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Original Research

Biochemical imbalance of pro- and anti-inflammatory cytokines as putative risk factors for atherosclerosis in patients with Hashimoto's thyroiditis – A cross-sectional study on South Indian population

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Abstract

Background and aims: Hashimoto's thyroiditis (HT) is a commonly observed autoimmune thyroid disease and a frequent cause of hypothyroidism. The nexus between hypothyroidism and the risk of cardiovascular diseases is of current interest globally. Chronic inflammation culminating in endothelial dysfunction is perceived as a link. The association of HT with cardiovascular diseases still needs to be clearly delineated. The inflammatory markers and their association with thyroid autoimmunity in HT patients are attempted herein. **Material and methods:** Eighty (80) HT patients and an equal number of controls were enrolled in this study. The serum concentration of TSH, FT4, FT3, anti-thyroperoxidase antibodies (Anti-TPO), interleukin-6 (IL-6), interleukin-10 (IL-10) and high sensitivity – C reactive protein (hs-CRP) were estimated. **Results:** A significant increase in the levels of IL-6 and hs-CRP in HT patients ($p<0.01$) was observed as against healthy controls. IL-10 levels significantly declined in HT patients. IL-6 was found to be positively correlated with both TSH and Anti-TPO ($p<0.01$) among HT subjects. **Conclusion:** An imbalance of pro- and anti-inflammatory cytokines prevalent in HT could be a valuable tool to assess cardiovascular risk.

Keywords: chronic inflammation, interleukin-6, interleukin-10, hs-CRP, cardiovascular disease.

Background and aims

Hashimoto's thyroiditis (HT) is the commonest of hypothyroidism [1] and is typified by diffuse infiltration of the gland with sensitized T lymphocytes. This in turn leads to gradual destruction and fibrous replacement of the thyroid parenchymal tissue. Elevated serum antithyroid antibodies resulting in thyroid dysfunction to varying degrees is a cardinal feature [2]. HT has a prevalence rate of 1–4% and is more common in women and increases with age [3, 4]. HT is believed to exert obnoxious effects on the cardiovascular system attributed to chronic inflammation [5]. Inflammation is implicated at all stages of the progression of atherosclerosis. The chief inflammatory markers involved are interleukin-6 (IL-6) and high sensitivity – C reactive protein (hs-CRP) [6]. IL-6 is an important marker of endothelial dysfunction, which is regarded appropriately as an early step in the pathogenesis of atherosclerosis [5]. IL-6 indirectly promotes atherogenesis by increasing the hepatic production of hs-CRP [7]. CRP is the

major protein of the plasma, whose serum concentration can be directly related to the magnitude of inflammation [8]. IL-6 and hs-CRP are considered to be potential tools for predicting atherosclerosis and cardiovascular events [6]. Although few studies have demonstrated the involvement of IL-6 and hs-CRP in HT pathogenesis [6, 7, 9, 10], others have reported a contradictory outcome [11, 12]. While pro-inflammatory cytokines are considered to have a detrimental role in atherosclerosis [13], IL-10 synthesized by regulatory T cells (Treg) has antagonistic effects on pro-inflammatory cytokines [14, 15]. IL-10 suppresses Th1- mediated immune response through downregulation of pro-inflammatory cytokine synthesis and also inhibits antigen-presenting cells by downregulating major histocompatibility complex – II (MHC-II) [16]. Though recent studies have reported the involvement of IL-10 in HT pathogenesis, it is still unclear, as to whether IL-10 promotes or ameliorates the inflammation in HT patients [15]. A proper balance between pro-inflammatory cytokines (IL-6) and anti-inflammatory cytokines (IL-10) plays a critical role in the development of autoimmune diseases [17]. Though the role of cytokines in HT pathogenesis has been investigated by various studies, there exists significant heterogeneity as well as ambiguity in their results. Thus, this study aims to probe into the inflammatory cytokines and their association with thyroid autoimmunity. The study was conducted on patients with HT in South India.

