Measurement of TSHR AB and IL-6 Levels and Its Role with Thyroid Dysfunction in Graves ' disease Patients in Najaf City

Qusay Abdulmuttaleb Al-Jabery[1] and Rasha Muzahem Hatem[2]
College of Science, Al-Qadisiya University, Iraq
[1]Sci.bio.mas.21.29@qu.edu.iq
[2]rasha.albukhlate@qu.edu.iq

ABSTRACT
Graves' disease (GD) is a common autoimmune disease that accounts for the majority of cases of hyperthyroidism in the developed world [1] Autoimmune thyroid Graves' disease is one of the most common autoimmune disease (AID), affecting 13 million people and targeting women seven times as often as men. It most often affects young to middle-aged adults but can occur at all ages.[2]. Aim of The study is determination of TSHR Ab levels and IL-6 and its correlation with thyroid dysfunction in Graves' disease patients. A Case - control study was done on 100 patients with GD diagnosed at three hospitals in Najaf city in Iraq, in Al-Sadr Teaching Hospital, Al-Hakim General Hospital, and Al-Najaf Teaching Hospitals, respectively, with group of 32 healthy individual as control. Blood sample was collected from all patients and control. TSHR Ab and IL-6 levels were measured by enzyme-linked immunosorbent assay (ELISA). While TSH, T3 and T4 hormones measured by Cobas e 411 analyzer. The result shown that female (89%) more than male (11%), and the mean age (36.86) were highest than other age. This result explains that TSHR Ab was the main responsible for Grave's disease. IL-6 were in this study, There were significant levels of TSHR Ab and IL-6 compared to the healthy control group.

Keyword: Graves' disease, TSHR Ab, IL-6, TSH, T3, T4 Hormones.

1. INTRODUCTION

Graves' disease, is an autoimmune disorder of the thyroid gland that occurs in genetically predisposed individuals. It is the most frequent cause of hyperthyroidism, with a clear female predominance. It is characterized by the presence of anti-TSH receptor antibodies [3]. GD is describe by diffuse goiter, thyrotoxicosis[3]. Graves' hyperthyroidism is caused by autoantibodies to the thyroid stimulating hormone receptor (TSHR Ab) that act as agonists and induce excessive thyroid hormone secretion, releasing the thyroid gland from pituitary control. TSHR autoantibodies also underlie Graves' orbitopathy (GO) and pretibial myxedema**.** [4], [5] The incidence of GD is 5–10 times greater in women than in men, and GD is uncommon in children[6]. The most prevalent reason for hyperthyroidism is Grave's disease. It is a complex illness with genetic, environmental, and endogenous variables all playing a role . The condition is most common between the ages of 30 and 50, but it is developed at every age, and women are more frequently affected than males.[6]. Interleukin-6 (IL-6) is a significant pleiotropic proinflammatory cytokine produced in various tissues such as bone, thyroid, and blood mononuclear cells. Lakatos et al. showed that serum concentrations of IL-6 produced by blood mononuclear cells in patients with toxic nodular goiter or GD were significantly higher than in controls, suggesting that IL-6 is involved in the pathogenesis of GD[7]. IL-6 is one of the cytokines that are produced by macrophages as well as B lymphocytes. It is important for cells where it participates in their differentiation, growth, expression and activity in the body's immune response. Interleukin I is able to regulate the immune system and participate actively in the immune response. It is also associated with a variety of autoimmune diseases.[8]

2. Exclusion criteria:

All patients under treatment, as well as pregnant women, were excluded from this study because this affects the results, its evaluation, and disease-related criteria.

3. METHODOLOGY:

3.1. Patients Selection Criteria:

Patients with Graves' disease were selected on the basis of the initial diagnosis made by the physician, as well as according to the preliminary data of hormonal and serological tests, which initially indicated hyperthyroidism, with TSH levels below normal and T3 and/or T4 above normal levels. As well as some of the signs and symptoms of the patient that necessitate sending him to the laboratory for confirmation.

3.2. Ethical Approval:

In this study, all necessary consents to conduct the research, as well as the questionnaire, were obtained from all patients and healthy controls. Also, we obtained all required agreements from Al-Qadisiya University and the formal healthy institutes.

