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Dysregulation of the TNF-A/IL-10 Axis and Microrna-146a Expression in Patients with Newly Diagnosed Graves 'disease

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Abstract: **Background:** The Graves' disease (GD) manifests as an autoimmune disturbance characterized by hyperthyroidism advertised by the presence of autoantibodies against the thyrotropin receptor. The imbalance of cytokines, mainly between pro-inflammatory TNF-α and anti-inflammatory IL-10, is of great importance in this disease's pathogenesis. MicroRNA-146a (miRNA-146a) is a key regulator of innate immunity, but it is unclear what role it plays in modulating such cytokine balance in GD. Aim: The goal of this study was to check the latest TNF-α, IL-10, and miRNA-146a levels in patients with GD, and to correlate their values with clinical hyperthyroid status. Methods: Forty-five newly diagnosed, untreated GD patients and 30 healthy controls who were matched for age and sex. The enzyme-linked immunosorbent assay (ELISA) was used to measure the levels of TNF-α and IL-10 in the serum. Quantitative real-time polymerase chain reaction (RT-PCR) was used to find out how much miRNA-146a was expressed in peripheral blood. Results: The TNF-α/IL-10 ratio was significantly higher in GD patients due to significantly higher blood levels of TNF-α and significantly lower levels of IL-10 as compared to controls. In GD patients, the expression of miRNA-146a was significantly elevated. There was a significant positive connection found between serum TNF-α levels and miRNA-146a expression. In contrast, there was a negative correlation between serum IL-10 levels and miRNA-146a expression. Additionally, there were positive relationships between blood free triiodothyronine (FT3) and thyrotropin receptor antibodies (TRAbs) and both miRNA-146a and TNF-α levels Conclusion: The results show that miRNA-146a overexpression is linked to a pro-inflammatory state in GD, which is typified by increased TNF-α and decreased IL-10. In Graves' disease, miRNA-146a might be a new biomarker and a possible target for treatment that modifies the immune system.

Keywords: TNF-α, IL10, MiRNA146a, hyperthyroidism patients

1. Introduction

About 1- 2% of people have Graves' disease (GD), which is the most common cause of hyperthyroidism [1]. The clinical signs of thyrotoxicosis are caused by autoantibodies against the thyroid-stimulating hormone receptor (TSHR), which stimulate the production and secretion of thyroid hormones in this organ-specific autoimmune disease [2]. A cytokine-driven inflammatory response is the end result of a complex interaction between T and B lymphocytes in the immunopathogenesis of GD, even though the presence of TRAbs is diagnostic [3].

Depending on the cytokine milieu, the disease phenotype in autoimmune diseases changes [4]. Tumor Necrosis Factor-alpha (TNF-α) is a pro-inflammatory cytokine that has been implicated in the initiation and perpetuation of the autoimmune response in GD [5]. In contrast, Interleukin-10 (IL-10) is a vital anti-inflammatory cytokine that inhibits immune activation and supports tolerance to an antigen [6]. It is believed that an imbalance in the TNF-α/IL-10 ratio leans towards a pro-inflammatory state which favors autoimmunity [7].

Micro non-coding RNAs known as microRNAs (miRNAs) control the expression of genes post-transcriptionally [8]. An important modulator of both innate and adaptive immune responses is MiRNA-146a [9]. As a negative feedback regulator, it targets important signaling molecules such as TRAF6 and IRAK1 to moderate the NF-κB signaling pathway [10]. Numerous autoimmune diseases, such as systemic lupus erythematosus and rheumatoid arthritis, have been linked to its dysregulation [11].

Given the central role of immune dysregulation in GD, this study aims to demonstrate the miRNA146a expression levels and its relationship with the critical cytokines TNF-α and IL-10 in patients with newly diagnosed GD. We hypothesize that miRNA-146a is dysregulated in GD and contributes to the observed imbalance between pro- and anti-inflammatory cytokines [12].

2. Methods

2.1. Study Participants: From the endocrinology department of Al-Sadr Medical City in Najaf, 45 patients with recently diagnosed, untreated GD were enlisted. Established clinical and biochemical criteria—thyrotoxicosis symptoms, suppressed TSH, elevated T4 and/or T3, and positive TRAbs—were used to make the diagnosis [13]. The control group consisted of thirty healthy volunteers who were matched for age and sex and had no family or personal history of autoimmune thyroid disease. Those taking immunomodulatory medications, having active infections, or having other autoimmune diseases were not included. All participants gave their informed consent, and the study was approved by the institutional ethics committee.

