Association of Interlukine-27 and Interlukine-37 serum levels with Autoimmune Thyroiditis in a sample of Iraqi patients

Ali Msahir Atshan[1], Ahmed Abdul-Hassan Abbas[2], Mahmood Shakir Khudhair[3]
[1]Department of Biology, College of Science, Thi-Qar University, Thi-Qar, Iraq
[2]Department of Microbiology, College of Medicine, Al-Nahrain University, Kadhimiya, Baghdad, Iraq
[3]Internal Medicine and Endocrinology, College of Medicine, Al-Nahrain University, Baghdad, Iraq
Email: msahir21@yahoo.com

Abstract

Background: Autoimmune thyroid diseases (AITD) are caused by environmental factors that alter self-tolerance in genetically susceptible individuals.Inappropriate cytokine expression appears to play a role in the genesis of multiple diseases, including autoimmune thyroid disorders. Aim: To evaluate the role of cytokines IL-27 and IL-37 in autoimmune thyroiditis. Methods: Fifty patients with Graves' disease and fifty patients with Hashimoto's thyroditis, both newly diagnosed, were enrolled in this study, along with fifty apparently healthy individuals to serve as a control group. Patients were gathered between November 2020 and December 2021 by the Endocrinology and diabetes consultant at Al-Imamain Al-Kadhymain Medical City in Baghdad and the Specialized Center for Endocrinology and Diabetes at the Thi-Qar health directorate. In the current investigation, Enzyme-Linked Immunosorbent Assay was used. Result: Serum levels of IL-37 and IL-27 were statistically significantly different between Graves disease patients and controls (p=<0.001). while only IL- 37 was significantly different between Hashimoto's disease patients and controls p=0.005. Conclusion: Patients with Graves disease and Hashimoto's thyroiditis have elevated IL-37 serum levels, but only Graves disease has an elevated IL-27 level, implying that these cytokines may play a role in the pathogenesis of autoimmune thyroiditis.

Keywords: AITD, Cytokines, ELISA

Introduction

Autoimmunity is the mechanism through which an organism fails to recognize its own constituent parts as'self,' hence provoking an immune response against its own cells and tissues. Depending on their clinical appearance, autoimmune disorders can be classed as systemic or organ-specific. (1).

AITDs are characterized by two types of symptoms: Graves' disease (GD) and Hashimoto's thyroiditis (HT). The most frequent kind of thyroid inflammation is HT, which is distinguished by unusually increased thyroglobulin antibodies (TGAb) and thyroid peroxidase antibodies (TPOAb). In clinics, it produces hypothyroidism and manifests clinically in the opposite way as GD (2). HT is more prevalent in middle-aged women (3). GD is one of the most prevalent causes of hyperthyroidism, marked by positive thyroid-stimulating hormone (TSH) receptor antibodies (TRAb), and is an organ-specific autoimmune disease defined by excessive thyroid hormone release (TH). TRAb can bind to the TSH receptor on thyroid follicular cells, thereby stimulating the production of thyroid hormones (4).

Cytokines are a class of polypeptides produced mostly by immune cells, but also by non-immune cells; they play a crucial role in initiating and coordinating inflammatory and immunological responses (5). IL27 is a heterodimeric cytokine produced primarily by antigen-presenting cells, such as DCs and monocytes/macrophages (6). IL27 is a pleiotropic cytokine with various functions within the immune system (7). It has been demonstrated that IL-37 is a natural inhibitor of innate immunity and inflammatory reactions. The high level of IL-37 expression in inflammatory tissues limits an excessive inflammatory response (8).

This study was chosen due to the importance of cytokines as major inflammatory mediators in autoimmune illnesses, as well as the shortage of previous research on the subject in Iraq.