3.3. Sample collection:
A 100 patient samples and 32 healthy samples were selected as a control group for the purpose of the required hormonal and immunological tests related to Graves' disease in the current study in the city of Najaf. The age of patients and healthy control ranged between (20 – 59) years. The Study type is Case – control study. 3 ml of blood was withdrawn using a single-use disposable syringe, after sterilizing the area well with 70% ethyl alcohol. Then the blood is transferred into a free of anticoagulant tube, it is a gel tube in which 3 ml of blood is placed, then allowed to clot by leaving it for 30 to 45 minutes at room temperature. Then the gel tube is taken and centrifuged at 4000 rpm for 10 minutes to separate the serum. After that, the serum is transferred to Eppendorf tubes and placed in the freezer at -20 degrees until it is used in the immunological tests required in this study.[9]

2.4. Statistics:
Analysis of data was performed by using Statistical Package for Social Science (SPSS) system/ version 24 and excel programs. Results expressed as mean ±S.D. and p-value.

3.4. Hormonal and Immunological Assays for the Patients and Control:
Hormonal tests were carried out using the Cobas e 411 Analyzer of German origin, which included each of TSH, T3 and T4 hormones for the purpose of determining their levels[10], through which the initial diagnosis of Graves' disease is made after the symptoms and signs that make the doctor suspect the disease, and based on them, the patient is sent for further examinations to confirm.[11]. Immunological tests were also carried out using the enzyme-linked immunosorbent assay (ELISA), it is sandwich method by Sunlong kit / China, which included in our study the TSHR Ab, which is essential in diagnosing autoimmune diseases of Graves' disease in particular, and have 98% sensitivity and 100% specificity for Graves' disease[11]. As well as the interleukin-6 test, which increases its production in the case of Graves' disease.[12]

5. RESULTS:
5.1. Demographic study:
Regarding the gender, the current study showed the female / male ratio according to the statistical analysis. This ratio explained in figure (1). The percentage of the female and male for patients were (89%), (11%) respectively as in figure (1 a) with a clear predominance for women (p-value=0.000), and for control group were (84%) , (16%) respectively as in figure (1 b). So, the statistical data showed a clear superiority among female patients compared to male patients, and this ratio was represented about (8:1),

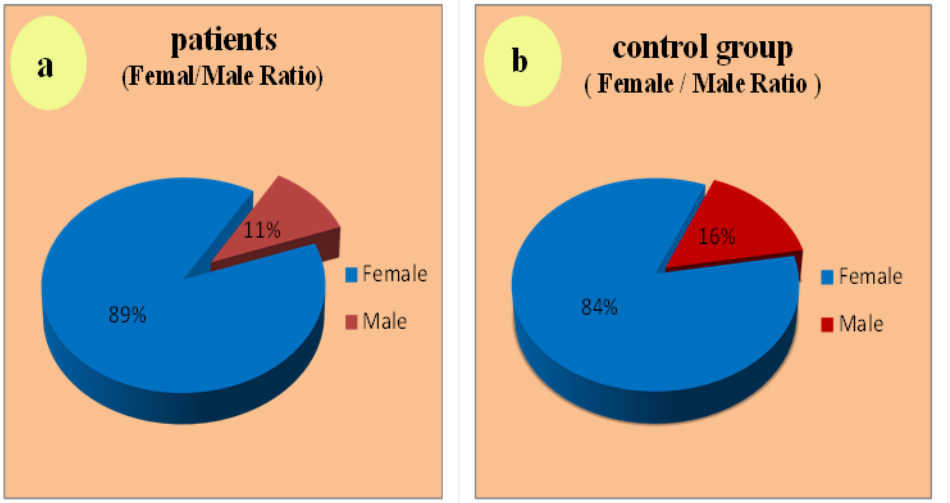

Figure (1): the ratio of female / male for the Graves' patients and control.

The gender did not show through the statistical analysis any significant for the patients compared to the control group, where the p-value was (0.25), which is similar to what was mentioned by Kwon in his study, where he mentioned that the gender did not show a significant difference between the study groups [13]. According the figure (3) which showed the percentage of female and male, so the ratio female/male in our study was 8:1, this ratio agreed with Ippolito et al. the ratio between 4:1 and 10:1 [14] and with Wiersinga, the ratio is 6:1 to 10:1 [15]. The basic immune responses differ between females and males. Studies focused on sex hormones and their role in autoimmunity, such as the hormones estrogen, progesterone, and testosterone as a primary mediator of gender differences [16].