Material and methods

Study design and patients

Inclusion criteria: This cross-sectional study was designed to include eighty (80) newly diagnosed and untreated HT patients and an equal number of patients with same age and gender-matched healthy controls who had attended the clinics at a tertiary care hospital in South India. The patients who had elevated serum TSH levels, with or without elevated fT3 and fT4, high serum anti-TPO and enlarged rubbery thyroid were categorized as Hashimoto's thyroiditis [18].

Exclusion criteria: Patients with diabetes mellitus, cardiovascular disease, pregnancy, thyroid cancer and any other autoimmune disease such as lupus erythematous and rheumatoid arthritis were excluded from the study.

All the participants were enrolled for the study following written informed consent. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institute Ethics Committee.

Anthropometric and laboratory data collection

Anthropometric measurements and laboratory assessments were carried out on all the participants, as per established protocol. Bodyweight and height were measured with a calibrated digital scale and stadiometer respectively. Waist circumference (WC) was measured midway between the lowest rib and iliac crest at umbilicus level and hip circumference (HC) was measured at the widest girth of the hip using a measuring tape in centimeters. The waist to hip ratio (WHR) was calculated by WC divided by HC. Blood pressure values were measured in the sitting position. Demographic information and family history of diabetes, hypertension, and any thyroid disorder were obtained from the participants.

Blood was collected from all the participants in the fasting state and subjected to centrifugation at 3000 rpm. The separated serum was used for the estimation of biochemical parameters. Serum concentrations of fT3, fT4, and TSH were measured using the chemiluminescence (CLIA) method adapted to Advia Centaur XP, Siemens, USA. Anti-TPO was determined by Medizym Anti-TPO Enzyme-Linked Immunosorbent Assay (ELISA) kit, Berlin, Germany. The circulating levels of IL-6 and IL-10 were estimated by Diaclone ELISA kit, France. The serum level of hs-CRP among HT patients and healthy controls was determined by Calbiotech ELISA kit, USA.

Statistical analysis

Statistical analysis was performed using licensed SPSS software 20.0 version. Data were

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presented as mean±standard deviation (SD) or median (inter-quartile range), wherever appropriate. Differences between the two groups were tested using the independent student's t-test for normally distributed variables and Mann-Whitney's U-test for non-normal distributed variables. Spearman's correlation was used to determine the correlation of TSH and Anti-TPO with other variables. p-Value <0.05 was considered to be statistically significant.

Results

A total of 160 subjects including 80 healthy controls and 80 Hashimoto's thyroiditis patients were enrolled in this study. Anthropometric parameters and thyroid profile of the two groups were compared in Table 1. Based on the data, there was a significant difference between the mean values of the Hashimoto's thyroiditis (HT) group and the control group in terms of WC, WHR, fT3, fT4, TSH, and Anti – TPO antibodies.

Table 2 shows a significant increase in serum levels of IL-6 and hs-CRP among the HT group as compared to healthy controls. Whilst, there was a significant decrease in the serum level of IL-10 in the HT group when compared to controls.

Correlation analyses of TSH and anti-TPO antibodies with other biochemical parameters were done. In correlation analysis, TSH was found to be negatively correlated with fT3 (r=-0.700, p<0.01), fT4 (r=-0.755, p<0.01) and positively correlated with Anti-TPO antibodies (Figure 1) and IL-6 (Figure 2).

Anti-TPO antibodies were positively correlated with TSH (r=0.795, p<0.01) and IL-6 (Figure 3), whereas, negatively correlated with fT3 (r=-0.591, p<0.01) and fT4 (r=-0.628, p<0.01). When IL-6 was subjected to linear regression analysis, it was found to be independently associated with Anti-TPO (Standard co-efficient beta=0.364, p<0.01).

Discussion

Hashimoto's thyroiditis is the most common cause of hypothyroidism and is suggested to be connected with higher cardiovascular risk independent of thyroid function. A chronic

Table 1: Comparison of anthropometric parameters and thyroid profile between healthy controls and Hashimoto's thyroiditis patients.