Materials and Methods

Current study is conducted From November 2020 to December 2021, this case-control study included 100 patients with newly diagnosed autoimmune thyroditis (50 Graves' disease and 50 Hashimoto's thyroditis) and 50 apparently as healthy controls. Each patient had 2 ml of whole venous blood drawn and collected in gel tubes for serum separation. Patients with newly diagnosed autoimmune thyroid disease are acceptable. People on therapy or with another autoimmune disease, patients with negative anti TSH Receptor antibody for Graves disease and negative anti thyroid peroxidase antibody for Hashimoto's thyroditis, patients with radioactive iodine or thyroidectomy are all excluded.

doi.org/10.31838/hiv22.02.601 Received: 29.04.22, Revised:11.07.22, Accepted: 27.08.22

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) version 26 was used for statistical analysis, categorical data were reported as counts and percentages, and the Chi-square test was used to describe the relationship between these data. The minimal statistically significant difference is less than or equal to 0.05..

Results

Graves disease patients, Hashimotoes thyroditis patients, and controls had mean ages of 35.50±12.80, 42.80±12.73 and 38.16±11.69, respectively. There were no statistically significant differences between the mean age of Graves disease patients and the control group (P=0.286), nor were there any between the mean age of Hashimotoes thyroditis patients and the control group (p=0.064), but there were statistically significant differences between the mean age of Graves disease and Hashimotoes thyroditis (p=0.004). (Figure 1)

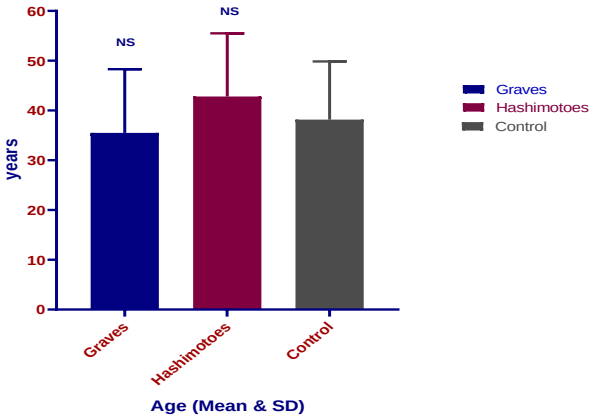

Figure 1: Mean age of patients and controls

Most Graves disease patients were between the ages of 20-40 (56.0%) while Hashimotoes thyroditis patients were >40 (60.0%). There was no statistically significant difference between Autoimmune thyroditis patients and controls across age groups (P=0.118) (Figure 2).

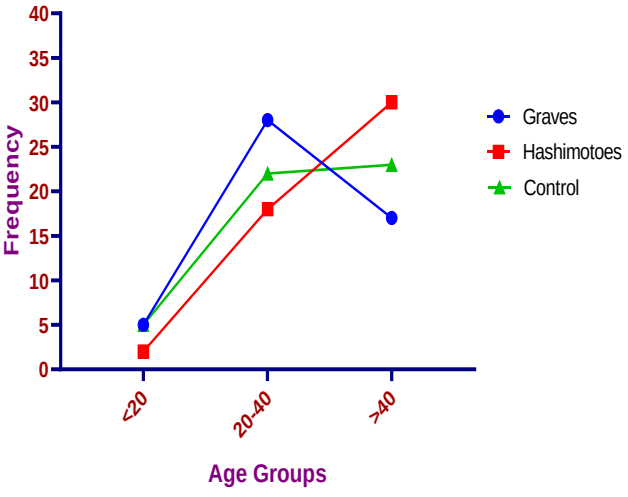

Figure 2: Distribution of age groups among patients and controls

In terms of sex distribution, 37 (74.0%) and 43 (86.0%) of Graves disease and Hashimoto's thyroditis patients were females, whereas 13 (26.0%) and 7 (14.0%) were males, respectively. There was also no statistically significant difference between the autoimmune thyroditis groups and the controls (P=0.144). (Figure 3).