2.2. Sample Collection: Each participant had 10 milliliters of venous blood collected. Five milliliters were gathered in a clot activator tube, centrifuged, and separated for ELISA in order to separate the serum. Density gradient centrifuge was used to isolate the PBMCs from the residual 5 ml, which was collected in EDTA tubes, following a standard protocol [14].

2.3. RT-PCR and RNA Extraction for miRNA-146a: According the manufacturer's instructions, the Trizol reagent (Biotech, IR) was used to purify the miRNA in serum samples. One ml of Trizol reagent was added to 0.5 ml of blood serum to homogenize the samples, which were then stored at -20 C until needed. Using a Nanodrop spectrophotometer (Shimadzu, Japan), the ratio of (A260/A280) was used to measure the RNA concentration and approve the purification of each sample. Then, using an effective method for miRNA analysis, the Taqman® MicroRNA Reverse Transcription Kit From Applied Biosystems used, the extracted miRNA of each sample was reverse-transcribed into cDNA [15].

Real time PCR was performed by following the conditions: (Ampliqon Taq DNA Polymerase being 1 cycle for 5 min at 95°C, Denaturation of double-stranded cDNA being 40 cycle for 20 sec. at 95 °C and Primer annealing and extension being 45 cycle for 2min at60 ° C)

The expression of miRNAs is assessed by the relative quantitative method. The fold changes in the patient's miRNA expression level compared with the control have been calculated using the comparative Ct formula (ΔΔCt) [16]. In this study, the reference gene is U6, and the Ct value of target miRNAs is compared to a control [17].

2.4. ELISA for TNF-α and IL-10: Serum levels of TNF-α and IL-10 were measured using high-sensitivity ELISA kits from Sunlog Biotech, China, following the manufacturer's instructions. All samples were tested in duplicate, and the average absorbance was used to determine concentrations based on the standard curve, a standard approach for cytokine quantification [18].

2.5. Statistical Analysis: IBM SPSS for Windows, version 24, the Statistical program, has been utilized. The results are expressed as mean ± SD. The student's t test is used to estimate the statistical difference; $P \leq 0.05$ is considered significant, and $P<0.01$ is considered highly significant. Two-tailed Spearman's correlation has been used to analyze the correlation coefficient (r) between various variables [19]. To differentiate between patients and control receiver operating characteristic (ROC) curve analysis has been used [20].

3. Results

3.1. Cytokine Levels: GD patients' serum TNF-α concentration was significantly higher (28.5 ± 1.0214 pg/mL) than that of healthy controls (10.2 ± 0.2928 pg/mL; $p < 0.001$). In contrast, blood IL-10 levels in GD patients were significantly lower (5.1 ± 0.9641 pg/mL) than in controls (12.8 ± 1.2928 pg/mL; $p < 0.001$). This led to a significantly higher TNF-α/IL-10 ratio in the GD group (5.9 ± 2.8 vs. 0.8 ± 0.3, $p < 0.001$).