	Control (n=80)	Hashimoto's thyroiditis (n=80)	p-Value
Age (years)	29.34±7.6	31.1±7.14	0.135
Gender (M/F)	3/77	2/78	-
WC (cm)	81.6±7.03	88.5±7.4*	<0.01
HC (cm)	92.6±6.6	93.9±4.4	0.162
WHR	0.88±0.03	0.94±0.05*	<0.01
Systolic BP (mmHg)	117.4±6.3	118.5±8.6	0.356
Diastolic BP (mmHg)	78.3±3.6	78.6±8.2	0.759
fT3 (pg/ml)	2.8±0.40	2.5±0.72*	<0.01
fT4 (ng/dl)	1.03±0.12	0.8±0.3*	<0.01
TSH (mIU/ml)	2.1 (1.5–2.9)	21.4 (9.1–28.9)*	<0.01
Anti thyroperoxidase antibodies (IU/ml)	29.5 (27–39)	1060 (867.5–1400)*	<0.01

WC: waist circumference, HC: hip circumference, WHR: waist hip ratio, TSH: thyroid stimulating hormone. The data are represented as mean±SD/Median (IQR). p-Value is calculated by independent t-test for normal distributed parameters and Mann-Whitney's U-test for non-normal distributed parameters.
*p-Value <0.01 is considered statistically significant.

Table 2: Comparison of Inflammatory parameters between the healthy controls and Hashimoto's thyroiditis group.

	Control (n=80)	Hashimoto's thyroiditis (n=80)	p-Value
IL-6 (pg/ml)	33.4±8.3	122.74±20.3	<0.01
IL-10 (pg/ml)	12.16±4.03	8.5±3.04	<0.01
hs-CRP (mg/l)	1.1 (0.4–2.0)	4.1 (2.5–6.8)	<0.01

IL-6: interleukin-6, IL-10: interleukin-10, hs-CRP: high sensitivity C-reactive protein. The data are represented as mean±SD/Median (IQR). p-Value calculated by independent t-test for normal distributed parameters and Mann-Whitney's U-test for non-normal distributed parameters. *p-Value <0.01 is considered statistically significant.

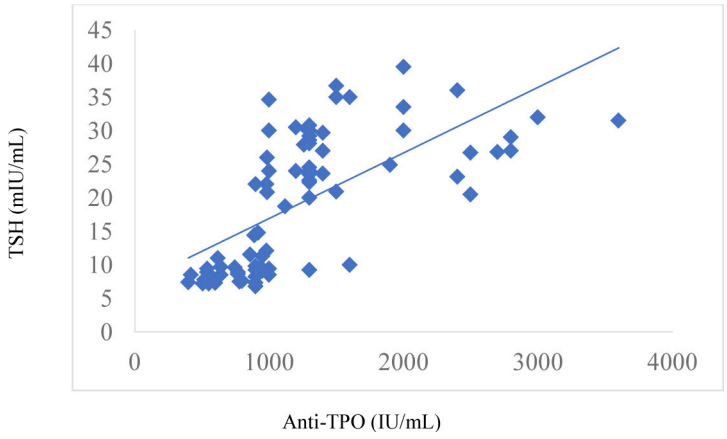

Figure 1: Correlation analysis between anti-TPO and TSH (r=0.795, p<0.01, done using Spearman's correlation analysis).