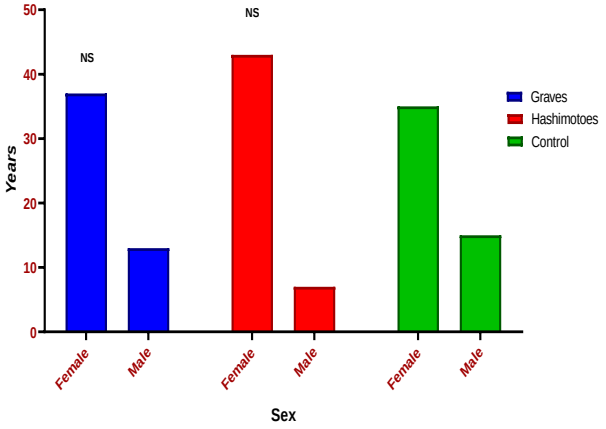

Figure 3: Distribution of Sex among patients and controls

The current study of IL-27 showed statistically significant difference between Graves' disease and control group (p=<0.001) while there was no statistically significant differences between Hashimotoes and control group (p=0.618) (Figure 4)

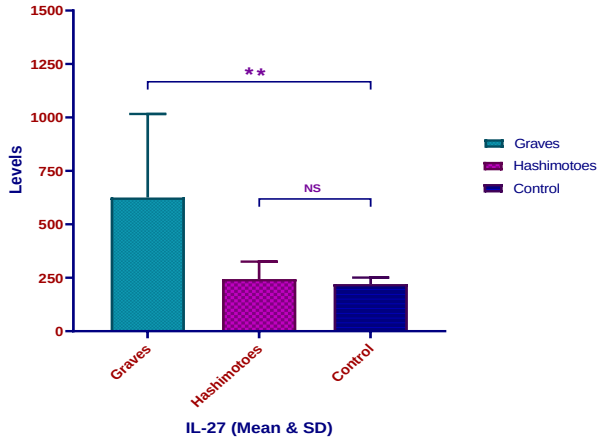

Figure 4: IL-27 levels in patients and controls

In terms of IL-37, the current study found a statistically significant difference between Graves' diseases patients and controls (p=0.001), as well as a statistically significant difference between Hashimoto's disease and controls (p=0.005) (Figure 5)

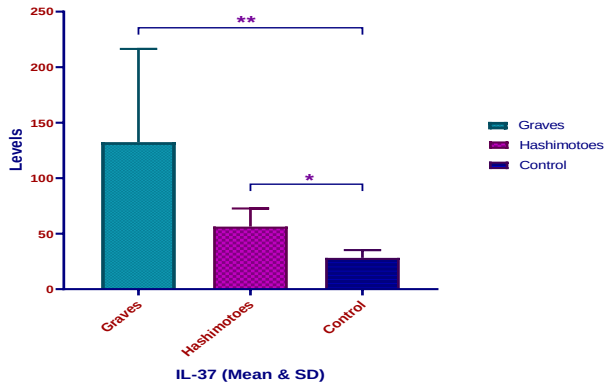

Figure 5: IL-27 levels in patients and controls

Discussion

The majority of Graves disease (GD) patients in the current study were between the ages of 20 and 40. A study by Peter Laurberg *et al.* revealed that the disease is most common in people between the ages of 20 and 50. While the majority of Hashimotoes thyroditis (HT) patients were over 40 (42.80±12.73), this finding is consistent with another study by Assaad *et al.* who found that the mean age was 40.40±12.06 years (9)

Concerning to the sex, the autoimmune thyroid diseases more occurrence in females than in males, this is probably due to differences between male and female immune systems (10), which are present in numerous animal species; in reality, males exhibit immunological suppression as opposed to females, which is associated with male sexual activity (11). Females show greater immune reactivity (12), and this enhanced immunological competency may translate into enhanced resistance to infectious and non-infectious diseases. Female immunoreactivity may predispose people to have an autoimmune disease. Females are more likely than males to suffer autoimmune disorders. Estrogen inhibits Th1-dependent disease but exacerbates Th2-dependent disease. Testosterone and estrogens have been found to reduce the severity of inflammatory diseases (13) (14), Differences in reproductive function may potentially explain sex differences in autoimmunity; parental inheritance, mitochondrial inheritance, genomic imprinting, and chromosomal inactivation may play a role as well.

Immunological alterations in females during pregnancy and their reversal in the postpartum period are decisive, despite the fact that the susceptibility of females to AITD is also documented in nulliparous women. Microchimerism is an endogenous component linked to AITD (15). The X chromosome has an abundance of genes involved in immunity (16),(17). Inactivation of the X chromosome has been hypothesized to contribute to the onset of autoimmune disease. Skewed X chromosome inactivation is reported in HT and GD, and the study revealed a considerably increased prevalence of skewed XCI in the blood cells of females with AITD (18). Compared to the X chromosome, the Y chromosome has received less attention in the context of human autoimmunity. The Y chromosome is lost with age in HT and GD, according to research (19). These findings suggest the significance of the X chromosome in autoimmunity (14).

