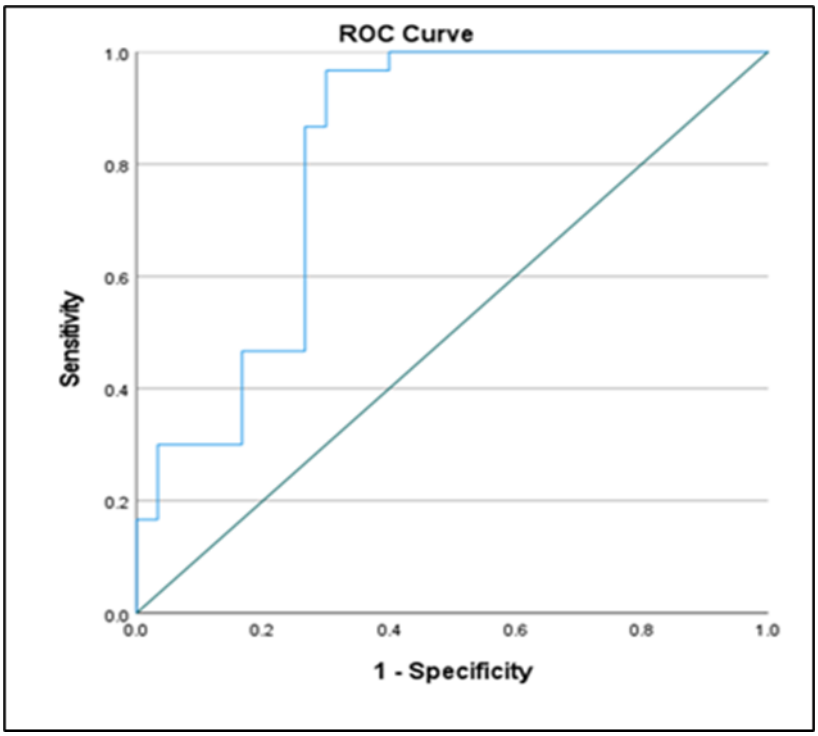

Table: 7 Analysis of VEGF-A Receiver Operating Characteristic in Hashimoto Disease

Parameter	AUC	S. E	P-value	Cutoff value	Sensitivity	Specificity	Cl (95%) of AUC
VEGF-A	0.818	0.058	0.000	29.88	0.97	0.700	0.704 – 0.931

Area Under Curve (AUC) and Standard Error (SE)

Conclusions

The chronic inflammation in HT patients may be reflects the elevated VEGF-A, in addition to enhancement of immune system in HT patients causes high expression of VEGF-A which related with disease severity, on the other side the HT patients will be

more exposure to abnormal blood vessel formation and thyroid tissue remodeling which then leading to disease development and fibrosis synthesis due to elevation of VEGF-A. Finally, need more research to explore role of VEGF-A in HT patients and may be used as a biomarker for disease activity or severity or as monitor tool to disease progression and treatment.

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