5.2. Hormonal Assays Results:

The results were represented by hormonal tests for thyroid hormones, which were ideal and gave clear indications of hyperthyroidism, as Graves' disease was one of the most important causes, but rather the most common cause.[17]. The samples of patients that were obtained had a level of TSH below normal, while the concentrations of T3 and T4 hormones were above normal levels (high levels), and therefore it is considered the first step for the diagnosis of Graves' disease [18] as in table (1).

Table (1): thyroid stimulation hormone (TSH), the mean and SD for patients and control groups:

Marker	Patients (No. = 100)		Control (No. = 32)		P Value (Sig.)
	Mean	SD	Mean	SD	
TSH (µIU/Ml)	0.08	0.10	2.64	1.02	0.000 (HS)
T3 (nmol/L)	4.65	1.36	2.15	0.57	
T4 (nmol/L)	216.37	36.07	114.73	31.58	

The table (1) showed the arithmetic mean and standard deviation of TSH were (0.08± 0.10) for patients, while for control groups were (2.64±1.02), regarding the triiodothyronine (T3) hormone were (4.65± 1.36) for patients and (2.15±0.57) for control groups, and relating to T4 hormone in current study has mean and standard deviation were (216.37±36.07) for patients and (114.73±31.58) for control group. Table (1) shows that there was high significance (HS) in thyroid stimulation hormone TSH, T3, T4 compared with the control groups, with T-test, P value was (<0.001). The Measurement and differences in thyroid stimulation hormone (TSH) concentration between patients and control groups. According to this table, there is a high significant decrease (P <0.000) in the level of TSH, Lei et al explains the same results in his study regarding the TSH[19]. The table (1) also explains that there was a high significant (HS) in the T3 hormone compared to the control groups by means of the T-test, where the p-value was (<0.001). Graves' disease is described and initially diagnosed by an elevation of T4 and T3 hormones, as well as a decrease in thyroid-stimulating hormone (TSH) levels.[20]. Similar research indicated the significance of T3 hormone in Graves patients, as mentioned by Mohammed in his study [10]. There are also clear significant differences between the two study groups for the T3 hormone [18]. With regard to the T4 hormone, its concentrations were clearly higher than the normal levels of the patients, and therefore it showed high significance in the T test, P-Value was (<0.001). These The results related to the T4 hormone in this study found similarity with that Rashied mentioned in his study[21]. Also in Mohammeds' study found that There was a significant differences between studied groups, also p-value was (<0.001)[10]. These results of hormones (TSH,T3 and T4) that appeared in the current study were also consistent with what Al-Ghazali mentioned about the presence of high significant levels of the mentioned hormones with regard to Graves' disease compared to the control group.[22]. The feedback between TSH and T3,T4 depends on the presence of the thyroid hormone receptor, (TH receptor), which binds to the promoters of the TRH genes and the TSH subunit and regulates gene expression.[23]

5.3. Immunological Assays Results:

The immunological tests in the current study were represented by TSHR Ab and IL-6. The percentage of the TSHR antibody was present in all patients with Graves' disease (100%), in different concentrations, which is an indication of the presence of Graves' disease, that can to distinguish autoimmune (Graves' disease) or non-autoimmune thyroid disease by this antibody, Which is one of the most important tests and biomarkers for diagnosing Graves' disease [24]. There was a significant in relation to the TSHR antibody in patients compared to the control group. The mean and standard deviation of (592.33±188.10) for patients, and (213.45±43.55) for control group, as in figure 2.