The current study showed IL-27 is statistically significant difference in Graves' disease as compared to control group p=<0.001, but elevated without any significant association in Hashimotoes thyroditis p=0.618. IL-27 which possesses both pro-inflammatory and anti-inflammatory activities (20). There were more than one study (21–23) conclude that IL-27 has a pathogenic role in T-cell-mediated disease by inhibiting anti-inflammatory cytokines and enhancing pro-inflammatory cytokines. Weiwei He *et al.* discovered that single-nucleotide polymorphisms supported the connection between IL-27 and autoimmune thyroid disorders (7). IL-27 is capable of inducing the proliferation of naive T cells, promoting TH1 immunological responses, and contributing to the onset of some autoimmune disorders (24).

The increased serum levels of IL-27 in GD patients compared to the control group suggests that these cytokines may play a pro-inflammatory function in GD. In contrast, IL-27 has been demonstrated to possess anti-inflammatory activity in autoimmune disease; however, the precise conditions that confer IL-27's dual functional characteristics have not yet been fully elucidated (25). IL27 has been found to function as a negative immune response regulator during infection and autoimmune inflammation (26). IL-27 levels were found to be lower in several autoimmune diseases compared to healthy individuals (27). This may be because it inhibits the synthesis of pro-inflammatory cytokines by CD4 T cells, such as IFN and IL-6, and regulates the production of anti-inflammatory cytokines, including IL-4, IL-10, and transforming growth factor (TGF)-ß.

Current study disagreed with Saeed *et al.* that found reduced serum levels of IL-27 in patients with GD (28). This differences may because the all patient in this study was newly diagnosed autoimmune thyroditis or may be due to that the IL-27 have dual function pro and anti inflammatory effect. Saeed M.H. *et al.* suggested that the dual role of IL-27 may be explained by the fact that this cytokine can be released from a variety of cells, such as antigen-presenting cells, under a variety of settings based on the types of disease, cytokine network, and dominant cytokine profile (28).

Concerning serum level of IL-37, present study finding statistically significant differences between Graves' disease and controls (p=<0.001). And also there are statistically differences in Hashimotoes thyroditis with healthy controls (p=0.005). This results in agreement with Yanqun Li *et al.* who discovered that the levels of IL-37, TNF-a, IL-6, and IL-17 in PBMCs and serum were considerably higher in patients with GD than in healthy controls (p=0.0006), this study shown that the levels of serum IL-37 and IL-37 expression in PBMCs were positively and tightly linked with the levels of pro-inflammatory cytokines TNF-a, IL-6, and IL-17 in GD patients. Increased levels of IL-37 in patients with GD are connected with TNF-a, IL-6, IL-17 and disease activity, and it protects against the inflammatory effect of GD by suppressing the synthesis of proinflammatory cytokines. Therefore, IL-37 may serve as a potential research target for the pathogenesis and treatment of GD. (29), same the study explain that the therapy with recombinant IL-37 significantly decreased the secretion and repressed the expressions of pro-inflammatory cytokines TNF-a, IL-6 and IL-17 in the PBMCs of GD patients. On the other hand Marcel F Nold *et al*, found the pro-inflammatory cytokines induce the production of IL-37 (30). Therefore, it is reasonable

to predict that the pro-inflammatory cytokines in GD patients may stimulate the development of IL-37 in PBMCs, and that IL-37 may mediate a negative feedback loop to inhibit excessive pro-inflammatory cytokines in GD inflammation. This results also is in agreement with Ruggeri *et al.* who discovered that the IL-37 levels were significantly higher in HT than in controls (P=0.018). IL-37 is up-regulated in HT and may play a protective role by combating oxidative stress and inflammation by inhibiting the effects of Th1/Th17-mediated responses. (31). Ni Yan *et al.* indicated that the significance of IL37 in the immune response is well-established. Thus, SNPs in this cytokine may have a role in the susceptibility to HT and GD, where the immune response is a prominent component (32). In another study of Cui-Ping Ren *et al.* results showed that the relative expression levels of IL-37 mRNA in HT patients were 4.4 times greater than those detected in healthy controls (33). These observations suggest that IL-37 may play a protective role in chronic inflammations and autoimmune disorders. IL-37 may therefore decrease the excessive inflammatory response and also serve as a protective cytokine in HT.