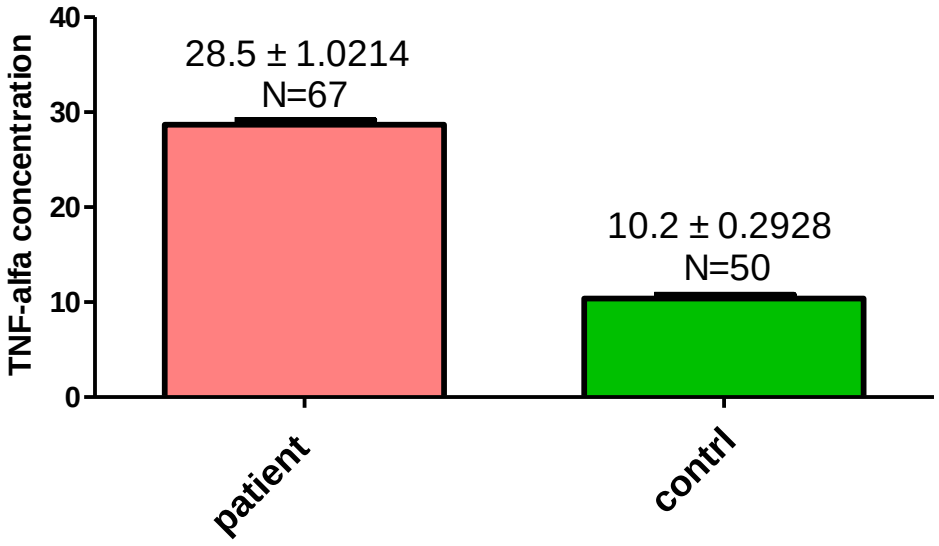

Figure 1 show TNF-α concentration

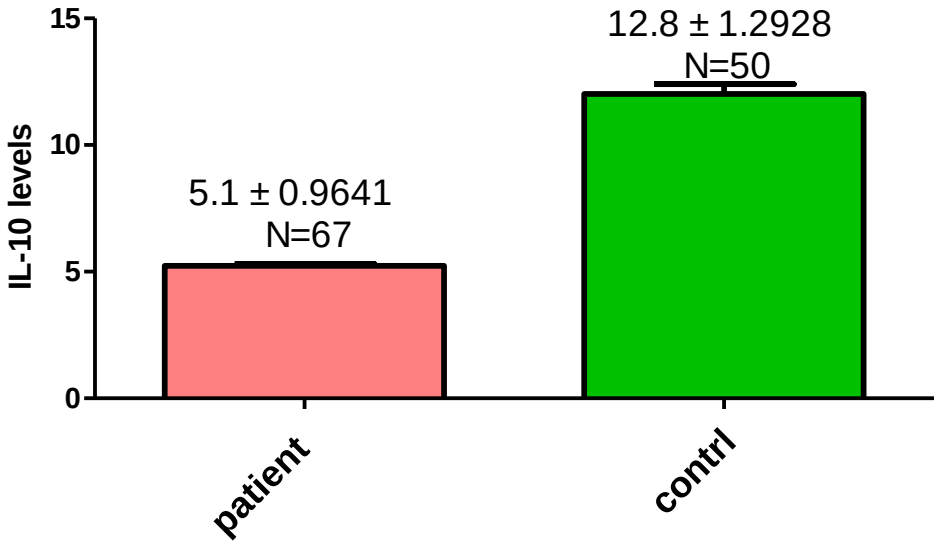

Figure 2 show blood IL-10 levels

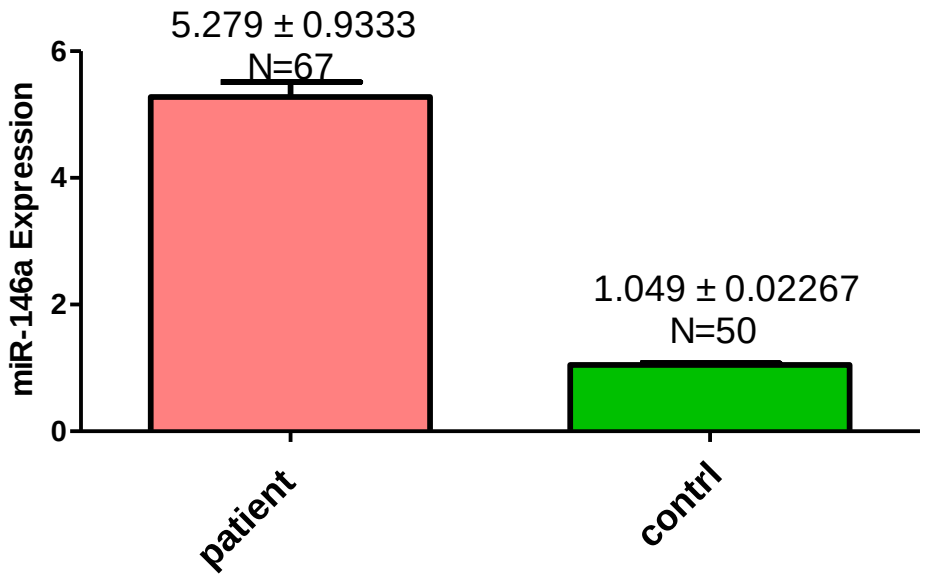

Figure 3 show Expression of miR-146a

3.2. Expression of miR-146a: GD patients' PBMCs exhibited a 4.8-fold higher relative expression of miR-146a than the control group ($p < 0.001$). miR-146a Expression

3.3. Analysis of Correlation:

Serum TNF-α levels and miR-146a expression were shown to be strongly positively correlated ($r = 0.752$, $p < 0.001$).

Serum IL-10 levels and miR-146a expression were shown to be significantly correlated negatively ($r = -0.613$, $p < 0.001$).

Clinical indicators such as FT3 (miR-146a: r=0.521, p<0.01; TNF-α: r=0.487, p<0.01) and TRAb titers (miR-146a: r=0.568, p<0.001; TNF-α: r=0.502, p<0.01) were positively correlated with both miR-146a expression and TNF-α levels.