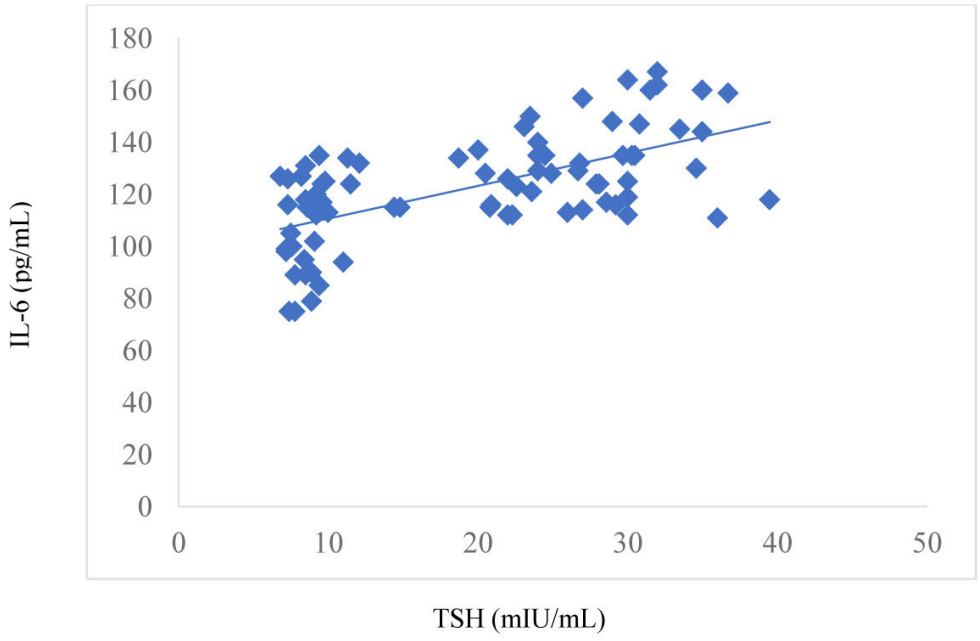

Figure 2: Correlation analysis between TSH and IL-6 (r=0.593, p<0.01, done using Spearman's correlation analysis).

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inflammation resulting from an imbalance between pro-inflammatory (IL-6) and anti-inflammatory (IL-10) cytokines could be a link [17]. Thus, in this study, we aimed to evaluate the inflammatory markers in HT subjects and their association with thyroid autoimmunity among the South Indian population.

The findings of the present study have depicted an increase in the pro-inflammatory cytokines and a decrease in the anti-inflammatory cytokines. We found an increase in serum levels of IL-6 and hs-CRP, whereas a decrease in IL-10 in HT subjects compared to healthy control. There was a strong positive correlation of IL-6 levels with TSH and Anti-TPO among HT subjects, which possibly demonstrates the role of thyroid autoimmunity.

Various studies have delineated the involvement of cytokines in the pathogenesis of HT, which are known to modify epithelium integrity and allow the infiltration of the thyroid by T cells and other immune cells. IL-6 is a marker of systemic inflammation, which has been found to be elevated in HT patients [5]. IL-6 plays an important role in the pathogenesis of atherosclerosis by promoting endothelial dysfunction, smooth cell proliferation and migration [16]. Turemen et al. [19] have observed an increase in the serum levels of IL-6, TNF-α and hs-CRP in HT patients and also a positive correlation of inflammatory markers with flow-mediated dilation which displays, low-grade chronic inflammation as a factor to promote atherosclerosis in HT. In the current study, there was a significant increase in IL-6 levels among HT subjects. This was in concordance with previous studies [19, 20]. Marchiori et al. [6] also showed similar results, in which they stated an increase in IL-6 levels in HT subjects, which started to decrease gradually after treatment. In contrast, Kammoun-Krichen et al. observed decreased IL-6 levels in the hypothyroid group and a significant increase of IL-6 in the hyperthyroid group [12]. Whereas, Mikos et al. showed no difference in IL-6 levels among hypothyroid and controls [21].

Recent advances in immunology led to the discovery of a new subset of T helper cells viz, Regulatory T cells (Treg) and IL-17 expressing T cells (Th17). Treg cells aids in the prevention of autoimmunity by the production of immunosuppressive cytokines such as IL-10 and TGF-β [22]. IL-10 exerts its anti-inflammatory effect by inhibiting the synthesis of inflammatory cytokines, such as IL-1, IL-2, granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor-alpha (TNF-a), IL-6, IL-8 and interferon-γ by lipopolysaccharide (LPS)-stimulated monocytes/macrophages. The present study demonstrated a significant decrease in the serum levels of IL-10 in HT patients in comparison to controls, which is in agreement with the study done by De La Vega et al. [23]. Though IL-10 plays a role in the prevention of thyroid autoimmune diseases, it's more evident only in the earlier stages of the disease. As the disease progresses, the effect of