References:

1. Jose J, Naidu RM, Sunil PM, Varghese SS. Pathogenesis of Autoimmune Diseases: A Short Review. Oral Maxillofac Pathol J [Internet]. 2014;5(1):434–6. Available from: https://www.researchgate.net/publication/260652505
2. Brix TH, Kyvik KO, Christensen K, Hegedüs L. Evidence for a major role of heredity in Graves' disease: A population-based study of two Danish twin cohorts. J Clin Endocrinol Metab. 2001;86(2):930–4.
3. Ajjan RA, Weetman AP. The Pathogenesis of Hashimoto's Thyroiditis: Further Developments in our Understanding. Horm Metab Res. 2015;47(10):702–10.
4. Burch HB, Cooper DS. Management of graves disease a review. JAMA - J Am Med Assoc. 2015;314(23):2544–54.
5. Ajjan RA, Weetman AP. Cytokines in thyroid autoimmunity. Autoimmunity. 2003;36(6–7):351–9.
6. Jung JY, Roberts LL, Robinson CM. The presence of interleukin-27 during monocyte-derived dendritic cell differentiation promotes improved antigen processing and stimulation of T cells. Immunology. 2015;144(4):649–60.
7. He W, Wang B, Mu K, Zhang J, Yang Y, Yao W, et al. Association of single-nucleotide polymorphisms in the IL27 gene with autoimmune thyroid diseases. Endocr Connect. 2019;173–81.
8. Pan G, Risser P, Mao W, Baldwin DT, Zhong AW, Filvaroff E, et al. IL-1H, an interleukin 1-related protein that binds IL-18 receptor/IL-1Rrp. Cytokine. 2001;13(1):1–7.
9. Assaad SN, Meheissen MA, Elsayed ET, Alnakhal SN, Salem TM. Study of Epstein–Barr virus serological profile in Egyptian patients with Hashimoto's thyroiditis: A case-control study. J Clin Transl Endocrinol [Internet]. 2020;20(March):100222.
10. Mccombe PA, Greer JM, Mccombe PA, Greer JM. Female reproductive issues in multiple sclerosis multiple sclerosis. 2012;
11. Mckean KA, Nunney L. Bateman's principle and immunity: phenotypically plastic reproductive strategies predict changes in immunological sex differences. 2005;59(7):1510–7.
12. Hewagama A, Richardson B. The genetics and epigenetics of autoimmune diseases. J Autoimmun [Internet]. 2009;33(1):3–11.
13. Lasrado N, Jia T, Massilamany C, Franco R, Illes Z, Reddy J. Mechanisms of sex hormones in autoimmunity: Focus on EAE. Biol Sex Differ. 2020;11(1):1–14.
14. Ngo ST, Steyn FJ, McCombe PA. Gender differences in autoimmune disease. Front Neuroendocrinol [Internet]. 2014;35(3):347–69.
15. Brix TH, Hegedu L. Twin studies as a model for exploring the aetiology of autoimmune thyroid disease. 2012;457–64.
16. Eleanor N. Fish. The X-files in immunity: sex-baseddifferences predispose immuneresponses. Nat Rev Immunol [Internet]. 2008;8(SEPTEmBER):737–44. Available from: www.nature.com/reviews/immunol.
17. Libert C, Dejager L, Pinheiro I. The X chromosome in immune functions: When a chromosome makes the difference. Nat Rev Immunol [Internet]. 2010;10(8):594–604.
18. Brix TH, Knudsen GPS, Kristiansen M, Kyvik KO, Ørstavik KH, Hegedüs L. High frequency of skewed x-chromosome inactivation in females with autoimmune thyroid disease: A possible explanation for the female predisposition to thyroid autoimmunity. J Clin Endocrinol Metab. 2005;90(11):5949–53.
19. Lleo A, Oertelt-Prigione S, Bianchi I, Caliari L, Finelli P, Miozzo M, et al. Y chromosome loss in male patients with primary biliary cirrhosis. J Autoimmun [Internet]. 2013;41:87–91.
20. Meka RR, Venkatesha SH, Dudics S, A B, Acharya, Moudgil KD. IL-27 increases the proliferation and effector functions of human naı ̈ve CD8 1 T lymphocytes and promotes their development into Tc1 cells. 2011;3:47–59.
21. Wang R, Han G, Wang J, Chen G, Xu R, Wang L, et al. The pathogenic role of interleukin-27 in autoimmune diabetes. Cell Mol Life Sci. 2008;65(23):3851–60.
22. Schmidt C, Giese T, Ludwig B, Mueller-Molaian I, Marth T, Zeuzem S, et al. Expression of interleukin-12-related cytokine transcripts in inflammatory bowel disease: Elevated interleukin-23p19 and interleukin-27p28 in Crohn's disease but not in ulcerative colitis. Inflamm Bowel Dis. 2005;11(1):16–23.
23. Honda K, Nakamura K, Matsui N, Takahashi M, Kitamura Y, Mizutani T, et al. T helper 1-inducing property of IL-27/WSX-1 signaling is required for the induction of experimental colitis. Inflamm Bowel Dis.