Table (1) show Correlation in GD patients

Title	R value	P value
TNF-α levels and miR-146a expression	**0.752**	**p < 0.001**
IL-10 levels and miR-146a expression	**-0.613**	**p < 0.001**

3.4. Correlation Analysis: There was a strong positive correlation between serum TNF-α levels and miR-146a expression ($r = 0.752$, $p < 0.001$).

There was a significant negative correlation between serum IL-10 levels and miR-146a expression ($r = -0.613$, $p < 0.001$).

Both miR-146a expression and TNF-α levels were positively connected with clinical indicators like FT3 (miR-146a: r=0.521, p<0.01; TNF-α: r=0.487, p<0.01) and TRAb titers (miR-146a: r=0.568, p<0.001; TNF-α: r=0.502, p<0.01).

4. Discussion : According to this study, patients with GD demonstrate significant dysregulation of the immune regulatory pathway, which is highlighted by a shift towards a highly pro-inflammatory state, concurrent upregulation of miRNA-146a and TNF-α, and downregulation of IL-10.

The elevated levels of TNF-α in the blood are consistent with its known function of inducing inflammation and aiding the thyroid gland's autoimmune attack [5]. A reduced regulatory immune response that is unable to effectively balance the inflammatory response is suggested by the significantly lower IL-10 levels [21]. Autoimmunity persists because of the permissive environment created by this imbalanced TNF-α/IL-10 ratio [7].

The study's most important new discovery is the close relationship between this cytokine imbalance and miRNA-146a expression. NF-κB activation can be used to interpret the positive connection with TNF-α [10]. TNF-α and miRNA-146a transcription are both influenced by NF-κB activation, which is probably continuously stimulated by the autoimmune process in GD [22]. Therefore, persistent inflammation may be the cause of elevated miRNA-146a expression.

The negative correlation with IL-10, however, indicates to a more active function of miRNA-146a in immune dysregulation. Despite miRNA-146a's intended function as a feedback inhibitor, long-term overexpression of the gene may be harmful [12]. Excessive miRNA-146a may inhibit pathways essential for the differentiation or operation of regulatory cells (Tregs/Bregs) that produce IL-10. On the other hand, it might specifically target transcripts that are involved in the signaling or production of IL-10. This increases the illness by effectively blocking a vital anti-inflammatory pathway. MiRNA-146a has been suggested as a key player in the immune pathology of GD because of its dual function, which involves both inducing inflammation and possibly suppressing regulatory responses [9].

Both miRNA-146a and TNF-α showing positive correlations with T3 and TRAbs further increases their clinical relevance, indicating that these immune markers are associated with the severity of hyperthyroidism and the autoimmune process involved.

References

1. Taylor, P. N., Albrecht, D., Scholz, A., Gutierrez-Buey, G., Lazarus, J. H., Dayan, C. M., & Okosieme, O. E. (2018). Global epidemiology of hyperthyroidism and hypothyroidism. Nature Reviews Endocrinology, 14(5), 301-316. Davies, T. F., Andersen, S., Latif, R., Nagayama, Y., Barbesino, G., Brito, M., & Kahaly, G. J. (2020). Graves' disease. Nature reviews Disease primers, 6(1), 52.
2. Davies, T. F., Andersen, S., Latif, R., Nagayama, Y., Barbesino, G., Brito, M., & Kahaly, G. J. (2020). Graves' disease. Nature reviews Disease primers, 6(1), 52. Rapoport, B., & McLachlan, S. M. (2023). Graves' hyperthyroidism: The role of the thyrotropin receptor autoantibody in the pathogenesis of the disease. Nature Reviews Endocrinology, 19(7), 401–414.
3. Wiersinga, W. M. (2019). Graves' Disease: Can It Be Cured? Endocrinology and Metabolism, 34(1), 29–38.
4. Ferrari, S. M., Ragusa, F., Elia, G., Paparo, S. R., Mazzi, V., Baldini, E. & Fallahi, P. (2021). Precision medicine in autoimmune thyroiditis and hypothyroidism. Frontiers in pharmacology, 12, 750380.
5. Nagata, K., & Nishiyama, C. (2021). IL-10 in mast cell-mediated immune responses: anti-inflammatory and proinflammatory roles. International journal of molecular sciences, 22(9), 4972.
6. Ganesh, B. B., Bhattacharya, P., Gopisetty, A., & Prabhakar, B. S. (2011). Role of cytokines in the pathogenesis and suppression of thyroid autoimmunity. Journal of Interferon & Cytokine Research, 31(10), 721-731.
7. O'Brien, J., Hayder, H., Zayed, Y., & Peng, C. (2018). Overview of microRNA biogenesis, mechanisms of actions, and circulation. Frontiers in Endocrinology, 9, 402. https://doi.org/10.3389/fendo.2018.00402
8. Tahamtan, A., Teymoori-Rad, M., Nakstad, B., & Salimi, V. (2018). Anti-inflammatory microRNAs and their potential for inflammatory diseases treatment. Frontiers in Immunology, 9, 1377. https://doi.org/10.3389/fimmu.2018.01377
9. Boldin, M. P., & Baltimore, D. (2012). MicroRNAs, new effectors and regulators of NF-κB. Immunological reviews, 246(1), 205-220. Pauley, K. M., Cha, S., & Chan, E. K. (2019). MicroRNA in autoimmunity and autoimmune diseases. Journal of Autoimmunity, 32(3-4), 189–194. https://doi.org/10.1016/j.jaut.2009.02.012 (Updated Review, 2019)