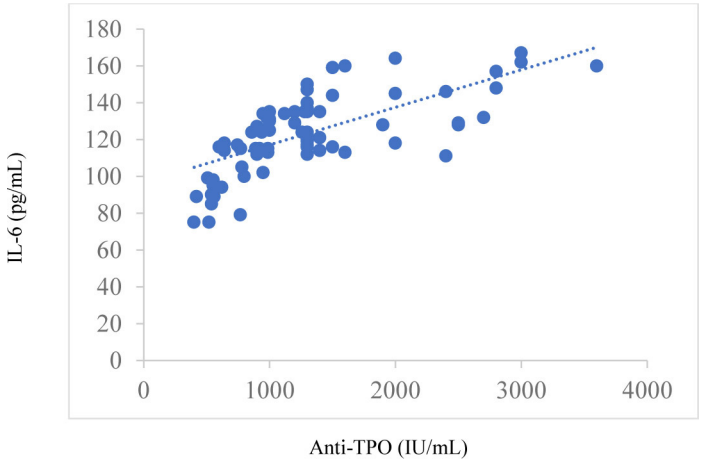

Figure 3: Correlation analysis between Anti-TPO and IL-6 (r=0.690, p<0.01, done using Spearman's correlation analysis).

pro-inflammatory cytokine overrides the effect of anti-inflammatory cytokine [23]. Increased IL-6 levels conceal the conversion of naïve CD4+ T cells to Treg cells, leading to decreased secretion of IL-10 [24]. But still, disparity prevails about the role of IL-10 in HT. While Gerenova et al. [15] observed increased IL-10 levels in HT subjects, Phenekos et al. [25] and Kristensen et al. [26] did not find any association of IL-10 with HT.

In the present study, IL-6 was found to be positively correlated with TSH and Anti-TPO. But we could not find any association of IL-10 with TSH and Anti-TPO. In a study done by Sieminska et al., IL-6 levels were higher in hypothyroid patients with positive Anti-TPO when compared to hypothyroid patients without Anti-TPO [5]. Whereas, El-Shenawy et al. did not find any correlation between IL-6 and Anti-TPO in HT subjects [7]. We found a positive correlation between Anti-TPO and TSH in our study. Though the exact role of Anti-TPO is unclear, it can be speculated that antibodies against thyroperoxidase positively correlated with TSH, might promote the release of a variety of cytokines like IL-6, TNF-α and IFN-γ [27].

As regards hs-CRP in the current study, it was significantly elevated in HT when compared to healthy controls. This is in agreement with previous studies [19, 20], where a significant elevation of hs-CRP is seen among HT patients. Interaction of IL-6 on TNF-α and IL-1 might increase serum levels of CRP among hypothyroid patients. Also, CRP clearance rate decreases due to the slower metabolic rate observed in hypothyroidism, which results in elevated serum CRP levels [28]. Taddei et al. in their study observed an increase in IL-6 and hs-CRP levels in HT subjects and concluded that chronic low-grade inflammation may cause endothelial dysfunction, promoting atherosclerosis. IL-6 may promote atherogenesis directly by endothelial-dependent mechanisms or else indirectly by stimulating hepatic production of CRP [29]. In turn, CRP hampers endothelial function by downregulating endothelial NO synthase and augmenting endothelin-1 synthesis, which is a potent vasoconstrictor [30]. Further studies, including subjects at different thyroid function levels with and without elevated anti-TPO and larger sample size, may throw more light and provide a deeper insight into the role of inflammatory cytokines and their association with cardiovascular risk in HT patients.

Conclusion

A chronic low-grade inflammation prevails in HT patients which has been attributed to a lack of proper balance between pro- and anti-inflammatory cytokines. This may lead to impending cardiovascular diseases. Therefore, assessment of inflammatory cytokines may help in attenuating the onset and progression of cardiovascular diseases in HT patients. Further elaborate studies dwelling into the molecular basis of the immunopathogenesis of HT would provide greater and renewed insights into several important *hitherto* unknown facets. This could open newer vistas for the development of novel targeted therapeutics and reinforced personalized medicine for the management of Hashimoto's thyroiditis.