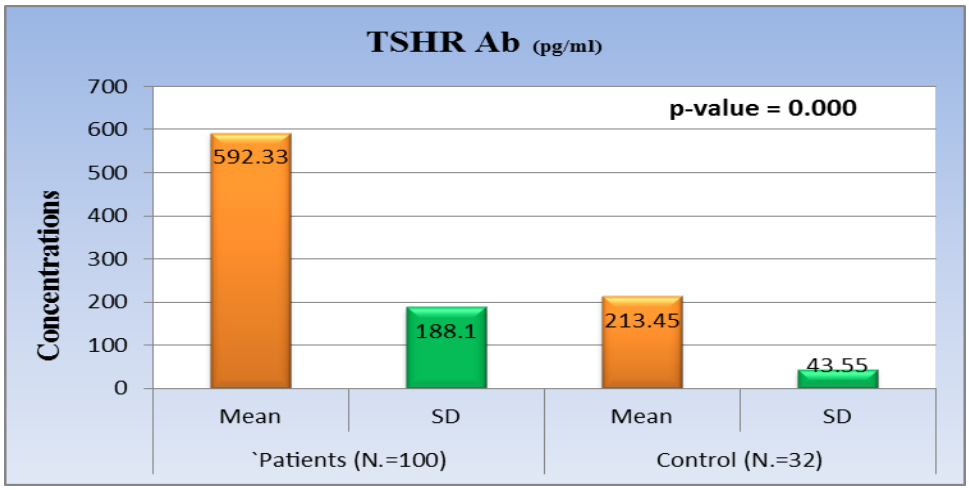

Figure (2): concentrations (mean and SD) of TSHR Ab, in patients and control.

According to this figure, and based on the results of the statistical analysis by means of the t-test, with a high significant(HS) on patients appeared, compared to the control group, where the p-value was (<0.001), which is consistent with a group of studies, such as, [9], [25] and so on. The TRAb antibody (TSHR Ab) binds to TSHR and mimics the TSH action and stimulating the production of hormones (T4, T3) continuously despite the low level of TSH (Graves' disease), and it may attack other organs such as the eye, the skin and joints because they contain TSH receptors[26]. Therefore, we chose interleukin 6 (IL-6) as an immune marker in Grave's' disease to determine its levels in patients and compare it with the control group. The result of the mean and standard deviation were as follows: (31.68±16.32) for patients and (8.9±6.7), and as shown in the following figure (Figure 3).

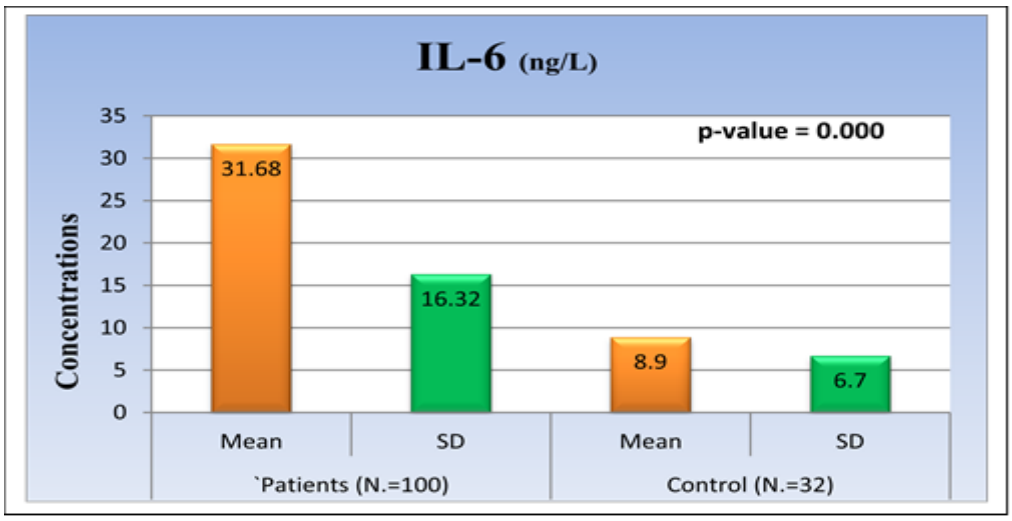

Figure (3): concentrations (mean and SD) of IL-6, in patients and control.

According to this figure, and based on the results of the statistical analysis by means of the t-test, with a high significant(HS) on patients appeared, compared to the control group, where the p-value was (<0.001), which is consistent with a group of studies, [27], [28]. IL-6 is a major inflammatory cytokine and its elevation is noted in a variety of conditions, including autoimmune diseases of the thyroid gland [29].

6. CONCLUSIONS:

Based on the aforementioned results that appeared and on the basis of their statistical analysis, the number of females outnumbered the number of males in the onset of the disease. All of these advanced criteria were of clear significance and are considered among the basic features of Craves' disease when conducting clinical examinations of the disease. It showed that there was a significant decrease in the levels of TSH concentrations for patients compared to the control group, and there were high significant differences, and the matter was similar with regard to T3 and T4 hormones. If it was high compared to the control group, considering the TSHR antibody, which is considered the main cause of the disease, and rise of

thyroid hormones, there was a clear increase in its concentration levels. In terms of interleukin 6, it is also considered as an inflammatory cytokine and important in the immune response. Its levels were also found to be high compared to the control group.