2005;11(12):1044–52.
24. Owaki T, Asakawa M, Fukai F, Mizuguchi J, Yoshimoto T, Alerts E. IL-27 Induces Th1 Differentiation via p38 MAPK/T-bet- and Intercellular Adhesion Molecule-1/LFA-1/ERK1/2-Dependent Pathways. 2015;
25. Rakeshchandra R. Meka a, Shivaprasad H. Venkatesha a, Steven Dudicsa B, Acharyaa, and Kamal D. Moudgila B. IL-27-induced modulation of autoimmunity and its therapeutic potential. Autoimmun Rev. 2016;14(12):1131–1141.
26. Wang H, Li Z, Yang B, Yu S, Wu C. IL-27 suppresses the production of IL-22 in human CD4+ T cells by inducing the expression of SOCS1. Immunol Lett [Internet]. 2013;152(2):96–103.
27. Li TT, Zhang T, Chen GM, Zhu QQ, Tao JH, Pan HF, et al. Low level of serum interleukin 27 in patients with systemic lupus erythematosus. J Investig Med. 2010;58(5):737–9.
28. Saeed MH, Kurosh K, Zahra A, Hossein DM, Davood R, Ataollahi MR. Decreased serum levels of il-27and il-35 in patients with graves disease. Arch Endocrinol Metab. 2020;64(5):521–7.
29. Li Y, Wang Z, Yu T, Chen B, Zhang J. Increased Expression of IL-37 in Patients with Graves ' Disease and Its Contribution to Suppression of Proinflammatory Cytokines Production in Peripheral Blood Mononuclear Cells. 2014;9(9):1–10.
30. Nold MF, Nold-petry CA, Zepp JA, Palmer BE, Bufler P, Dinarello CA. IL-37 is a fundamental inhibitor of innate immunity. Nat Publ Gr [Internet]. 2010;11(11):1014–22.
31. Ruggeri RM, Cristani M, Vicchio TM, Alibrandi A, Giovinazzo S, Saija A, et al. Increased serum interleukin - 37 (IL - 37) levels correlate with oxidative stress parameters in Hashimoto ' s thyroiditis. J Endocrinol Invest [Internet]. 2018;37(March 2020).
32. Yan N, Meng S, Song R, Qin Q, Wang X. Polymorphism of IL37 gene as a protective factor for autoimmune thyroid disease. 2015;(September):1–31.
33. Ping C, Li R, Feng S, Liu C, Lin C, Miao Z, et al. Potential role of IL - 37 signaling pathway in feedback regulation of autoimmune Hashimoto thyroiditis. Histochem Cell Biol [Internet]. 2019;(0123456789).