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References

1. Garber, J. R., Cobin, R. H., Guarib, H., et al. (2012). Clinical practice guidelines for hypothyroidism in adults: cosponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association. *Endocr Pract*. 18:989–1028.
2. Ganesh, B. B., Bhattacharya, P., Gopisetty, A., Prabhakar, B. S. (2011). Role of cytokines in the pathogenesis and supression of thyroid autoimmunity. *J Interferon Cytokine Res*. 31:721–731.
3. Catarina, A. C., Celestino, N., JoãoSérgio, N., et al. (2017). Cardiovascular risk factors in patients with autoimmune thyroiditis. *Rev Port Endocrinol Diabetes Metab*. 12:133–141.
4. Sood, N., Nigam, J. S. (2014). Correlation of fine needle aspiration cytology findings with thyroid function test in cases of lymphocytic thyroiditis. *J Thyroid Res*. 2014:430510.

 https://doi.org/10.46389/rjd-2021-1058

5. Siemińska, L., Wojciechowska, C., Walczak, K., et al. (2015). Associations between metabolic syndrome, serum thyrotropin, and thyroid antibodies status in postmenopausal women, and the role of interleukin-6. *Endokrynol Pol*. 66(5):394–403.
6. Marchiori, R. C., Pereira, L. A., Naujorks, A. A., et al. Improvement of blood inflammatory marker levels in patients with hypothyroidism under levothyroxine treatment. *BMC Endocr Disord*. 15:32.
7. El-shenawy, S. Z., Hemi, M. H., Attia, H. (2011). Serum levels of pro-inflammatory cytokines (interleukin 6 and interleukin 15) and adiponectin in hashimoto's thyroiditis with different thyroid function states. *J Am Sci*. 7:1156–1162.
8. Sahar, H. A. Al-Hindawi. (2019). Analysis of serum Il-6 and CRP levels among autoimmune and non-autoimmune hypothyroid patients. *Indian J Public Health Res Dev*. 10:1439–1445.
9. Tuzcu, A., Bahceci, M., Gokalp, D., Tuzun, Y., Gunes, K. (2005). Subclinical hypothyroidism may be associated with elevated high-sensitive C-reactive protein (low grade inflammation) and fasting hyperinsulinemia. *Endocr J*. 52(1):89–94.
10. Savas, E., Sahin, A. Z., Aksoy, S. N., Tascan, A., Sayıner, Z. A., Ozkaya, M. (2016). Serum levels of inflammatory markers in patients with thyroid dysfunction and their association with autoimmunity status. *Int J Clin Exp Med*. 9(2):4485–4490.
11. Pearce, E. N., Bogazzi, F., Martino, E., et al. (2003). The prevalence of elevated serum C-reactive protein levels in inflammatory and non-inflammatory thyroid disease. *Thyroid* 13:643–648.
12. Kammoun-Krichen, M., Bougacha-Elleuch, N., Mnif, M., et al. (2012). IL-1β a potential factor for discriminating between thyroid carcinoma and atrophic thyroiditis. *Eur Cytokine Netw*. 23:101–106.
13. Libby, P. (2012). Inflammation in atherosclerosis. *Arterioscler Thromb Vasc Biol*. 32:2045–2051.
14. Nicolae, O. B. (2017). Immunological mechanisms implicated in the pathogenesis of chronic Urticaria and Hashimoto thyroiditis. *Iran J Allergy Asthma Immunol*. 16:358–366.
15. Julieta, G., Irena, M., Spaska, S. (2016). Impact of IL-10 and IL-12B single nucleotide polymorphisms on circulating cytokine level in development of Hashimoto's thyroiditis. *Biotechnol Biotechnol Equip*. 30:713–720.