REFERENCES:

[1] R. R. Rich and T. A. Fleisher, *Clinical Immunology (Fifth Edition) Principles and Practice*. 2018.

[2] X. G. Vos, N. Smit, E. Endert, J. F. Brosschot, J. G. P. Tijssen, and W. M. Wiersinga, "Age and stress as determinants of the severity of hyperthyroidism caused by Grave's disease in newly diagnosed patients," *Eur. J. Endocrinol.*, vol. 160, no. 2, pp. 193–199, 2009, doi: 10.1530/EJE-08-0573.

[3] M. Anvari *et al.*, "Graves' disease and gene polymorphism of TNF-α, IL-2, IL-6, IL-12, and IFN-γ," *Endocrine*, vol. 37, no. 2, pp. 344–348, 2010, doi: 10.1007/s12020-010-9311-y.

[4] T. F. Davies *et al.*, "Graves' disease," *Nat. Rev. Dis. Prim.*, vol. 6, no. 1, 2020, doi: 10.1038/s41572-020-0184-y.

[5] B. S. Prabhakar, R. S. Bahn, and T. J. Smith, "Current Perspective on the Pathogenesis of Graves' Disease and Ophthalmopathy," *Endocr. Rev.*, vol. 24, no. 6, pp. 802–835, 2003, doi: 10.1210/er.2002-0020.

[6] M. Nabi, R. Noor, A. Zahid, T. Zulfiqar, A. Khalid, and S. Ri, "Grave's Disease: Pathophysiology of a Model Autoimmune Disease," *Arch. Microbiol. Immunol.*, vol. 06, no. 02, pp. 149–164, 2022, doi: 10.26502/ami.93650083.

[7] T. OMMA, Ç. YÜCEL, E. SERTOĞLU, S. N. FIRAT, C. ÇULHA, and T. ÖZGÜRTAŞ, "The role of IL-6 and osteoprotegerin in bone metabolism in patients with Graves' disease," *Turkish J. Med. Sci.*, vol. 52, no. 2, pp. 338–345, 2022, doi: 10.55730/1300-0144.5320.

[8] C. E. Lee, S. H. Choi, and J. S. Yoon, "Chemokine expression during adipogenesis and inflammation in orbital fibroblasts from patients with graves' orbitopathy," *Korean J. Ophthalmol.*, vol. 34, no. 3, pp. 192–202, 2020, doi: 10.3341/kjo.2020.0002.

[9] A. J. Mohammed Ali and M. J. M. Hamoud, "Assessment of the Correlation Between Vitamin D and T3, T4, FT3, FT4 and TSH Among Patients with Graves' Disease," *Pakistan J. Med. Heal. Sci.*, vol. 16, no. 5, pp. 1500–1502, 2022, doi: 10.53350/pjmhs221651500.

[10] H. Ali, J. Altaf Baig, and J. M. Alam, "Frequency distribution of anti-thyroid peroxidase and anti-thyroglobulin antibodies in relation to TSH, T3 and T4 levels," 2017.

[11] Z. W. Liu, L. Masterson, B. Fish, P. Jani, and K. Chatterjee, "Thyroid surgery for Graves' disease and Graves' ophthalmopathy," *Cochrane Database Syst. Rev.*, vol. 2015, no. 11, 2015, doi: 10.1002/14651858.CD010576.pub2.

[12] L. J. De Groot, "Graves ' Disease and the Manifestations of Thyrotoxicosis," *Http://Www.Endotext.Org/*, no. April, pp. 1–77, 2015, [Online]. Available: http://creativecommons.org/licenses/by-nc-nd/2.0/

[13] H. Kwon, J. kyu Kim, W. Lim, B. I. Moon, and N. S. Paik, "Increased risk of postoperative complications after total thyroidectomy with Graves' disease," *Head Neck*, vol. 41, no. 2, pp. 281–285, 2019, doi: 10.1002/hed.25484.

[14] S. Ippolito *et al.*, "Change in newly diagnosed Graves' disease phenotype between the twentieth and the twenty-first centuries: meta-analysis and meta-regression," *Journal of Endocrinological Investigation*, vol. 44, no. 8. pp. 1707–1718, 2021. doi: 10.1007/s40618-020-01479-z.

[15] W. M. Wiersinga, "Thyroid autoimmunity," *Endocr. Dev.*, vol. 26, pp. 139–157, 2014, doi: 10.1159/000363161.