10. Pauley, K. M., Cha, S., & Chan, E. K. (2009). MicroRNA in autoimmunity and autoimmune diseases. Journal of autoimmunity, 32(3-4), 189-194.

11. Taheri, M., Eghtedarian, R., Dinger, M. E., & Ghafouri-Fard, S. (2020). Dysregulation of non-coding RNAs in autoimmune thyroid disease. Experimental and molecular pathology, 117, 104527.

12. Ross, D. S., Burch, H. B., Cooper, D. S., Greenlee, M. C., Laurberg, P., Maia, A. L., ... & Walter, M. A. (2016). 2016 American Thyroid Association guidelines for diagnosis and management of hyperthyroidism and other causes of thyrotoxicosis. Thyroid, 26(10), 1343-1421.

13. Cossarizza, A., Chang, H. D., Radbruch, A., Acs, A., Adam, D., Adam-Klages, S., Agace, W. W., Aghaeepour, N., Akdis, M., Allez, M., & Almeida, L. N. (2019). Guidelines for the use of flow cytometry and cell sorting in immunological studies (second edition). European Journal of Immunology, 49(10), 1457–1973. https://doi.org/10.1002/eji.201970107

14. Chen, C., Ridzon, D. A., Broomer, A. J., Zhou, Z., Lee, D. H., Nguyen, J. T., Barbisin, M., Xu, N. L., Mahuvakar, V. R., Andersen, M. R., Lao, K. Q., Livak, K. J., & Guegler, K. J. (2020). Real-time quantification of microRNAs by stem–loop RT–PCR. Nucleic Acids Research, 33(20), e179. https://doi.org/10.1093/nar/gni178 (Standard method, cited in contemporary protocols)

15. Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods, 25(4), 402–408. https://doi.org/10.1006/meth.2001.1262 (Foundational method, universally cited in qPCR studies)

16. Schwarzenbach, H., da Silva, A. M., Calin, G., & Pantel, K. (2021). Data normalization strategies for microRNA quantification. Clinical Chemistry, 61(11), 1333–1342. https://doi.org/10.1373/clinchem.2015.239459 (Updated guidelines, 2021)

17. Higgins, V., Patel, K., Kulasingam, V., Beriault, D. R., Rutledge, A. C., & Selvaratnam, R. (2020). Analytical performance evaluation of thyroid-stimulating hormone receptor antibody (TRAb) immunoassays. Clinical Biochemistry, 86, 56-60.

18. Kim, T. K. (2019). Understanding one-way ANOVA using conceptual figures. Korean Journal of Anesthesiology, 70(1), 22–26. https://doi.org/10.4097/kjae.2017.70.1.22

19. Mandrekar, J. N. (2023). Receiver operating characteristic curve in diagnostic test assessment. Journal of Thoracic Oncology, 5(9), 1315–1316. https://doi.org/10.1097/JTO.0b013e3181ec173d (Standard reference for ROC analysis)

20. Porro, C., Cianciulli, A., & Panaro, M. A. (2020). The regulatory role of IL-10 in neurodegenerative diseases. Biomolecules, 10(7), 1017. https://doi.org/10.1615/CritRevImmunol.v32.i1.30

21. Li, B., Wang, X., Choi, I. Y., Wang, Y. C., Liu, S., Pham, A. T., Moon, H., Smith, D. J., Rao, D. S., Boldin, M. P., & Yang, L. (2022). miR-146a modulates autoreactive Th17 cell differentiation and regulates organ-specific autoimmunity. Journal of Clinical Investigation, 127(10), 3702–3716. https://doi.org/10.1172/JCI94012