16. de Waal, M. R., Yssel, H., de Vries, J. E. (1993). Direct effects of IL-10 on subsets of human CD4C T cell clones and resting T cells. Specific inhibition of IL-2 production and proliferation. *J Immunol*. 150:4754–4765.
17. Hackett, T. L., Holloway, R., Holgate, S. T., Warner, J. A. (2008). Dynamics of pro-inflammatory and anti-inflammatory cytokine release during acute inflammation in chronic obstructive pulmonary disease: an ex vivo study. *Respir Res*. 9:47.
18. Tina, M., Faranak, S., Koorosh, K. (2014). Insulin resistance in hypothyroid patients under Levothyroxine therapy: a comparison between those with and without thyroid autoimmunity. *J Diabet Metabol Dis*. 13:103.
19. Türemen, E. E., Çetinarslan, B., Şahin, T., Cantürk, Z., Tarkun, İ. (2011). Endothelial dysfunction and low grade chronic inflammation in subclinical hypothyroidism due to autoimmune thyroiditis. *Endocr J*. 58:349–354.
20. Khaled, A., El-Hefnawy, Hanaa, H. E. (2019). Assessment of some inflammatory markers and lipid profile as risk factors for atherosclerosis in subclinical hypothyroid patients. *Egyptian J Intern Med*. 31:115–121.
21. Mikos, H., Mikos, M., Rabska-Pietrzak, B., Marek, N. M. (2014). The clinical role of serum concentrations of selected cytokines: IL-1β, TNF-αand IL-6 in diagnosis of autoimmune thyroid disease (AITD) in children. *Autoimmunity* 47:466–472.
22. Xue, H., Yu, X., Ma, L., et al. (2015). The possible role of CD4□CD25(high)Foxp3□/CD4□IL-17A□ cell imbalance in the autoimmunity of patients with Hashimoto thyroiditis. *Endocrine* 50:665–673.
23. De La Vega, , Vilaplana, , Biro, A., Hammond, L., Bottazzo, , Mirakian, R. (1998). IL-10 expression in thyroid glands: protective or harmful role against thyroid autoimmunity? *Clin Exp Immunol*. 113:126–135.
24. Yang, X., Gao, T., Shi, R., et al. (2014). Effect of iodine excess on Th1, Th2, Th17, and Treg cell subpopulations in the thyroid of NOD.H-2h4 mice. *Biol Trace Elem Res*. 159:288–296.
25. Phenekos C, Vryonidou A, Gritzapis AD, Baxevanis CN, Goula M, Papamichail M. Th1 and Th2 serum cytokine profiles characterize patients with Hashimoto's thyroiditis (Th1) and Graves' disease (Th2). *Neuroimmunomodulation* 11:209-13, 2004.
26. Kristensen, B., Hegedüs, L., Lundy, S. K., Brimnes, M. K., Smith, T. J., Nielsen, C. H. (2015). Characterization of regulatory B cells in Graves' disease and Hashimoto's thyroiditis. *PLoS One*. 10(5):e0127949.
27. Nielsen, C. H., Brix, T. H., Leslie, R. Q., Hegedus, L. A. (2009). A role for autoantibodies in enhancement of pro-inflammatory cytokine responses to a self-antigen, thyroid peroxidase. *Clin Immunol*. 133:218–227.
28. Czarnywojtek, A., Owecki, M., Zgorzalewicz-Stachowiak, M., et al. (2014). The role of serum C-reactive protein measured by high-sensitive method in thyroid disease. *Arch Immunol Ther Exp. (Warsz)* 62:501–509.
29. Taddei, S., Caraccio, N., Virdis, A., Dardano, A., et al. (2006). Low-grade systemic inflammation causes endothelial dysfunction in patients with Hashimoto's thyroiditis. *J Clin Endocrinol Metab*. 91:5076–5082.
30. Yu, Y. T., Ho, C. T., Hsu, H. S., et al. (2013). Subclinical hypothyroidism is associated with elevated high-sensitive C-reactive protein among adult Taiwanese. *Endocrine* 44:716–722.