[16] M. M. Klote and R. J. M. Engler, "Response to sex differences in autoimmune disease [2]," *Lupus*, vol. 16, no. 6. Ohio State University College of Medicine and Public Health, USA, p. 457, 2007. doi: 10.1177/0961203307078732.

[17] L. Smith, T. J., & Hegedüs, "Graves' disease.," *Am. Pract. Dig. Treat.*, vol. 2, no. 7, pp. 488–493, 2016, doi: 10.1056/nejmra1510030.

[18] S. A. Morshed, R. Ma, R. Latif, and T. F. Davies, "How one TSH receptor antibody induces thyrocyte proliferation while another induces apoptosis," *Journal of Autoimmunity*, vol. 47. pp. 17–24, 2013. doi: 10.1016/j.jaut.2013.07.009.

[19] Y. Lei, J. Yang, H. Li, H. Zhong, and Q. Wan, "Changes in glucose-lipid metabolism, insulin resistance, and inflammatory factors in patients with autoimmune thyroid disease," *J. Clin. Lab. Anal.*, vol. 33, no. 7, 2019, doi: 10.1002/jcla.22929.

[20] L. N. Guerra, S. Moiguer, M. Karner, M. del C. R. Del Carmen Ríos de Molina, C. M. Sreider, and J. A. Burdman, "Antioxidants in the treatment of graves disease," *IUBMB Life*, vol. 51, no. 2, pp. 105–109, 2001, doi: 10.1080/15216540152122102.

[21] R. M. Rashied, A. M. Sameen, and I. A. Shabeeb, "Comparing the values of anti-TPO and IL-17 among patients with thyroid disorders and comparing them with healthy controls in Ramadi City," *Int. J. Health Sci. (Qassim).*, pp. 2500–2508, 2022, doi: 10.53730/ijhs.v6ns5.9188.

[22] M. E. Al-Gazally, M. A. Al-shalah, and S. A. Muttaleb, "The Role of Thyrotropin Hormone Receptor Antibody (TRAb) in Distinguishing between Autoimmune and non-Autoimmune Disease.," *Med J Babylon*, vol. 10, no. 1, 2013.

[23] F. E. Chiamolera, M. I., & Wondisford, "Thyrotropin-Releasing Hormone and the feedback 2009.pdf." Endocrinology, March 2009, 150(3):1091–1096, U.S.A., 2009. doi: 10.1210/en.2008-1795 Received.

[24] R. Tozzoli, F. D'Aurizio, D. Villalta, and L. Giovanella, "Evaluation of the first fully automated immunoassay method for the measurement of stimulating TSH receptor autoantibodies in Graves' disease," *Clin. Chem. Lab. Med.*, vol. 55, no. 1, pp. 58–64, 2017, doi: 10.1515/cclm-2016-0197.

[25] J. M. Cruse, MD, PhD, and R. E. Lewis, *Atlas of Immunology, Third Edition*. 2010. [Online]. Available: https://books.google.co.uk/books/about/Atlas_of_Immunology_Third_Edition.html?id=kNI5Lk2z37sC&pgis=1

[26] B. Pandiyan, S. J. Merrill, F. Di Bari, A. Antonelli, and S. Benvenga, "A patient-specific treatment model for Graves' hyperthyroidism," *Theor. Biol. Med. Model.*, vol. 15, no. 1, pp. 1–25, 2018, doi: 10.1186/s12976-017-0073-6.

[27] L. Lv, H. Jia, H. Zhang, and Y. Hu, "IL-2, IL-6 and TGF-β in elderly patients with goiter and hyperthyroidism." pp. 4680–4686, 2017.

[28] M. Niyazoglu *et al.*, "Association of PARP-1, NF-κB, NF-κBIA and IL-6, IL-1β and TNF-α with Graves Disease and Graves Ophthalmopathy," *Gene*, vol. 547, no. 2, pp. 226–232, 2014, doi: 10.1016/j.gene.2014.06.038.

[29] F. Kutluturk, S. Yarman, F. Sarvan, and C. Kekik, "Association of Cytokine Gene Polymorphisms (IL6, IL10, TNF-α, TGF-β and IFN-γ) and Graves' Disease in Turkish Population," *Endocrine, Metab. Immune Disord. Targets*, vol. 13, no. 2, pp. 163–167, 2013, doi: 10.2174/18715303113139990001.